Deadline for Proposals for SOT 2013 Annual Meeting Sessions: April 30, 2012

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 emerging fields and how they apply to toxicology.

2013 Thematic Approach and Continuing Education Target Areas

Themes
- Application of Systems Biology to Toxicology
- Biomarkers for Exposure Assessment, Safety Evaluation, and Translational Medicine
- Effects of Nanomaterials on Biological Systems
- Molecular Basis of Genetic Variability and Susceptibility to Toxicants
- Regulatory Science: Advancing New Approaches for Hazard Identification and Risk Assessment

Continuing Education Target Areas
- Developmental Origins of Health and Disease (DOHaD)
- Molecular Imaging
- Personalized 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
- Regional Interest Session—Central topics of relevance that describe public health and/or ecological problems of a particular region

Submit your proposal online at www.toxicology.org
Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 51st Annual Meeting of the Society of Toxicology, held at the Moscone Convention Center, March 11–15, 2012.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 591.

The issue also contains a Key Word Index (by subject or chemical) of all the presentations, beginning on page 617.

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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This abstract book has been produced electronically by ScholarOne, Inc. Every effort has been made to faithfully reproduce the abstracts as submitted. The author(s) of each abstract appearing in this publication is/are solely responsible for the content thereof; the publication of an article shall not constitute or be deemed to constitute any representation by the Society of Toxicology or its boards that the data presented therein are correct or are sufficient to support the conclusions reached or that the experiment design or methodology is adequate. Because of the rapid advances in the medical sciences, we recommend that independent verification of diagnoses and drug dosage be made.
Over the last two decades, alternatives to animal testing were strongly driven by animal welfare considerations. A culture of organotypic cell models, quality assurance and validation developed, which resulted in a number of novel approaches for regulatory testing. Progress to replace especially the systemic and chronic types of tests has been limited. Novel programs to assess large number of substances such as existing chemicals (REACH and the emerging TSCA e生态圈化), nanoparticles or mixtures, as well as new products such as biologicals and cell therapies now add to the need to move to another approach for toxicology testing. Additionally, interest in health effects like endocrine disruption, developmental neurotoxicity, immunotoxicity, obesity, attherosclerosis or childhood asthma require extensive and new types of testing. This is often referred to as Toxicity Testing for the 21st Century (Tox-21c), after the respective NAS vision document from 2007, which has been made EPA’s toxicity testing strategy in 2009. The central change is moving from apical “black box” animal models to mechanism or pathway of toxicity (PoT). The biotechnology and bioinformatics revolution of recent years has made it possible to develop systems biology, here systems toxicology, approaches. The experiences from the field of alternative methods now prove to be the most important to implement a new regulatory approach. Standardization and validation of cell cultures is crucial for PoT identification as well as the implementation of high-throughput type of tests based on PoT. The first projects to systematically map the entirety of human PoT, the human Toxome, have started. The validation of these novel tests represents an enormous challenge. It is proposed to follow the role model of evidence-based medicine. For this purpose, the evidence-based toxicology collaboration was started at SOT 2011 and is currently shaping its procedures and governance.

Biomarkers serve as quantitative measures of chemical exposures and biologically effective doses, provide early warning signals of biologic effect, predict outcome in a patient with disease, and identify who will respond to an intervention and whether the intervention is working. The current era of scientific discovery has brought seemingly limitless opportunities for improvements in medical care. Translational biomarkers that can be measured in blood or urine in both experimental animals and man are of particular interest. Given the importance to the clinical, pharmaceutical, and regulatory communities motivated by more specific and timely diagnoses, early intervention and safer therapies, clinically useful biomarkers have evolved over time, reflecting the scientific and technologic progress made over the centuries. An increasing number of clinically relevant tests and procedures are available to estimate organ injury and guide treatment. The use of molecular signals in the assessment of health and disease is not new; however, the concept of what constitutes a useful biomarker has evolved considerably in the past two to three decades given the advanced enabling technologies, deeper molecular understanding of disease, and the advent of a regulatory framework for biomarker qualification. Our panel experts will highlight the potential of these molecular signals in the assessment of health and disease, and the advent of a regulatory framework for biomarker qualification. Our panel experts will highlight the potential of these molecular signals in the assessment of health and disease, and the advent of a regulatory framework for biomarker qualification. Our panel experts will highlight the potential of these molecular signals in the assessment of health and disease, and the advent of a regulatory framework for biomarker qualification. Our panel experts will highlight the potential of these molecular signals in the assessment of health and disease, and the advent of a regulatory framework for biomarker qualification. Our panel experts will highlight the potential of these molecular signals in the assessment of health and disease, and the advent of a regulatory framework for biomarker qualification.

Embryonic and fetal development in mammalian species is a complex process which is sensitive to the effects of maternal and environmental factors. The timing of development of the major organ systems varies between humans and other animal species, but the basic biology of development is similar in all species thus allowing extrapolation of animal testing results for xenobiotics to humans. The course will begin by providing an overview that highlights developmental biology from fertilization of the gametes to normal maturation of a full term placenta and fetus including examples of developmental toxicants and teratogens with known modes of action. Subsequently, applied toxicology concepts for evaluation of the potential for bioaccumulation of chemicals to affect pregnancy and embryofetal development will be discussed. Global regulatory strategies and requirements to minimize health effects on women and unborn children will also be addressed. Finally, key information will be presented to provide for a better understanding of the biological and toxicological basis of prenatal developmental toxicity testing and the impact of various outcomes on drug development, chemical use, environmental impact, and human health risk.

Skin is the largest external organ and serves as a living, dynamic protective envelope surrounding the body. As such, it is constantly exposed to environmental hazards, including hazardous compounds; these exposures account for a major portion of all reported industrial illnesses. Skin exposures may also occur from pharmaceuticals or consumer products that are intentionally applied. In vitro methods are important as a first step to estimate skin permeation, and the potential of skin irritation and senescent chemical sensitization for compounds or mixtures of compounds that are directly toxic to the skin or systemically toxic. In exploration of these issues we will provide an overview of the current status of in vitro models for cutaneous toxicity safety evaluations and the regulatory requirements for establishing the nonclinical safety of dermal drug products. This important topic has relevance to toxicologists involved in safety evaluations and risk assessments for chemicals that contact the skin.

With the integration of open source programs, in silico tools, and bioinformatics, the role of the computer continues to transform daily activities and work for the modern scientist. Furthermore, the call for reduced animal testing in toxicity evaluation led to an expansion of in silico resources, quantitative structure-activity relationship (QSAR) programs, chemoinformatics systems, and predictive metabolism tools. Regulatory authorities, pharmaceutical, chemical, and food industries are actively using such tools in the safety evaluations of novel drug candidates, food additives, environmental contaminants and consumer products. We will begin by providing a basic introduction to various in silico tools, specifically predictive toxicology and metabolism platforms, and how their algorithms compute results and influence the decision process. A composed set of didactic lectures will be used to introduce the basic concepts and activities surrounding the use of in silico tools in ADME/Tox and safety studies. In follow-up participants will be provided with a brief summary of various platforms with practical tutorials of the computational toxicology platforms discussed.

The WHO/IPCS harmonized guidance document is the first compendium guidance document for risk assessment in toxicology. Immunotoxicity risk assessment of chemicals is an evaluation of the potential for unintended effects of chemical exposure on the immune system. These effects manifest as four principal types of immunotoxicity which are categorized as immunosuppression and stimulation, autoimmunity, and sensitization. We will provide an overview of the methods used to detect and characterize immunotoxicity and the potential consequences of unintended immunomodulation. It is well established that xenobiotic-related immunomodulation can lead to reduced resistance to infections and certain neoplastic diseases. Exposure to xenobiotics has been shown to be associated with development or worsening of autoimmune disease. It has been established that xenobiotics can
elicit hypersensitivity responses directly as an allergen, or they can enhance the in-duction or severity of allergic sensitization to pollen or dust mites. The determina-tion of risk associated with immunostimulation may be more difficult, but unex-pected stimulation should not be disregarded as it may result in nonspecific inflammation or the skewing of normally protective immune responses to favor in-duction or exacerbation of autoimmunity and hypersensitivity. The fundamental concepts of risk assessment as they apply to the evaluation of immunotoxicity as well as the application of the guidance will be highlighted. We will begin by re-viewing case studies which include data that focuses on different areas of immuno-toxicity—suppression, sensitization, and autoimmunity. The studies will demon-strate application of the guidance, particularly the development of weight of evidence conclusions from the available data. Finally, we will illustrate that risk as-sessment for a given chemical should consider the full range of immune effects for that chemical, and data should be evaluated separately for evidence of suppression, stimulation, autoimmunity, and sensitization.

7 STEM CELLS IN TOXICOLOGY.
E. J. Tokar and M. P. Waalkes, NIEHS, Research Triangle Park, NC.

Stem cells are revolutionizing toxicological research and remain an area with tremendous potential. Recently, research on stem cells has generated tremendous public and professional interest. However, some areas of toxicological research have lagged behind in the integration of stem cells as a concept in toxicant-induced disease etiology. We will describe the utility and suitability of the assorted types of stem cell models (i.e. embryonic, fetal, progenitor, induced pluripotent, immortal-ized stem cell lines, etc.) for various research purposes, including disease modeling, drug discovery, and toxicity testing in order to describe the potential applications of stem cells in toxicological research. This important overview of stem cells will high-light their nomenclature, properties, and their roles in the genesis of various dis-eases.

8 CONCEPTS OF GREEN CHEMISTRY AND ITS ROLE IN THE IDENTIFICATION AND DESIGN OF SAFER CHEMICALS AND PRODUCTS.
P. J. Spencer1 and J. Warner2, 1DuPont Chemical Company, Midland, MI and 2Warner Babcock Institute for Green Chemistry, Wilmington, MA.

Hazard identification, dose-response characterization, and exposure potential are the underpinning of product safety assessments. These basic principles help regula-tory agencies, manufacturers, and formulators determine the conditions for safe use of chemicals, raw materials, and products for a given application to reduce adverse impacts to human health and the environment. Today, as a part of the growing in-terest in green chemistry, the pendulum is shifting. The large number of companies engaging in sustainability initiatives coupled with increased consumer demand for greener products is driving a new process where impacts of chemical products and processes are included as design criteria. Reducing intrinsic chemical hazards up front is a strategy used in developing safer alternatives to existing chemicals. Thus, green chemistry is raising the bar for chemical safety assessments. Our panel of ex-perts will begin with a background of green chemistry, its basic principles, why it is useful and highlight key certification programs/tools used to identify safer alterna-tives including their methods and criteria with specific emphasis on the Green Screen for Safer Chemicals alternatives assessment tool. There are unique opportu-nities for toxicologists to assist molecular designers in reducing the intrinsic hazards of their molecules by providing insight into toxicological mechanisms and data that support the application of green chemistry principles in the design of new chemi-cals and products. To underscore the importance of this issue, we will illustrate how principles of green chemistry are applied in a consumer products and a chemical company. The caveats and challenges will be addressed by using case studies. The exploration of this topical area will provide an understanding of green chemistry, awareness of the tools and programs immediately available and how to access and use them as well as an appreciation for some of the practical challenges associated with implementing principles of green chemistry into product development and as-sessments of safer alternatives.

9 INNATE IMMUNITY AND ITS RELEVANCE TO TOXICOLOGY.
W. J. Freebern1 and J. M. Shenton2, 1Immunotoxicology, Bristol-Myers Squibb, North Brunswick, NJ and 2MedImmune, Inc., Cambridge, United Kingdom.

The innate immune system is the host’s first line of defense against infection. Thus, knowing the what, why, how, and when of innate immune function assessment in toxicology evaluations is important. This course will introduce the components of the innate immune system and its role in host defense, discuss clinical observations resulting from inhibition or stimulation of innate immune function in non-clinical species, provide case examples where understanding intentional or inadvertent ef-fects on the innate immune system has had utility in toxicity testing, and explain the what and how of innate immune measurements and the gaps in capabilities thereof. Innate immunity assessments to be discussed include bacterial killing assays and an array of macrophage, neutrophil, and natural killer cell activity assessments which will be described within the context of various target organs, animal models, and toxicity programs. In addition, investigating innate immune function on a mole-cular level through evaluating cell signaling molecules and regulated expression of anti-microbial peptides, chemokines, and cytokines will be discussed. In closing, the application of innate immunity testing in the clinic and translatability of non-clinical findings to the clinic will be examined. This course should be of broad in-te rest to Toxicologists with a desire to learn about the complex innate system and how innate immune evaluations can be applied to toxicology testing. In addition, the course will appeal to scientists who are interested in learning methodologies of innate immune function testing and applicability thereof.

10 MICRORNAS IN BIOLOGY AND TOXICOLOGY.
N. Aluru1 and C. J. Marsili2, 1Biology, Woods Hole Oceanographic Institution, Woods Hole, MA and 2Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH.

MicroRNAs (miRNAs) constitute a critically important class of non-coding, small RNA molecules which post-transcriptionally regulate gene expression. miRNAs, approxi-mately 18-24 nucleotides (nt) in length, regulate gene expression by binding to 3’untranslated regions (UTR), coding sequences or 5’UTR of target messenger RNAs (mRNAs), and leading to inhibition of translation or mRNA degradation. It is estimated that miRNAs regulate approximately 30% of the human protein-coding genome. miRNAs control the expression of genes involved in several biological processes, including apoptosis, proliferation, differentiation, and metastasis. Given the prominent role miRNAs play in organismal function, it is not surprising that the aberrant expression of miRNAs can lead to a wide range of human diseases and disorders, including cancer, neurodegenerative diseases, diabetes and a variety of cardiovascular and hepatic disorders. In addition to contributing to the underlying cause of a particular disease, miRNAs can also represent potential therapeutic tar-gets and diagnostic biomarkers. The recently discovered of circulating miRNAs are promising biomarker candidates since they can be detected from readily attainable blood samples. Because of the critical role that miRNAs play in biological function and the diverse range of applications in which miRNA analysis is of value, signifi-cant effort has been invested over the past decade to develop new detection meth-ods. We will provide an overview of existing and emerging tools for miRNA analy-sis, with particular emphasis placed on the current state of the art and important developments in this emerging field.

11 REGULATORY SCIENCES: PRECLINICAL DRUG DEVELOPMENT FROM SMALL MOLECULES TO BIOLOGICS.
T. Wang1 and W. McGuinness2, 1Preclinical Safety, Novartis Pharmaceuticals, Emerpixelle, CA and 2US FDA, Silver Spring, MD.

Drug development is a highly regulated but science-driven process from early dis-cover to marketing. Once the drug candidate has been discovered, preclinical safety evaluations are required to ensure the safety of the drug during clinical trials, ultimately bridging the gap between drug discovery and marketing. The preclinical development of four types of drugs, small molecular-weight drugs, biologics, oligonucleotide-based therapeutic drugs and antibody drug conjugates (ADCs), will be illustrated to emphasize the cross-functional nature of regulatory sciences. In exploration of this important topic, we will begin with a focus on small molecular-weight drug candidates, which require a sliding scale for the degree of development from relatively minimal regulatory requirements for oncology drug development to the much more stringent requirements for the development of drugs for non-life-threatenning conditions and chronic treatment. Next the focus will shift to large molecule biologics and will provide illustrations of the unique challenges in regula-tory safety assessment and clinical development that biologic drug candidates pres-ent. Our panel of experts will then focus on oligonucleotide-based therapeutics since many of the overarching oligonucleotide class-based properties have been well estab-lished, but unique considerations remain for each subclass of oligonucleotide. This talk will discuss the preclinical development of oligonucleotide-based therapeu-tic drugs, including antisense, siRNA, immunostimulatory and aptamer applica-tions. At last this course will also cover ADCs, which are comprised of mono-clonal antibodies (biologics) conjugated with drugs or cytotoxins (small molecules). Standard approaches for preclinical safety evaluation of each of the individual com-
in utero and postnatal development is uncertainty regarding the actual exposure in the target subpopulation. We will demonstrate the value of PBPK modeling in quantitative health risk assessments for infants and children by providing a scientifically sound tool to predict the target tissue dose in the young. A thorough understanding of dynamic changes in physiological and biochemical factors is essential to predict the target tissue exposure during development. Factors that influence the kinetic behavior of chemicals in early life include ontogeny in metabolizing enzymes, changes in transporter expression, maturation of biological barriers such as the blood brain barrier, differential growth of tissues, and distinct exposure patterns compared to adults. PBPK modeling provides a means to integrate these factors in the proper context and thus reduce uncertainty in conducting risk/safety assessment for early life. The presentations will provide an overview of pharmacokinetic factors affecting early life sensitivity and two case studies of PBPK approaches for gestation/lactation and childhood exposures, plus a demonstration of how PBPK modeling of development can be used to evaluate neonatal epidemiological results. The course participants will get an in-depth understanding of the value of PBPK modeling in addressing issues of potential sensitivity in infants and children and the possible application scenarios of this valuable tool.

**15 DIETARY SUPPLEMENT ADULTERATION–A CLINICIAN’S PERSPECTIVE.**


A 12-year-old child develops bleeding from his gums and a diffuse rash. A 30-year-old man presents to the hospital with a new onset seizure that is resistant to therapy. A 77-year-old man is found in cardiac arrest after taking an herbal medication for his shortness of breath. What do all of these cases have in common? Each of these patients was using a dietary supplement that contained a toxic ingredient, though in each case the intent was markedly different. Herbal or cultural products may be intentionally adulterated with conventional medications known to be therapeutic for a given disorder in an effort to assure effectiveness. An example is the inclusion of an analgesic for a pain syndrome, Indomethacin and phenylbutazone are just two examples of nonsteroidal anti-inflammatory drugs that have been implicated in many adverse clinical outcomes. Herbal medications may contain a small quantity of a known toxic principle added for a purported benefit that has a historical, not scientific, basis. True to the tenet that “dose makes the poison” these compounds are themselves directly toxic following more substantial exposures. Due to the relatively poor quality control standards required of largely unregulated dietary supplements, dangerous exposures may occur. Examples include the use of mercury in Chinese herbs or lead in Ayurvedic (Indian) medications. Furthermore, excessive exposure to a pharmacologically active ingredient from a plant may result in an unanticipated side effect. An example is exposure to diethylene glycol from Digitalis plants or aconitine from Monkshood (Aconitum sp.), both used in the practice of classical herbal medicine and both associated with cardiac arrhythmias. These examples highlight the potential risks in the use of poorly regulated dietary supplements, and should focus efforts on the amelioration of the conditions that allow this dangerous situation to persist.

**16 DIETARY SUPPLEMENT ADULTERATION: CANADIAN REGULATOR’S PERSPECTIVE.**


In the age of a global economy, and the widespread international movement of products, poor quality ingredients manufactured in one country can impact on a multitude of finished products elsewhere in the world. The intentional manufacture of adulterated dietary supplements has become a significant global problem which now only creates risks to the health of consumers, but also consumer uncertainty and suspicion directed towards dietary supplements. Proper quality control and ingredient testing by manufacturers plays a crucial role in protecting the public from adulterated dietary supplements. Another key activity in the protection against adulterated products is the surveillance of adverse reactions (ARS) reported to domestically available products. It is also useful to monitor the activities of foreign regulatory agencies, to detect issues of product adulteration in other countries, which may only become relevant if consumers import these products or if they are sold in Canada. Health Canada carries out routine monitoring of both the domestic and international situations, and informs the Canadian public as necessary. ARs are reported to Health Canada through the Canada Vigilance program. Issues of product adulteration have been detected during the monitoring of AR information. In these situations, suspicion of adulteration may lead to the collection of the product in question, and analysis by Health Canada. Examples where domestic adverse reactions actions have led to the detection of product quality issues include adulteration of sexual enhancement products (sildenafil/analogaues), sleeping aid products (estazolam), and anti-inflammatory/arthritis products (dexmethasone). In these situations, Health Canada issues risk communications to inform the public, and to reduce the risk associated with use of these products. Health Canada also actively monitors risk communications issued by foreign regulatory agencies, related to adulterated products.
US REGULATORY PERSPECTIVES ON THE INTENTIONAL ADULTERATION OF DIETARY SUPPLEMENTS.


FDA has, since 2008 identified almost 300 tainted products marketed as "dietary supplements" that contain undeclared or mislabeled drugs or other chemicals. The hidden ingredients include various combinations of prescription drugs and analogues, controlled substances, drugs withdrawn for safety reasons, IND drugs, foreign drugs not approved in the US and other untested chemicals. FDA has received numerous reports of serious injury and even deaths associated with these products. As announced in April, 2011, more than half of all Americans take a dietary supplement. Thus the potential for a large scale public health catastrophe with intentionally adulterated products is significant. The agency is using tools at its disposal to address the issue and developing new targeted strategies and techniques to prevent further intentional adulteration that places public health at risk.

QUALITY STANDARDS: SPECIFICATIONS ON PURITY AND AUTHENTICITY.

J. C. Griffiths, Global Services, US Pharmacopeia, Rockville, MD.

Quality standards or compendial standards provide an independent, science-based measure of criteria regarding purity and authenticity to industry, the regulators and by extension, the consumers. An international compendium of quality specifications for dietary supplement ingredients and dosage forms in commerce would support current Good Manufacturing Practices (cGMP) initiatives, and supply a neutral measure of authenticity, purity, strength of these frequently adulterated products. Compendial standards are comprised of relevant acceptance criteria defining the authenticity, appropriate analytical methods to verify these criteria, and in many cases, authenticated physical reference standards to verify the appropriate execution of the test method. A compendium providing all three components to everyone involved in the supply chain of dietary supplements, will enhance product safety through a predictable and stable supply chain of verifiable authentic products to everyone involved in the supply chain of dietary supplements, will enhance product safety through a predictable and stable supply chain of verifiable authentic materials to an industry that ultimately aims at producing healthy and safe products to consumers. One such example, the Dietary Supplements Compendium (DSC) prepared and published by the United States Pharmacopeia (USP) will be detailed, demonstrating the information provided and the processes by which additional dietary ingredients and dietary supplement dosage forms are added.

DNA DAMAGE RESPONSES AND REPAIR.

R. S. Paules1 and L. D. Samson2, 1NIEHS, Research Triangle Park, NC and 2Massachusetts Institute of Technology, Cambridge, MA.

Exposure to agents that have the potential to damage DNA occurs every day and is in fact a normal part of life. As a consequence, extremely efficient processes have developed that protect the genome from deleterious alterations by recognizing DNA damage in what is referred to as the DNA Damage Response (DDR) and initiating a series of cellular signal transduction pathway responses that lead to a coordination of regulation of cell cycle proliferation and repair of DNA lesions, if possible, or to the initiation of a cell death process, if appropriate repair is not possible. DNA damage can arise from both endogenous and exogenous sources, and, if not repaired properly, may lead to mutations, development of diseases such as cancer, cellular, or tissue toxicity, or ultimately to the death of the organism. Activation of the DDR results in the activation of the DNA damage response sensors ATM and ATR, which can then initiate an inhibition of cell cycle progression through the activation of a DNA damage checkpoint, in order to allow time for the repair of DNA lesions and to prevent the transmission of damaged or incompletely replicated genetic material. By repair of the DNA damage and restoration of the integrity of the DNA duplex, genomic stability can be maintained. DNA repair mechanisms that function to maintain genomic stability and to avoid toxicity include base excision repair, nucleotide excision repair, double-strand break repair, as well as translesion DNA synthesis. We will present important updates on the progress made in elucidating the consequences of genetic and pharmacological modifications of the levels and functions of DNA repair and response genes on both toxicities of exposures, as well as exciting possible new therapeutic approaches. We will focus on recent advances in understanding the molecular mechanisms of the DDR and DNA repair, both to provide molecular insight into toxicity and disease processes, and also potentially to provide novel targets for therapeutic strategies for treating individuals with certain diseases such as cancers.

IDENTIFYING TRANSLATIONAL COMPONENTS OF THE DNA DAMAGE RESPONSE.

T. Begley1, J. Aguirre-Ghiso1, P. Dedon1, M. Soledad Sosa2, U. Begley3, C. Chan4, M. Dyavahla5, Y. Estrada2, A. Patil6, A. Avivar-Valderas7 and K. Taghizadeh1, 1College of NanoScale Science and Engineering, University at Albany, SUNY, Albany, NY, 2Division of Hematology and Oncology, Mount Sinai School of Medicine, New York, NY, 3Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA, 4Department of Biomedical Engineering, Columbia University, New York, NY, and 5Laboratory of Structural Biology, NIEHS/NIH, Research Triangle Park, NC.

Transcriptional and post-translational signals are known mechanisms which promote efficient responses to DNA damage. Using high throughput screening and small molecule analysis approaches we have identified translational components of the DNA damage response, which operate through the modification of RNA. Our data indicates that enzyme catalyzed modification of RNA can be dynamically regulated after exposure to DNA damaging agents and we have developed and tested computational models to demonstrate their signaling role. Specifically we have shown that mRNA methylation of chromosome 9 (Trm9) from budding yeast and humans enhances the translation of transcripts over-represented with specific arginine and glutamic acid codons. In budding yeast, we have shown that translation of the DNA damage response transcripts ribonucleotide reductase 1 & 3, which are over-represented with specific arginine and glutamic acid codons, is dependent on Trm9 catalyzed RNA modifications. In human systems we demonstrate that Trm9 optimizes codon-specific translation and plays important roles in stress signaling, preventing toxicant-induced cell death and responding to environmental conditions. In addition we demonstrate that Human Trm9 is epigenetically silenced in colorectal cancer and that reactivating this gene by responding stress signaling. Ultimately our studies highlight a translation-associated mechanism that regulates gene expression during times of cellular stress and DNA damage.

COMPLEX RESPONSES OF MICE TO ALKYLATION AND INFLAMMATION-INDUCED TOXICITY.

L. D. Samson, Biological Engineering Department and Biology Department, Center for Environmental Health Sciences, MIT, Cambridge, MA.

In the last few years we have been characterizing the effects of modulating the level of DNA base repair enzymes on the sensitivity of various tissues to the effects of model alkylating agents, to inflammation. Human and rodent cells exposed to such agents from a variety of endogenous and exogenous sources, and we have simulated such exposures in knockout and transgenic mouse models. Surprisingly, we find that for wild type mice, specific cells in some tissues (for example certain postmitotic neurons in the retina and the brain) are extremely sensitive to alkylination-induced damage, while the same cells are completely resistant in animals that are knocked out for repair mediated by the Aag DNA repair glycosylase. Moreover, we find that the same is true for specific cells in other tissues that are undergoing an inflammatory response as a result of ischemia and reperfusion. These counter-intuitive findings are supported by the fact that these cells in Aag-transgenic mice, expressing higher than wild type levels of Aag, become super-sensitive to induced damage. We will discuss these findings in the context of the large inter-individual differences in AAG levels in human populations.
23 DAMAGE-SPECIFIC PATHWAYS FOR THE REGULATION OF POSTREPLICATION REPAIR.

Postreplication repair (PRR) pathways play important roles in restarting stalled replication forks and regulating mutagenesis. In yeast, Rad5-mediated damage avoidance and Rad18-mediated translesion synthesis (TLS) are two forms of PRR. Rad18 promotes PCNA monoubiquitination and the recruitment of TLS polymerases, while Rad5 forms lysine 63-linked ubiquitin chains on monoubiquitinated PCNA to promote damage avoidance through an unknown process. In mammalian cells, PCNA is also ubiquitinated in a Rad18-dependent manner, and two Rad5-related proteins, SHPRH and HLTF, have also been identified. However, the specific functions of these two proteins in response to DNA damage have not been clearly addressed. We have been investigating the roles of SHPRH and HLTF in PRR. Surprisingly, we find that SHPRH and HLTF act in non-redundant, damage-specific ways to suppress mutagenesis. SHPRH suppresses mutagenesis induced by MMS treatment, while HLTF suppresses mutagenesis induced by UV treatment.

These findings suggest that SHPRH and HLTF can discriminate between different types of DNA damage at a stalled replication fork. Our data also suggest that SHPRH and HLTF specifically recruit distinct, damage-specific TLS polymerases to stalled forks. The use of SHPRH versus HLTF appears to be regulated, at least in part, by a switch between Rad18-HLTF and Rad18-SHPRH complexes that is induced by DNA damage and the damage-specific degradation of HLTF. We therefore propose that different types of DNA damage specify the recruitment of the most appropriate TLS polymerase(s) by controlling the relative abundance of the different Rad18-Rad5 complexes at the fork. We will discuss this work and more recent findings about the mechanisms underlying damage-specific regulation of HLTF and SHPRH. Our work may have important implications for damage-specific mutagenesis and may also provide insight into how mutations in HLTF and SHPRH may influence cancer progression and treatment.

24 REDUNDANCY AND CROSS-TALK IN PATHWAYS AND FUNCTIONS IN DNA DAMAGE RESPONSES TO THE HIGHLY TOXIC DNA DOUBLE-STRAND BREAK.
R. S. Paules1, S. S. Paliit2, Y. Cui1, J. E. Hesse1, C. L. Innes1 and B. P. Sleckman2.
Environmental Stress & Cancer Group, Laboratory of Toxicology & Pharmacology, NIEHS, NIH, Research Triangle Park, NC and 2Department of Pathology & Immunology, Washington University School of Medicine, St. Louis, MO.

DNA double strand breaks (DSBs) are the most lethal form of DNA damage and can be generated by both endogenous and exogenous sources. In the DNA damage response (DDR), most cells have the capacity to activate a cell cycle checkpoint response to delay proliferation in order to allow time for repair of the DNA lesions or to activate a programmed cell death process. We and others have demonstrated substantial cross-talk between DDR pathways mediated by members of the PI3 kinase family, i.e. ATM, ATR, and DNA-PKcs. Using lentiviral expression of shRNAs, we have generated normal human mammary epithelial cell lines that have either stable or inducible knockdown of ATM, ATR and CHK1. As expected, depletion of either ATM, ATR or CHK1 by more than 90% results in impaired G2 checkpoint control and lower viability. Interestingly, CHK1 deficiency also results in enhanced phosphorylation of multiple DNA damage proteins following IR-treatment, most notably phosphorylation of CHK2 on Thr68, and this effect is modulated by both ATM and DNA-PKcs. Furthermore, these cell lines have allowed us to investigate a negative feedback from CHK1 to the ATM kinase involving protein phosphatases and potentially regulation by microRNAs. The results of these studies could be exploited to enhance the efficacy of therapeutic regimens for certain cancers. In addition, our genomic studies with mouse pre-B cells that DSBs, generated by physiologically relevant sources, arrest not only DDR pathways but also lymphocyte developmental pathways, a finding that could help to explain the immunotoxic effects of certain genotoxic agents.

This research was supported by the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences.

25 THE THICK AND THIN OF NUCLEAR RECEPTORS IN DIABETES AND OBESITY.
A. L. Slitt, Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI.

Xenobiotic nuclear receptors, such as CAR, PXR, AHR, FXR, and NRF2 are best known for transcription regulation and induction of Phase I and Phase II biotransformation enzymes, as well as transporters. These receptor pathways are of interest because they are activated by diverse chemicals—drugs, chemicals of environmental exposure, as well as endogenous chemicals such as hormones and bile acids. It is well established that there are chemical-receptor interactions—especially in the liver, which lead to transcriptional upregulation of gene batteries and result in coordinate regulation of metabolism and transport. However, recent evidence points to nuclear receptors and the role for these receptors in diseases related to metabolic syndrome, dyslipidemia, and glucose homeostasis. As the incidence of obesity and obesity-related diseases, which culminate in metabolic syndrome, increase worldwide, understanding the mechanisms of obesity is of high interest for human health. As these receptors are targeted by drugs and environmental chemicals, it is of importance to understand how activators of these xenobiotic receptors affect aspects of adipocyte differentiation, adiposity, and pancreatic function in adults, as well as sensitivity variations, specifically pregnant women and children. Our findings will be presented for various receptor-mediated pathways with regard to various obesity and diabetes models. Presentations will cover nuclear receptor-mediated gene induction and research findings related to nuclear receptors with regard to obesity and diabetes. Finally, findings will be presented regarding what is currently known regarding nuclear receptor function in caloric restriction, the practice most recommended to combat obesity and obesity-related disorders. The information presented will be useful to those interested in nuclear receptors, aspects of metabolic syndrome, mouse models of obesity and diabetes, aspects of metabolic syndrome and drug interactions, therapeutic drug targets, and environment-obesogen effects.

26 A NOVEL FUNCTION OF THE XENOBIOTIC RECEPTOR CAR IN OBESITY AND TYPE 2 DIABETES.
W. Xie. Department of Pharmaceutical Sciences, University of Pittsburgh School of Pharmacy, Pittsburgh, PA.

The constitutive androstane receptor (CAR) was initially characterized as a xenobiotic receptor. We have recently uncovered an unexpected endobiotic role of CAR in preventing obesity and alleviating type II diabetes. We showed that treatment of wild type mice with the CAR agonist TCPOBOP efficiently prevented diet-induced obesity from happening or reversed pre-induced obesity. Treatment with TCPOBOP improved insulin sensitivity in diabetic mice. In contrast, CAR null mice maintained on chow diet showed spontaneous insulin insensitivity. The metabolic benefits of CAR activation may have resulted from the combined effect of inhibition of lipogenesis, VLDL secretion and export of triglycerides, gluconeogenesis, and increases in brown adipose tissue energy expenditure and peripheral fat mobilization. In summary, our results have revealed an important metabolic function of CAR and may establish this “xenobiotic receptor” as a novel therapeutic target for the prevention and treatment of obesity and type II diabetes.

27 PARADOXICAL ROLES OF NRF2 ACTIVATION IN ARSENIC-INDUCED PANCREATIC ß-CELL DYSFUNCTION AND INSULIN RESISTANCE.
L. Pi. The Hammer Institute, Research Triangle Park, NC.

There is growing evidence that chronic exposure of humans to inorganic arsenic (iAs), a potent environmental oxidative stressor, is associated with the incidence of Type 2 Diabetes (T2D). Nuclear factor E2-related factor 2 (Nrf2) is a CNC-bZIP transcription factor that is well established as a master regulator in the cellular adaptive response to oxidative stress. Although cytosolic, reactive oxygen species (ROS) also function as important intracellular signaling molecules to activate cellular responses to a variety of physiological stimuli, including glucose-stimulated insulin secretion (GSIS) in pancreatic β-cells and insulin action in insulin responsive cells. Therefore, we propose that Nrf2-mediated antioxidant response plays paradoxical roles in β-cell function and insulin signaling transcription: (1) It protects the cells from oxidative damage and possible cell death, thus minimizing oxidative damage-related impairment in insulin secretion and action; (2) Since ROS signaling triggered by glucose could be an important component involved in insulin secretion and action, the induction of endogenous antioxidants in the presence of oxidative stress may blunt the signals, resulting in reduced GSIS and insulin resistance. iAs and its methylated trivial metabolites are potent oxidative stressors and robustly activate Nrf2-mediated antioxidant response, but at the levels typically observed in human exposures, they are not likely to reach cytosolic concentrations sufficient to cause overt oxidative damage, especially when endogenous antioxidant enzymes can be actively induced. Therefore, blockade of ROS signaling in pre-mice is potentially more relevant to the etiology of T2D in the context of low-level environmental iAs exposure, whereas premice 1 might be associated with protecting cells from acute toxicity induced by high doses of arsenic.
Bile acids play an important role in maintaining lipid, glucose and energy homeostasis. Our recent study shows that transgenic mice overexpressing Cyp7a1 are resistant to western high fat diet-induced obesity and diabetes. Bile acid signaling may activate insulin signaling to maintain glucose and lipid homeostasis. In diabetes, hyperglycemia stimulated CYP7A1 gene expression by epigenetic mechanisms and increased bile acid bile acid pool and serum bile acid concentrations. Bile acid derivatives may be potential therapeutics for diabetes, fatty liver diseases, and obesity.

Evolving, previously approved drugs are now serendipitously being found to have coepigenetics is still relatively young. As greater understanding of the epigenome is developed, therapeutic intervention in these disease areas have been identified. While pharmaceutical mechanisms have become more extensively characterized, new opportunities for epigenetic interventions have emerged. These include: (1) using small molecules to reverse epigenetic changes and (2) using pharmacological intervention in these epigenetic changes to modulate gene expression.

In recent years, epigenetic factors have been implicated in the etiology of various disorders. This talk will provide an overview of published studies that focus on the role of the above presented nuclear receptors in mediating processes involved in weight loss. Given the emerging role for the Nrf2-Keap1 pathway regarding diabetes and obesity, targeting the Nrf2-Keap1 pathway may be of therapeutic benefit to promote weight loss or augment weight loss during caloric restriction (CR). Studies have been conducted to evaluate the effect of CR on Nrf2 expression and activation in lean and obese mouse models. Overall, findings will be presented from CR studies carried out using Nrf2-null, Keap1-Knockdown, and transgenic ARE-hPAP reporter mice. Overall the findings will summarize use of these transgenic/knockout models to address the role of Nrf2-Keap1 in weight loss, gluconeogenesis, glucose tolerance, and insulin sensitivity. The talk will conclude with discussion about the potential for targeting nuclear receptors as weight loss therapies.

Methylation at the 5-position of DNA-cytosine (5mC), the histone code and non-coding RNAs contribute to epigenetic regulation. Additionally, a sixth DNA base, 5-hydroxymethylcytosine (5hmC) was identified recently. It is generated by the Ten-eleven Transcription Location (Tet) family of proteins through oxidation of 5mC. Thus, Tet modules DNA methylation levels. Furthermore, Tet exhibits a dual function in that these proteins can also play a role in regulating transcription. Additionally, components of the core promoter recognition complex change in a cell-specific fashion to turn on transcriptional program while turning off others; this provides a novel epigenetic mechanism. Interest in understanding the extent to which epigenetic changes might underlie toxicity/disease (as a causative or susceptibility factor), including adverse transgenerational effects, is increasing. This will provide a basis for discussing issues to contemplate when considering epigenetics and safety assessments, (e.g., parameters to be evaluated, genomic region(s) to monitor, methodological (ies), biological endpoint(s),) and what tools and model systems might be employed? Attention should be paid to fundamental principles of toxicology, (e.g., dose-response, criteria for a maximum dose, normal variability, and change vs. an adverse effect). Might some alterations be beneficial? Additionally, the background provided will facilitate conversations regarding identification of epigenetic "targets" that might be amenable to pharmacological manipulation.

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Multiple signaling pathways contribute to the regulation of hepatobiliary transporter expression and function. Altered expression of uptake and efflux transporters influences hepatic drug disposition and susceptibility to chemical-induced toxicity. In response to hyperglycemia, the Nrf2 transcription factor mediates up-regulation of ABC efflux transporters in livers of mice. Induction of these transporters is in part regulated by hepatocyte proliferation. Interestingly, up-regulation of ABC efflux transporters during diabetes is attenuated in mice in response to pregnancy, likely through disrupted FXR-Shop signaling. In this presentation, the physiological and transcriptional pathways regulating ABC transporter expression during diabetes and pregnancy, as well as the implications for drug therapy and xenobiotic toxicity, will be discussed.

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Weight loss and "dieting" is an effective therapy to reverse obesity and obesity-related disorders. This talk will provide an overview of published studies that focus on the role of the above presented nuclear receptors in mediating processes involved in weight loss. Given the emerging role for the Nrf2-Keap1 pathway regarding diabetes and obesity, targeting the Nrf2-Keap1 pathway may be of therapeutic benefit to promote weight loss or augment weight loss during caloric restriction (CR). Studies have been conducted to evaluate the effect of CR on Nrf2 expression and activation in lean and obese mouse models. Overall the findings will be presented from CR studies carried out using Nrf2-null, Keap1-Knockdown, and transgenic ARE-hPAP reporter mice. Overall the findings will summarize use of these transgenic/knockout models to address the role of Nrf2-Keap1 in weight loss, gluconeogenesis, glucose tolerance, and insulin sensitivity. The talk will conclude with discussion about the potential for targeting nuclear receptors as weight loss therapies.

In recent years, epigenetic factors have been implicated in the etiology of various diseases including cancers, psychiatric disorders, and diabetes/obesity. As epigenetic mechanisms have become more extensively characterized, new opportunities for therapeutic intervention in these disease areas have been identified. While pharmaceutical inhibitors of epigenetic targets such as histone deacetylases and DNA methyltransferases have recently gained regulatory approval, the field of pharmacoeigenetics is still relatively young. As greater understanding of the epigenome is evolving, previously approved drugs are now serendipitously being found to have epigenetic modulatory properties and are currently under evaluation for new indications. With the rapid emergence of multiple novel epigenetic targets for pharmacological inhibition, new considerations for the field of pharmacoeigenetics are becoming apparent as well. In order to address the potential toxicological consequences associated with pharmacological inhibition of potential therapeutic epigenetic targets, toxicologists are addressing the need for new models and endpoints to be considered in the safety assessment of epigenetic targets. These important toxicological issues, which may be unique to epigenetic targets, will be addressed.

Epigenetics refers to heritable mechanisms superimposed on DNA base sequence that regulate gene expression (thus, the term epi- (Greek: over, above) genetics). Methylation at the 5-position of DNA-cytosine (5mC), the histone code and non-coding RNAs contribute to epigenetic regulation. Additionally, a sixth DNA base, 5-hydroxymethylcytosine (5hmC) was identified recently. It is generated by the Ten-eleven Transcription Location (Tet) family of proteins through oxidation of 5mC. Thus, Tet modules DNA methylation levels. Furthermore, Tet exhibits a dual function in that these proteins can also play a role in regulating transcription. Additionally, components of the core promoter recognition complex change in a cell-specific fashion to turn on transcriptional program while turning off others; this provides a novel epigenetic mechanism. Interest in understanding the extent to which epigenetic changes might underlie toxicity/disease (as a causative or susceptibility factor), including adverse transgenerational effects, is increasing. This will provide a basis for discussing issues to contemplate when considering epigenetics and safety assessments, (e.g., parameters to be evaluated, genomic region(s) to monitor, methodological (ies), biological endpoint(s),) and what tools and model systems might be employed? Attention should be paid to fundamental principles of toxicology, (e.g., dose-response, criteria for a maximum dose, normal variability, and change vs. an adverse effect). Might some alterations be beneficial? Additionally, the background provided will facilitate conversations regarding identification of epigenetic "targets" that might be amenable to pharmacological manipulation.
The epigenome can be passed through the germ line to influence transgenerational inheritance. Recent studies suggested that chemical exposure of pregnant rodents can cause epigenetic changes in the (F1) concepts, which can be inherited by subsequent generations. To further explore this phenomenon, groups of 25 pregnant F0 CD-1 mice were administered diethylstilbestrol or estradiol from gestation day 9 to lactation day 20. Offspring were mated for two additional generations, with F1-F3 offspring evaluated for uterotrophic effects and vaginal patency, and a subset of animals evaluated at 18 months for uterine tumor incidence. This presentation provides an overview of our, and others, transgenerational epigenetic data for areas of concordance and discordance, as well as provides thoughts on how these findings are placed within the context of product safety assessment.
will provide a single unified data repository and analysis system that compiles available information on a substance, including: chemical structures and properties, regulatory records, toxicity studies, biological screening assays, (QSAR) models and application programming interfaces (APIs) to commercial (QSAR) tools. In cases where no information is available for a particular substance, CERES provides tools to identify potential safety concerns by applying mode-of-action-driven (QSAR) prediction models, computer models using structural alerts and, in the future, modified TTC categories, and for identification and analysis of data on structural and biological analogs (read-across). The presentation will describe the use of (QSAR) tools at OFAS and the future directions of (QSAR) within OFAS through the CERES system.

W 42 APPLICATION OF IN SILICO APPROACHES IN COSMETICS—JUNIPER BERRY OIL.
T. Re L’Oréal, Clark, NJ.

Natural, plant-derived botanical ingredients are increasingly being used in cosmetic and personal care products. Safety evaluation of these complex mixtures is a challenge due to factors such as a high degree of variability in composition. Where direct evidence of safety does not exist, it may be necessary to use Threshold of Toxicological Concern (TTC) and a weight-of-evidence (WoE) approach to evaluate safety. The first step in the safety evaluation of natural ingredients is to obtain a complete as possible characterization of chemical constituents. For each component, its maximum known concentration, its molecular weight, and LogP are used to estimate skin penetration potential and to estimate a maximal daily systemic exposure. Nominal exposure values are then compared with their respective TTC class, known NOAELs and/or food intake values of “no safety concern.” In cases where systemic exposure exceeds these values, additional in silico evaluation may be done. Read across using procedures such as the JECFA congeneric model and/or computer modeling to identify similar structures with known toxicity data may be used. In addition, potential structural alerts of individual components can be evaluated using commercially available databases – DEREK, OECD toolbox, Lead Scope, etc.). Therefore, a weight-of-evidence approach based on available toxicology data, extrapolated NOELs, and/or predictive in silico data can be used to evaluate the safe cosmetic use of complex plant-derived materials.

W 43 HIGH-THROUGHPUT IN VITRO TOXICITY TESTING: A MIDDLE-COURSE ASSESSMENT OF PREDICTIVE ACCURACY FOR IN VIVO ENDPOINTS AND USE IN DECISION-MAKING.
R. S. Thomas1 and W. D. Pennie,1 The Hamner Institutes for Health Sciences, Research Triangle Park, NC, and2 Pfizer, Inc., Groton, CT.

Over the past five years there has been a broad-based discussion on the future direction of toxicology and how safety testing is performed. This discussion has spawned multiple research efforts looking to use high- and medium-throughput in vitro screening data in identifying chemical hazards. For industrial and agricultural chemicals, research efforts in United States and Europe have characterized the in vitro biological activity of chemicals using multiple in vitro assays and technologies to predict in vivo toxicity and prioritize compounds for conventional toxicity testing. For pharmaceuticals, high-throughput in vitro screening assays have been integrated early into preclinical drug development to identify toxic components and guide medicinal chemistry efforts. In both cases, the application of in vitro toxicity screening for prioritization and hazard identification relies on its ability to accurately predict the results of in vivo laboratory animal studies and humans. The efforts in the United States and Europe are now several years old and a significant amount of data has been collected to provide an evaluation of the strengths and limitations of the using high- and medium-throughput screening for predicting in vivo animal responses. This session will be of broad interest to investigators and regulators looking to use in vitro assays for toxicological testing and safety assessment of environmental, industrial, and pharmaceutical chemicals.

W 44 A COMPREHENSIVE STATISTICAL ANALYSIS OF THE IN VITRO-TO-IN VIVO PREDICTIVE CAPACITY OF HIGH-THROUGHPUT TOXICITY SCREENS.
R. D. Wolfinger, SAS Institute Inc., Cary, NC.

The release of the US Environmental Protection Agency’s ToxCast Phase 1 dataset has provided an excellent opportunity to evaluate the ability of high-throughput in vitro screening assays to predict in vivo toxic endpoints. A broad-based statistical classification analysis was performed using a suite of different predictive modeling methods. The predictors include the results from the nearly 400 in vitro cell-based and biochemical assays and structural chemical descriptors. The in vitro effects to be predicted include hundreds of endpoints from the ToxRef database. Analysis of the results demonstrated that the in vitro cell-based and biochemical assays provided similar predictive performance compared with the structural chemical descriptors across the majority of the in vivo endpoints. The combination of in vitro cell-based and biochemical assays and structural chemical descriptors provided increased predictive performance over either type of data alone. For the in vitro cell-based and biochemical assays themselves, broad evaluation of predictive performance showed limited sensitivity, but high specificity for predicting the in vivo effects. These results offer mid-course insights for their utility in toxicity testing and decision making.

W 45 FEASIBILITY OF PREDICTING IN VIVO MODE OF ACTION USING PATHWAY-BASED IN VITRO SCREENS.
R. S. Judson, National Center for Computational Toxicology, US EPA, Research Triangle Park, NC.

The US EPA is carrying out a large scale project called ToxCast, one goal of which is to build toxicity signatures. These are intended to be predictive models that link in vitro assay results for chemicals with whole animal in vivo toxicity outcomes. From Phase I of ToxCast (309 data rich chemicals), we have developed signatures for several cancer endpoints (e.g., liver and thyroid), reproductive fitness and developmental toxicity endpoints. Phase II data (700 chemicals combing data rich and data poor subsets) is allowing us to test the forward predictive power of the original signatures and to make further refinements. This talk will describe signature development approaches including hurdles to successful development of highly predictive models. These issues include multiplicity of modes of action (e.g. for cancer) leading to statistical power issues; problems with lack of metabolism in most in vitro assays; the need for pharmacokinetic modeling; inherent noise in in vitro assays; and the lack of realistically organotypic cell systems. Despite these inherent limitations, we have examples of useful signatures that link specific key molecular events that can be probing in vitro assays to predict in vivo modes of action. We illustrate this linkage using the example of pathways that help drive angiogenesis, which play roles in both chemically-driven developmental defects and cancer progression.

W 46 EVALUATING THE EFFECT OF DOSIMETRY ON THE IN VITRO-TO-IN VIVO PREDICTIVE CAPACITY AND RELATIVE SENSITIVITY OF THE TOXCAST SCREENS.
B. A. Wetmore, The Hamner Institutes for Health Sciences, Research Triangle Park, NC.

The current ToxCast Phase I project has evaluated 309 chemicals across 500 in vitro cell-based and biochemical assays. The results from the assays have been summarized based on the half-maximal activity concentration (AC50) or lowest effective concentration (LEC) which characterizes the responses based on nominal chemical concentrations in the well. The AC50 and LEC summary values are being used to predict the in vivo toxic endpoints; however, the use of nominal chemical concentrations does not account for pharmacokinetic factors in predicting in vivo toxicity and no overall evaluation has been performed to assess the relative sensitivity of the in vitro assays compared to in vivo responses. In this study, metabolic clearance was measured in rat primary hepatocytes and plasma protein binding was measured in rat plasma for a subset of 59 ToxCast chemicals. Computational in vitro-to-in vivo extrapolation methods and reverse dosimetry were then used to estimate rat oral equivalent doses required to produce steady state in vivo concentrations equivalent to in vitro AC50 and LEC values in each of the ToxCast assays. For each chemical, the rat oral equivalent dose from the most sensitive in vitro assay was compared with the low effect level from the most sensitive endpoint in the in vivo studies in the ToxRef database. The results suggest that the most sensitive in vitro assay is 10- to 15-fold more sensitive than the apical responses in vivo. When the oral equivalent doses for all the in vitro assays were used in a partition tree classification model to predict the in vivo endpoints, an increase in model performance based on the area under the curve (AUC) of the ROC curve was observed for more than half of the endpoints. However, the increase in the AUC was relatively small suggesting the gain in performance was minimal. Inherent pharmacodynamic differences between the in vivo human bioactivities and in vivo rat endpoints assessed are being explored as factors that may be compromising the analyses.

W 47 PREDICTING IN VIVO TOXICITY USING IN VITRO ASSAYS AND CHEMICAL PROPERTIES IN PRECLINICAL DRUG DEVELOPMENT.

The incorporation of predictive toxicity approaches in early preclinical drug development will be important for reducing the attrition rate within the industry. To identify potential toxic compounds and guide medicinal chemistry efforts, targeted
in vitro assays have been integrated with structure activity-relationship rules and physicochemical properties to predict in vivo effects. The results have demonstrated that selecting appropriate cell systems that predict the mechanisms of toxicity are key factors that determine the successful prediction of in vivo effects while predicting organ-specific toxicity remains a challenge. In profiling drug-like molecules, we have considered multiple mechanistic endpoints (cell death, apoptosis, promiscuity against secondary targets, microchimeric endpoints, etc.) in cell lines of different tissue origins (e.g. transformed cell line of hepatic or cardiac origin, or using stem cell derived specific cell lineages). In these experiments, we have established not only the technical performance of mechanistic cell-based assays, but also the impact of cellular background on predictivity. In building predictive models particular challenges exist in the careful annotation of the training data set and building data bases of well annotated and accessible data. Finally, the need to balance an understanding of transporter interactions, metabolism and intended dose of the compound are all shown to have an effect on predictive model performance. In this regard, we have built computational models that incorporate dose prediction as well as chemical properties in an attempt to guide early medicinal chemistry to safer chemical space.

W 48 USING IN VITRO MODELS TO PREDICT REPRODUCTIVE AND DEVELOPMENTAL TOXICITY: A MIDCOURSE ASSESSMENT OF THE EUROPEAN REPROTECT PROGRAM.

M. Schwarz, Institute of Experimental and Clinical Pharmacology and Toxicology, University of Tuebingen, Tuebingen, Germany. Sponsor: R. Thomas.

An integrated project called ReProTect (www.reprotect.eu) was run from 2004 to the end of 2009, which was funded by the European Commission within the 6th Framework Program for research. The project assembled 33 European partners from academia, small and medium-sized enterprises, governmental institutions and industries. The aim of ReProTect was to develop or improve in vitro assays for their integration into a testing strategy suited to provide detailed information on the hazard of compounds to the mammalian reproductive cycle. At the end of the project more than 20 different tests were developed, predicting effects on spermatogenesis, folliculogenesis, germ cell maturation and fertilization, steroidogenesis, the endocrine system, the pre-implantation embryo, placentaion, uterus function, and embryonic development. Standard operating procedures for each of the tests have been produced and more than 150 peer-reviewed reproducitive toxicants have been tested. In the final year of the project, a ring trial, termed the “Feasibility Study”, was conducted in order to challenge the in vitro tests of ReProTect. A set of 10 test chemicals were investigated under blinded conditions by the participants of the Feasibility Study in their respective assays. The outcome of the study revealed that this preliminary test battery was able to correctly predict most of the in vitro effects on fertility and embryonic development, while only few predictions were incorrect for explainable reasons. The Feasibility Study of ReProTect has constituted a paradigm of alternative testing batteries and will guide future approaches in this field. Its success is a major step towards reduction and replacement of animal experiments and portrays the main achievements of the EU project.

W 49 THE EPIDIDYMIS, THE FORGOTTEN TARGET OF TOXICANTS.

D. Cry1 and R. E. Chapin2, Institute Armand-Frappier, INRS, Laval, QC, Canada and Drug and Safety Development, Pfizer, Groton, CT.

The epididymis is the major component of the testicular excurrent duct system. Testicular input to the tissue is conveyed via the efferent ducts, which anastomose to form a single, highly convoluted epididymal duct. The epididymis can be divided into two main compartments: the epithelium and the lumen. In the lumen, the contents of sperm that are bathed in luminal fluid whose composition varies markedly along the tissue. The blood-epididymis barrier, formed by epithelial principal cells, regulates this luminal environment and distinguishes it from blood. Functional sperm maturation in the epididymis is the result of their exposure to this luminal environment. Thus, the ability of the epididymis to provide the appropriate milieu for sperm maturation is critical. This is created by several processes, most notably the highly absorptive and secretory activities of the epithelial cells that line the duct. Many epididymal functions are either androgen or estrogen-dependent. The critical functions of the epididymis for sperm maturation and its reliance on hormonal regulation make it a prime target for toxic action. Several studies have shown that endocrine-disrupting chemicals, such as phthalates, can alter the development of the epididymis, and, in extreme cases, lead to its absence. Other chemicals, such as dioxins, affect sperm maturation via alterations to epididymal functions. However, epididymis frequently is ignored in toxicity studies. Yet, posttesticular and idiopathic male infertility represents a significant problem, suggesting that alterations in epididymal sperm maturation may have greater significance than previously thought. This session will provide an overview of epididymal functions and regulation and show examples of how environmental toxicants may alter male fertility by targeting the epididymis.
The final stages of sperm maturation occur in the epididymis, where sperm acquire the ability to swim and fertilize. Since sperm are transcriptionally inert, they must rely on the epididymis for protection from chemical insult. This is accomplished in part by an extensive blood-tissue barrier that limits the passage of molecules between cells and the epididymal lumen. The blood-epididymis barrier is composed of occludin and claudins, which form tight junctions between adjacent principal cells of the epididymal epithelium. The barrier is, however, more complex involving a series of chemical and ionic transporters that permit creation of specific microenvironments, required for sperm maturation, within the lumen, along the length of epididymis. In addition to these transporters, specific pores formed by claudins within the tight junction allow rapid and selective passage of ions across the tight junction. The barrier also contains transporters that can protect sperm from chemical toxicity. Alkylphenol ethoxylates, such as octylphenol (OP) have been reported to exert toxicity on sperm motility at high concentrations. We have shown that, at environmentally relevant doses, octylphenol does not accumulate in either the testis or epididymis, and is in fact rapidly eliminated, suggesting an active transport mechanism. We have shown the presence of a functional ABC-B1 transporter in the epididymis, which is induced by octylphenol and may be responsible for preventing (OP) accumulation in the epididymis. Surprisingly, we also observed that during epididymal transit, sperm acquire a functional ABC-B1 transporter critical to the detoxification process protecting sperm from chemical insult. Thus the blood-epididymis barrier is not only critical to epididymal physiology but acts as an essential structure for protecting maturing spermatozoa from chemical insult. Supported by CIHR and NSERC.

In evaluating the risk of neoplasia posed by selective immunomodulatory drugs, preclinical models, by providing data on hazard identification and characterization, can play an important role in a weight of evidence-based approach to risk assessment. However, their predictive value in quantitative risk assessment is limited. This is particularly true for genetically deficient animals and studies conducted with surrogate molecules. This presentation will focus on the available preclinical models and discuss their utility in informing risk assessment of immunomodulation and cancer.

Risk assessment for carcinogenic potential of selective immunomodulatory drugs requires a strategy based on a weight-of-evidence approach. This presentation will describe how a Product Specific Assessment (PSA) is formulated, including a review of relevant data from a variety of sources, such as published data, information on class effects, target biology and mechanism of action, as well as in vitro, chronic toxicity, and clinical data. In some cases, the available information may be insufficient to address carcinogenic potential and inform clinical risk without additional nonclinical studies, while in other cases the sponsor should design a strategy to address the potential hazard, which may include additional studies that could mitigate the mechanism-based concern or the PSA may be used to communicate risk and provide input to the risk management plan along with labeling proposals, clinical monitoring, postmarketing surveillance, or a combination of these approaches.

Communication of risk of potential carcinogenicity of a drug product is a key component to any product label. Regulatory agency’s and product innovators are challenged to provide useful and understandable information to the clinician. This presentation will discuss the product labeling for several US FDA-approved immunomodulatory compounds and how the existing clinical and nonclinical information ultimately impacted the final product labeling and discuss how a detailed product-specific assessment could impact future drug product labeling.
60 IMAGING-BASED MULTISCALE MODELS OF THE RESPIRATORY SYSTEM THAT ACCOUNT FOR REGIONAL HETEROGENEITY IN HEALTH AND DISEASE.

D. R. Einstein, R. E. Jacob, S. Kabilan, A. P. Kuprat, J. P. Carson, K. R. Minard and R. A. Corley, Pacific Northwest National Laboratory, Richland, WA.

Historically, computational fluid dynamic (CFD) models of the respiratory system have been unable to account for mechanical variation in the lung. However, local deviations from nominal heterogeneity and compliance in disease states such as emphysema and fibrosis have both important clinical and pathological implications. Moreover, by altering site-specific flow, these deviations are likely to influence the dosimetry and responses to drugs, gases or particulates. To investigate the influence of regional heterogeneity and compliance on airflow, multiscale CFD models of the respiratory airways of control and elastase treated rats were constructed from high-resolution computed tomography (CT) images. Time-dependent CT images were acquired in live, mechanically-ventilated Sprague-Dawley rats at eleven time points over the breathing cycle. Three-dimensional (3D) deformation fields were calculated for each set of images by non-linearly warping each image to a reference image. From these deformation maps, 3D volumetric strain was computed by solving for non-linear strain based on a finite-element discretization. Model geometries were derived from high-resolution CT images of post-mortem lung casts. Lower-dimensional models of distal lung mechanics, including resistance, inductance and compliance were parameterized from the live and cast images and bi-directionally coupled to each CFD outlet. Boundary conditions for the multiscale models consisted of atmospheric pressure at the nose and time dependent regionally specific volumetric strain – ventilation – at the terminus of each lower-dimensional model. Compared to the control models, predications from the elastase-derived models indicated that disease specific mechanics profoundly altered flow. These models are the first to our knowledge to incorporate detailed mechanics of the parenchyma and form the basis for an investigation of airway toxicity in health and disease. Funded by NHLBI R01 HL073598.

61 AN IN SILICO MODEL OF ENDOTOXIC SHOCK MEDIATORS.

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Biologically-based in silico models of pathogen-host interactions are being designed in our lab to predict the time-course of pathogenic infection in humans. Macrophages respond to lipopolysaccharides (LPS), including the release of potent lipid autacoids, causing a cascade of events leading to endotox shock. However, animals have been shown to vary in response and susceptibility to E. coli endotox: guinea pig > hamster > mouse. To establish a sound basis for interspecies extrapolation, a pathogenesis model is being extended to encompass endotox shock. Exposing experimental animals to aerosols of LPS elicits bronchoconstriction, activation of alveolar macrophages, and recruitment of inflammatory cells into airways. These effects have been attributed to a potent lipid autacoid, platelet-activating factor (PAF). Species differences in the biomodulatory effects and mechanisms of PAF are similar to those seen with endotoxin. In guinea pigs, PAF (2 ug/kg IV) causes bronchoconstriction and hypotension in seconds and lethality within 25 minutes. In rats, however, 3 ug/kg of PAF had a negligible impact on heart rate. Therefore, a dynamic model for PAF was developed to link a pathogen’s kinetics and host response. The current model focuses on kinetics and receptor binding of PAF and its antagonist ginkgolide B (GB). The kinetic models include plasma, red blood cell, lung, heart, and rapidly and slowly perfused tissues, with IV and inhalation exposure routes, and pathways for binding and elimination of PAF. Kinetic parameters were from the literature. The model was used to simulate experimental exposures to PAF and GB, revealing potential explanations for species differences in sensitivity to PAF. Internal dose metrics were generated and correlated with observed signs of infection and lethality in an attempt to identify the most appropriate metrics for predicting adverse effects. This model of pathogen kinetics and these dose metrics help to elucidate mechanisms of host response dynamics and improve cross-species extrapolation of data.

62 COMPARATIVE EXPOSURE ASSESSMENT: USE OF PBPK MODELING FOR THE ESSENTIAL ELEMENT MANGANESE.

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Most methods for exposure and risk assessment cannot capture the typical biophysical response of an essential element. Investigations have been conducted to assess the potential for adverse health impacts from exposure to Mn present in air from occupational sources. These analyses have not considered the essentiality of Mn and its function in key biological processes. Furthermore, the lack of an integration of Mn-specific kinetic and experimental data has resulted in the application of uncertainty factors that may not be necessary for the derivation of a ‘safe’ inhalation exposure level. PBPK models were applied to determine target tissue concentrations of Mn following occupational and measured ambient environmental exposures. These data were then compared to determine margins of safety and to evaluate the biological plausibility of applying uncertainty factors to derive acceptable air concentrations. Blood concentrations reported in the occupational studies allowed for human verification of the PBPK models, increasing confidence in the estimates. The results of modeling considering both dietary and inhalation intake of Mn suggested that marginal exposure levels only when exposure is to levels of Mn in ambient air far higher than those currently measured in Canada or the United States. Margins of Safety were greater than three orders of magnitude, indicating how much of an increase in the ambient levels would be required to produce the target tissue level at or below which no subclinical neurotoxicological effect would be expected. This application of PBPK modeling for an essential element, such as Mn, clearly demonstrates that the application of default factors to “convert” an occupational exposure to a continuous environmental exposure, followed by the application of uncertainty factors is not scientifically defensible in the case of Mn. It also suggests a maximum uncertainty factor on the order of 10, rather than several orders of magnitude.

63 TRANSLATIONAL PHARMACOKINETIC-PHARMACODYNAMIC MODELING OF QTc EFFECTS IN DOG AND HUMAN.

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Prolongation of the QT interval is used as a biomarker for the arrhythmia Torxsdes de Pointes. In contrast, less is known about safety implications of QT shortening despite a recent increase preclinically in the number of drugs with this effect (1). A pre-clinical assessment of the relationship between concentration and QT change is therefore crucial to assure drug safety. The aim of this investigation was to perform a retrospective pharmacokinetic-pharmacodynamic (PKPD) analysis of dog and man data and compare the effects of 2 proprietary small-molecules, one increasing and one decreasing the heart-rate corrected QT interval (QTc) in both species. The preclinical data were collected in a conscious dog telemetry model while the clinical data were collected during Phase I trials in healthy volunteers. 2 or 3 compartmental models were used to describe the PK of drugs in dog and man, respectively. Final PK parameter estimates were then used as an input for the PKPD model. An effect compartment was introduced to derive the concentration-effect relationships. The QTc response from dog and man at matching free concentrations were then plotted against each other. The initial plot showed a clear translational relationship between dog and man QTc response, confirming that the conscious dog model is effective for predictions of drug-dependent QTc effects in man. To confirm our findings, we have included PKPD data for known QT prolongers (moxifloxacin & dofetilide). The resulting plot closely matched initial results and had a slope of 1.3 ms. Based on this relationship, QTc change of ~7 ms in dog would correspond to 10 ms change in man. Such relationships could be therefore useful to predict the clinical outcome on QTc interval change using preclinical dog data and will thus improve future decision-making. (1) Holbrook M et al 2009, JPTM, 59:21-28

64 DEVELOPMENT OF A HUMAN GESTATION PHYSIOLOGICALLY-BASED PHARMACOKINETIC/PBPK MODEL FOR COEXPOSURE OF POLYCHLORINATED BIPHENYLS (PCBS).

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Exposure of pregnant women to persistent organic pollutants such as PCBs can lead to toxic effects in mothers and fetuses via blood transfer through the placenta. Concentrations of PCBs measured in human biomonitoring data from expectant mothers exposed to background levels of PCBs, and the transfer of PCBs from mother to fetus were assessed using PBPK modeling. A human gestation PBPK model for the coexposure of PCBs was developed to simulate the disposition of a
maximum of ten PCB congeners within a pregnant mother and fetus throughout the entire 40 week gestational period. A five compartment maternal model, and four compartment sub-model was used to describe the pharmacokinetics of PCBs in the mother and fetus, respectively. Quantitative Structure Activity Relationships were applied to calculate the chemical-specific parameters, while the physiological parameters were taken from the literature. Model evaluation was performed by comparing estimated daily intakes and tissue concentrations of PCBs from published literature with the model predicted tissue concentrations. The model was capable of simulating the pharmacokinetics of PCBs during pregnancy in a sample of NHANES biomonitoring during 1999-2000 and 2001-2002. The model correlation coefficients values were 0.87, 0.81, and 0.85 for the first, second and third trimester, respectively. Hence, this model can be used as a screening tool that can provide useful information for mothers and the in utero exposure of PCBs.

Physiologically-based pharmacokinetic (PBPK) models are compartmental models that describe the uptake and distribution of drugs and chemicals throughout the body. They can be structured so that model parameters (i.e., physiological and chemical-specific) reflect biological characteristics. Bayesian methods can be used to combine prior information derived from the literature (e.g., mean values, chemical structure, and in vitro data) in the form of probability distributions on PBPK model parameters with new information from in vivo data to determine parameter estimates and model uncertainties. These methods can also be used to evaluate and refine biological modeling assumptions underlying PBPK models. Using a PBPK model developed for permethrin in rats, we combine prior information from the literature on several of the PBPK model parameters (e.g., blood flows, partition coefficients, chemical-specific parameters) with measured concentrations of both the cis- and trans-isomers of permethrin in various tissues for uncertainty analysis and model evaluation. We evaluate biological assumptions used to describe partition coefficients via Schmitt's predictive model (2008) by comparing the model fits of two different permethrin PBPK models. In the first version of the model, partitioning is characterized by the explicit use of standard tissue-specific partition coefficients as PBPK model parameters. In version two of the permethrin PBPK model, we use Schmitt's 2008 model based on tissue composition, solubilities, and binding properties to compute the partition coefficients for each tissue or compartment within the permethrin PBPK model. We illustrate how Bayesian methods provide a useful statistical framework in which to determine how well the underlying biological assumptions used to model partitioning describe the distribution kinetics of permethrin. This abstract does not necessarily reflect US EPA policy.

There has been considerable interest in the development of in vitro tests for the characterisation of chemical allergens, such as cytokine production by dendritic cell (DC)-like cell lines. These methods are often limited by toxicity and delivery of lipophilic chemical allergens in culture and lack of dynamic range. We have measured IL-8 production following 24h culture of the human monocytic THP-1 cell line with various allergens or with nonsensitising irritants. Treatment of THP-1 cells with a range of water soluble and lipophilic allergens of diverse chemistries (dinitrohalobenzenes, potassium dichromate, resorcinol and aminophenol) resulted in increased IL-8 expression. Although the concentrations at which cytokine secretion was stimulated spanned a relatively narrow range (generally doses that caused 10-30% decrease in cell viability), IL-8 production was selective for allergens, such that culture with a number of irritants (at doses causing equivalent levels of cell death) failed to upregulate this cytokine. Furthermore, an impressive dynamic range was revealed for THP-1 cell IL-8 expression. Thus, the reference lipophilic allergen dinitrochlorobenzene (DNCB) stimulated the production of 1-4ng/ml (equating to a 15-fold increase in IL-8 compared with background levels). Its water soluble analog, dinitrobenzenesulfonic acid (DNBS), induced levels of 12ng/ml. In addition, analysis of IL-8 expression in both supernatants and cell lysates revealed that allergens (but not irritants) stimulated increases in both secreted and intracellular levels of this cytokine. These data suggest that analysis of the production of IL-8 by THP-1 cells may provide a useful tool in the identification of chemical allergens, possibly involving a metric where total (secreted and intracellular) IL-8 is measured.
The production of cytokines such as interleukin (IL)-8 by cultured dendritic cell (DC)-like cells has been suggested as a potential endpoint for the in vitro identification of chemical allergens. It is of some interest that allergen-induced IL-8 production appears to be related to cell injury and cell death. We have demonstrated that for several cell types (including the HaCaT keratinocyte cell line), allergen-induced IL-8 production is closely associated with the profile of toxicity (with only subtoxic doses stimulating IL-8). However, the induction of cell death by other means, including osmolality-induced lysis or culture with a number of different chemical irritants (e.g. sodium lauryl sulfate, Tween 20 and benzene sulfonic acid), did not show the same relationship between cellular toxicity and IL-8 expression. Furthermore, this relationship was observed only for IL-8 expression, and not for other cytokines (IL-6 or IL-1β). Other cytokine inducing reagents (reactive oxygen species [ROS] activators) that provoke activation of the inflammatory immune system have also been examined. The ROS activators uric acid, aluminium hydroxide, ATP and silica had little effect on cell viability and provoked relatively modest increases in IL-8 but vigorous production of IL-1β. Hydrogen peroxide did cause marked cell death, but IL-8 expression was recorded even at doses at which >50% cell death was observed. Thus ROS activating agents also stimulate a very different pattern of cytokine expression, and show a very different relationship to cell death compared with that observed for the chemical allergens. The impact of diphenylethenoclonium chloride (DPI), an inhibitor of nicotinamide adenine dinucleotide phosphate-oxidases, on IL-8 expression and show a very different relationship to cell death compared with that observed for the chemical allergens. The inhibition of dienophylenedononium chloride (DPI), an inhibitor of nicotinamide adenine dinucleotide phosphate-oxidases, on cytokine production was examined. The inhibitor had some impact on IL-8 production induced by allergen, but was unable to completely block cytokine production. These data suggest that ROS generation is playing a partial role in IL-8 production, at least by some types of allergen.

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donor with the allergic reaction are incubated with the suspected drug and cytokines (IL-2, IL-5, IL-7, IL-10, IFNgamma) and green fluorescent protein (GFP) reporter transgenic liver supernatant. The objective of these studies was to evaluate incubation times and the ability of the mLTT to reproducibly detect positive responses against sulfamethoxazole (SM) with cryopreserved PBMC from an SM allergic donor. In addition, specificity was determined by evaluating the cytokine responses of PBMC from the SM allergic donor when incubated with an irrelevant drug (amoxicillin). Peak levels of IL-2, IL-5, IL-13, IFNgamma, and granzyme B were observed between days 3 to 7. Experiment-to-experiment variability for IL-2, IL-5, IL-13, IFNgamma, and granzyme B release was high but the mLTT was able to detect a positive response in 83% of 12 separate experiments (a positive is defined as 3 out of 5 endpoints having at least 2-fold higher signal with SM treatment than the signal with no drug experiment (stimulation index)). The specificity of the response was 90% since only 1 of 10 experiments gave a positive response with amoxicillin exclusion. In conclusion, the mLTT assay performance appears to be robust based on repeated experiments with PBMC from one SM allergic donor. Further evaluation of assay performance is needed using samples from additional donors with allergic reactions to different drugs.

74 DEVELOPMENT OF A DELAYED-TYPE HYPERSENSITIVITY ASSAY TO CANDIDA ALBICANS IN JUVENILE RATS: COMPARISON OF IN VIVO AND EX VIVO METHODS.

Delayed-type hypersensitivity (DTH) is a memory T cell-mediated, antibody-independent immune response that may be used for immunotoxicity testing in non-clinical species. However, a number of current DTH assays are confounded by the production of antibodies to the antigen. We are currently modifying a DTH model, previously established in mouse, for use in juvenile rats to assess the effect of immunosuppressive drugs on the developing rat immune system. The assay measures footpad swelling induced by subcutaneous footpad injection of *Candida albicans* (*C. albicans*) chitosan in rats previously immunized with *C. albicans*. Antibodies to chitosan are not produced in this model. Large inter-animal variability inherent in the footpad swelling assay makes it difficult to precisely quantify the magnitude of the immune response and inhibition by immunosuppressants. We have developed an *ex vivo* assay to assess DTH in rats using IFN-gamma production by splenocytes of rats previously sensitized with *C. albicans* as the quantifiable measure of DTH response. Rats dosed with dexamethasone, a known immunosuppressive compound, exhibited immunosuppression as evidenced by a reduction in IFN-gamma production in response to *C. albicans* chitosan. Current data indicate that the in vitro based DTH assay is more sensitive than the conventional footpad swelling assay due to lower background response and smaller variability. Our *ex vivo* based rat DTH assay offers a highly sensitive and quantitative alternative to the footpad swelling assay, and may be used to assess the immunosuppressive properties of drugs. The increased sensitivity of the *ex vivo* DTH assay may be useful for identifying smaller changes in response to immunosuppressive drugs, and also for studying the responses in juvenile rats that do not yet have a fully functional immune system.

75 SPECIFICITY PROTEINS LINK ONCOPICENMICRORNAMIRB14 SIGNALING AXIS TO EZH2 REGULATION.
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The Enhancer of Zeste 2 (EZH2) protein is a histone methyltransferase that mediates epigenetic silencing through chromatin remodeling. EZH2 plays a pivotal role in cell self-renewal and tumorigenesis and is frequently overexpressed in many cancers. Thus, it is important to identify pathways that regulate EZH2 expression and to develop strategies to target EZH2. Oncogenic microRNAs (miRs), including miR-106b and miR-93, target ZBTB4 which functions as a specificity protein (Sp) repressor through competitive binding to GC-rich sequences. Increased ZBTB4 expression by oncogenic miR depletion resulted in downregulation of Sp1, Sp3 and Sp4 proteins and Sp-dependent oncogenes, including survivin, VEGF and Wnt5a, suggesting a tumor suppressive role for ZBTB4. Bioinformatics analysis further revealed that EZH2 is a downstream target of ZBTB4 and in fact, ZBTB4 overexpression repressed EZH2 expression through downregulation of Sp5 proteins in MCF-7 and MDA-MB-231 breast cancer cells. Sp1, Sp3 and Sp4 knockdown by RNA interference decreased EZH2, indicating that Sp proteins are necessary for EZH2 expression. Chromatin immunoprecipitation and gel shift assays confirmed that Sp proteins bond GC-Rich Sp binding sites on the EZH2 promoter and ZBTB4 compete Sp protein binding at these sites. Furthermore, transfection of miR-106b antagonir increased ZBTB4 and decreased EZH2 expression and this was accompanied by enhanced ZBTB4 binding to the GC-rich sites in the EZH2 gene promoter. Moreover, betulinic acid (BA), a phytochemically previously reported to downregulate Sp proteins in various cancer cell lines also decreased EZH2 expression by modulating the oncogenic miR/ZBTB4 axis. These results underscore Sp proteins as “non-oncogene addiction” proteins essential to maintain oncogenic function and cancer phenotype during tumorigenesis. Furthermore, this is the first study showing that Sp proteins connect the oncogenic miR-ZBTB4 pathway to EZH2 regulation and that inhibition of Sp proteins by BA will be a novel therapeutic approach that targets EZH2.

76 METHYLESELENOCYSTINE RESETS CIRCADIAN RHYTHM IN MAMMARY EPITHELIAL CELLS BY ENHANCING NAD+-DEPENDENT SIRT1 ACTIVITY.
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We previously demonstrated that a chemopreventive regimen of dietary methyleselenocysteine (MSC) can reset and enhance the circadian expression of major clock genes (Per2) and circadian-controlled genes (CCGs) persistently disrupted by carcinogenic N-methyl-N-nitrosourea (NMU) during early stages of mammary tumorigenesis in rat. Here we show that dietary MSC significantly enhanced the intracellular circadian oscillations in the NAD+/NADH ratio, an indicator of REDOX status, with a peak value occurring at Zeitgeber Time (ZT8), resulting in an increased NAD+-dependent Sirt1 activity in the mammary glands of rats at ZT12. Moreover, dietary MSC specifically decreased acetylation of the Bmal1 (AcBmal1) transcription factor associated with E-box element within the promoter of the Per2 gene between ZT12 and ZT16, which negatively corresponded to the transcriptional phase of Per2 gene. The increase of NAD+/NADH and Sirt1 activity was also temporally correlated with significantly increased oscillations in the acetylation of lysine 9 in the histone 3 proteins (AcH3K9) associated with both E-box and Eson1 sequences within Per2 promoter regions in mammary glands of rats. The activating effect of MSC on NAD+-dependent Sirt1 activity was also observed *in vivo* when normal mammary epithelial cells (MCF10A) exposed to carcinogen were treated with MSC for 72 hrs. As was the case in vivo, the increased intracellular NAD+/NADH and increased Sirt1 histone deacetylation in vitro might be responsible, at least partially, for the increased cellular AcH3K9 level observed in mammary epithelial cells. These results suggested that MSC-enhanced circadian cycling of intracellular redox status increases the Sirt1 activity *in vitro* and *in vivo*. Moreover, these findings indicated that the resulting decrease in AcBmal1 and increase in AcH3K9 at E-box contributes to the induction of carcinogenic Per2 expression during chemoprevention. (Supported by NIH Grants: U19ES011387, P080ES05022, P30ES007083)

77 EPIGENETIC ALTERATIONS UNDERLIE THE MECHANISM OF N, N-DIETHYLITRITOSAMINE AND CARBON TETRACHLORIDE CARCINOGENESIS IN THE MOUSE LIVER.
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Human hepatocellular carcinoma (HCC), which develops mostly under conditions of liver fibrosis and cirrhosis, has been linked to changes in the cellular epigenome. However, despite the large body of evidence demonstrating profound cancer-linked epigenetic abnormalities in HCC, the role of these epigenetic changes may play in hepatocarcinogenesis is less understood. This study used a mouse model of genotoxic carcinogenesis in a fibrotic liver to test the hypothesis that epigenetic events play a role in liver carcinogenesis. Male B6C3F1 mice were injected intraperitoneally (i.p.) with either N,N-diethylitritosamine (DEN, single dose of 1 mg/kg) or PBS vehicle at 15 days of age. Carbon tetrachloride (CCL4) treatment (0.2 ml/kg, i.p.; 2/week) was initiated at 8 weeks of age and continued for 9 or 14 weeks. Treatment with DEN alone resulted in few histopathological changes, whereas treatment with CCl4 led to a severe bridging fibrosis and the development of liver adenomas and carcinomas. In the DEN+CCl4 group, a strong synergistic effect was observed, as evidenced by a marked increase in liver tumor incidence. Histomorphological changes in livers of mice from CCl4 and DEN+CCl4 groups were accompanied by the loss of global and repetitive element DNA methylation. Hypermethylation of the Cdkn2a, Mgmt, Socs1, Cdh1, and Riz1 gene promoters was also found in non-tumorous liver tissue in these groups; however, only hyper-
methyltransferase of Riz1 was associated with gene silencing. Changes in DNA methylation were associated with a decrease in histone H3 lysine 9 trimethylation, which may be a subsequent effect of epigenetically-induced inhibition of the Riz1 gene expression. The latter correlated with tumor incidence. These findings suggest that liver fibrosis is acting as a tumor promoter and is also affecting the cellular epigenome during genotoxic-induced liver carcinogenesis in the mouse.

Data from the National Lung Screening Trial (NLST) have suggested that computed tomography (CT) annual screening of at-risk patients can decrease lung cancer mortality by 20%. Concerns remain regarding the long term effects of radiation exposure to the damaged lung cells of heavy smokers and ex-smokers. We previously reported that exposure of transgenic mice expressing a mutant Ki-ras⁶¹G12 gene to multiple whole-body CT doses approximating the NLST screening protocol had a significant (p<0.01) 43% increase in tumor multiplicity. Using Affymetrix gene chip expression profiling followed by gene ontology analysis, we identified clusters of genes under the categories “immune response” and “oxidative phosphorylation” that were significantly up- and down-regulated, respectively, in mutant Ki-ras⁶¹G12 expressing mice 10 min after exposure to a single 50 mGy dose of CT radiation compared to controls. We now confirm these findings in mice treated at 8 weeks of age with 4-(methylthio)acetophenone-1-(3-pyridyl)-1-butanone (NNK) followed one week later by 4 weekly doses of 0, 10, 30, or 50 mGy of whole-body CT radiation. Eight months after the last fraction of radiation, mice treated with NNK and exposed to radiation exhibited significant 1.5 to 2-fold increases in radiation-induced tumors compared to mice treated with NNK alone. Treatment of female mice with 0.7% N-acetylcysteine in the diet starting 3 days prior to the first CT exposure and continuing for a total of 5 weeks inhibited the CT-induced increases in lung tumor multiplicity back to levels seen in NNK treated, unirradiated mice (p<0.05; one tailed t-test). Our data indicate that: 1) exposure to CT radiation in sensitive populations increases the risk of tumorogenesis, 2) this effect may be mediated by immune response and oxidative stress mechanisms, and 3) pre-treatment with an anti-oxidant may prevent the long term carcinogenic effects of low dose radiation exposure. (Supported by NIH grant CA136910)

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**80 EXPRESSION OF PROTO-ONCOGENE CFMS IN LUNG SQAMOUS CELL CARCINOMA AND ADENOCARCINOMA.**

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cFMS proto-oncogene encodes the tyrosine kinase receptor for macrophage colony-stimulating factor 1 (CSF-1). Previous studies have shown that CSF-1 and cFMS are co-expressed in tumor cells and tumor-associated stromal cells in gynecological cancers and are involved in invasiveness and metastasis through autocrine and paracrine mechanisms. It was also reported that glucocorticoids induced cFMS expression in the choriocarcinoma cell line through glucocorticoid receptor (GR). Although CSF-1 level increased in the serum and tumor cells of patients with non-small lung carcinomas, little is known about the expression of cFMS and GR in patients with lung cancers. We postulated that cFMS expression is increased in lung cancers through GR, an action that may promote tumor progression. To test our hypothesis, we performed immunohistochemistry for cFMS on tissue microarrays (TMA)s of human normal lung, lung squamous cell carcinoma (SSC) and adenocarcinoma (Ade) by using a validated anti-human cFMS specific antibody. cFMS was uniquely stained in tumor stromal cells but not stromal cells in normal lung tissue, normal epithelium or tumor epithelium. Evaluation of lung SCC (n=63) and Ade (n=71) samples revealed stromal cell cFMS staining in approximately 59% (37 out of 63) and 37% (26 out of 71) of the tumor samples, respectively. To investigate whether stromal cFMS expression in lung cancers was associated with glucocorticoid signaling, GR distribution was investigated in human lung SCC and Ade TMA's by immunohistochemistry. Stromal cell GR staining was observed only in 18% (2 out of 11) of lung SSC and 6% (1 out of 17) of lung Ade samples. These results suggest that: 1) cFMS expression is elevated in patients with lung cancers, 2) cFMS expression is localized to stromal cells rather than tumor cells, and 3) cFMS expression is not likely to be mediated by glucocorticoids due to the absence of GR staining in tumor stromal cells.

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**81 THE ROLE OF PPARγ IN MAMMARY SECRETORY EPITHELIAL CELLS DURING DBMA-INDUCED BREAST TUMOURIGENESIS.**

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Previously, we showed PPARγ normally suppresses 7,12-dimethylbenz(a)anthracene (DBMA)-induced breast tumour progression. We are now clarifying which cell types and mechanisms are involved. Mammary secretory epithelial (MSE) cells rapidly proliferate before pregnancy, and apoptose and/or transdifferentiate into adipocytes following pregnancy. Given PPARγ plays a critical role in fat cell differentiation, we hypothesized that deleting MSE-specific PPARγ enhances DBMA-mediated breast tumourigenesis. MSE-specific PPARγ knockout (MSE KO) mice were generated, and 12-week-old post-lactational female MSE KO and congenic wildtype (WT) controls were treated p.o. once/week for 6 weeks with 1mg DMBA. At week 7, mice were randomized to continue on a normal chow diet (DMBA Only: WT, n=15; MSE KO, n=25) or one supplemented with a PPARγ activating drug rosiglitazone (ROS, 4mg/kg/day; DMBA+ROS: WT, n=17; MSE KO, n=24) for 25 weeks. Compared to DMBA Only-treated WT mice, MSE KO had a significant decrease in median survival (>25 vs 17 weeks respectively, p<0.05), and significantly earlier median onset of mammary tumours (20 vs 15 weeks respectively, p<0.01). Compared to DMBA Only-treatment, DMBA+ROS-treated mice in either group had a modest increase in median survival and a 4-fold decrease in mammary tumour volumes. Further, DMBA+ROS-treated MSE KO had delayed mean mammary tumour onset versus DMBA Only-treated mice. Breast tumour analyses suggest MSE-specific PPARγ signaling stops mammary tumour growth, in part, by decreasing cyclin D1 expression. MSE cells lacking PPARγ express decreased Bax protein, which may provide a cell survival advantage after tumorigenic initiation. Together, these studies are the first to highlight...
The protective role of M-se-specific PPARγ expression and signaling in breast tu-
mourigenesis, and add support for a chemopreventive role of PPARγ activation in
breast cancer treatment.

**PL 82** COMPREHENSIVE MAPPING OF TRANSCRIPTIONAL AND EPIGENETIC PERTURBATIONS IN VITRO IDENTIFIES NONCODING RNAs AS NOVEL BIOMARKERS FOR LIVER TUMOR PROMOTION.

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The temporal events leading to tumorigenesis are still poorly understood. Long-
lasting epigenetic perturbations in gene regulation may take place at the earliest
stages of nongenotoxic carcinogenesis and their characterization will help to iden-
tify early mechanisms-based biomarkers with great impact for biomedical research.

We have used genome-wide transcriptional, microRNA, DNA methylation, chromo-
atin modification and phosphoprotein profiling approaches anchored to histopathological analyses to characterize the dynamics of molecular perturbations in a 7 time-point in vivo study using a well characterized rodent liver tumor pro-
motion model (phenobarbital (PB) treatment of B6C3F1 mice). We observed dy-
namic and progressive changes in the transcriptome and DNA methylation that highlight temporal perturbations of biological pathways at early stages of tumor promotion. Transcriptomic and DNA methylation perturbations show only limited cor-
relations, whereas chromatin modification perturbations were strongly corre-
lated with transcriptional changes and showed specificity to drug metabolism genes. This integrated profiling approach has revealed a progressive increase in liver expression levels of long noncoding RNAs (ncRNA) and microRNAs that have pre-
viously been associated with stem cell pluripotency in mice and various neoplasms
in humans. We show that the expression of selected liver ncRNA biomarkers is de-
pendent both on the constitutive androstanone receptor and beta-catenin pathways
and is maintained in PB promoted liver tumors. Investigation of the cell-type speci-
ficity and functions of these novel ncRNA biomarkers is ongoing, together with an
assessment of their potential perturbation by rodent nongenotoxic carcinogens
with distinct modes of action.

**PL 83** NANOMATERIAL (NM) BIOACTIVITY PROFILING BY TOXCAST HIGH-THROUGHPUT SCREENING (HTS).

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Rapidly increasing numbers of new NMs and their uses demand efficient tests of
NM bioactivity for safety assessment. The EPA ToxCast program uses HTS assays to
 prioritize for targeted testing, identify biological pathways affected, and aid in
 linking NM properties and potential in vivo effects. NMs (Au, Ag, CeO2, CNT, CuO, TiO2, SiO2, or ZnO core) and their ion and micro counterparts were tested for
cytotoxicity in various cell types, for transcription factor (TF) activation in
 HepG2 cells, and by protein expression in 8 BioMAP® human primary cell sys-
tems. Tested concentrations were from below the in vitro equivalents of the MMPD model–predicted lung retention (mass/surface area) after 24h exposure to reported
NM concentrations in air to about the equivalent of 45yr exposure. Assay/cell sen-
tivity and specificity was assessed for characterization of toxicity. Signific-
tant differences were observed with effects (10-12 in vitro) of AgNPs after 24h exposure in HepG2 cells. Mitochondrial toxicity was observed in HepG2 cells and A549
cells (AgNP) in a concentration-dependent manner.

**PL 84** BIOAVAILABILITY AND TOXICITY OF SILVER NANOPARTICLES AFTER TWENTY-EIGHT-DAY ORAL EXPOSURE IN RATS.


We investigated whether silver nanoparticles (AgNPs) can pass the intestines, accu-
mulate in tissues and induce toxicity. For this, rats (n=5) were exposed to ~20 nm,
non-coated or ~15 nm PVP-coated AgNPs ([Ag]=20 mg/kg bw) for 28 days. Since -
10% of the Ag in the AgNP solutions is present in an ionic form, a third group
obtained Ag ions (AgNO3) equal to this fraction. As negative control, rats obtained
the carrier solution. Dissection was either at day 29 or for studying the wash-out at
day 36 or 84. Ag measurements by AAS demonstrated presence of Ag in all organs
examined with highest levels in the liver and spleen for each of the three treatments.

The Ag content was decreased at day 84 in most organs but remarkably not in the
brain and testis. The AgNP- and Ag ion-treated groups had equal tissue Ag con-
tents, indicating that in AgNP exposed rats mainly dissolved Ag+ and to a much
lesser extent AgNPs passed the intestines. SP-ICPM was used to detect AgNPs in
gastro-intestinal (GI) tract, liver, spleen, kidney, lungs and bones. Remarkably,
AgNPs were not only found in the GI tract and liver of AgNP exposed rats, but also
in the Ag ion exposed rats, demonstrating formation of AgNPs from Ag+ in vivo.

To test this, CD1 male mice were injected of vehicle and 1 mg/kg spherical AgNPs of 10nm diameter via the tail vein every 3 days, five times, beginning at postnatal day 45.

**PL 85** INTRAVENOUS ADMINISTRATION OF SILVER NANOPARTICLES IN MALE MICE ALTERs LEYDIG CELL FUNCTION AND INCREASES SERUM TESTOSTERONE, BUT DOES NOT PERTURB SPERMATOGENESIS.

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Spermatogenic stem cells (SSCs) provide the foundation for spermatogenesis and
are vital for the perpetuation of species. Previous studies from our lab examined the
in vitro effects of silver nanoparticles (AgNPs) on an SSC cell line. Our data showed
that AgNPs interfered with SSC proliferation in a dose and size-dependent manner
and that AgNPs disrupted components of the GDNF signaling pathway, a pathway
critical for self-renewal of SSCs in vivo. If AgNPs were to induce a similar effect in
vivo, spermatogenesis and fertility would be severely impaired. To test this, CD1 male mice were given injections of vehicle and 1 mg/kg spherical AgNPs of 10nm diameter via the tail vein every 3 days, five times, beginning at postnatal day 45.

This in vitro dose is roughly equivalent to the lowest in vivo dose with effects (10
gg/ml) from our previous studies, and ~2,000 times greater than that found in the
blood in a previous AgNP inhalation study. Body and testis weights were unaffected
at 15, 60, and 120-days post-injection trial. Although serum levels of LH, FSH, and
Inhibin B were unaffected at 15, 60, and 120 days, testosterone was signifi-
cantly increased, and significant changes in Leydig cell nucleus and individual size
(morphology) were observed at 15 and 60 days. Sperm analyses demonstrated no
changes in cauda epididymal sperm/ml and all sperm motility parameters were
unaffected in AgNP mice at 15, 60, and 120 days. Likewise, the pregnancy rate and
delivery success of female mice, which mated with treated males for 1 week beginning
at 15 and 60 days, did not differ from those that mated with controls. Taken to-
gether, these results demonstrate that AgNPs do not perturb SSCs in vivo as seen in our
previous in vitro studies, and despite a significant increase in testosterone, sperm quantity and quality, and the overall fertility of male mice was unaltered
under the experimental conditions. Supported by T32ES007326.
Molecular Toxicology, Oregon State University, Corvallis, OR and 2Chemistry, Laboratory FA8650-05-1-5041. Nanoparticles can interact with and perturb developmental progression. This research demonstrates that exposures to 1.5 nm TMAT AuNPs impair vertebrate eye development, inducing apoptosis and reducing pigmentation, indicating that specific nanoparticles can interact with and perturb developmental progression. The results demonstrate that monolayers of 1.5 nm TMAT AuNPs grown on coverslips or in transwell chambers. Microscopy (confocal, TEM) indicated that nanoparticles translocate through barrier cells at different rates (30 min - 3 hr) without disrupting their tight junctions. Changes in transcellular electrical resistance (TERS) indicated that surface coating (PVP-citrate) and small aggregate size (10 nm-75 nm) facilitated this movement (15-30 min). Reporter genes (AP-1, ARE), translocated into RBC4 cells were significantly stimulated by 10 nm PVP-capped nanoparticles. The genomic response of the cells to each nanoparticle was also examined using Illumina BeadChips. PCA analysis indicated that the number and types of transcripts activated by 10nm PVP were significantly distinct from the other experimental treatment. Bioinformatic analysis (KEGG analysis, IPA) indicated that pathways activated by 10nm PVP 10 nm nanoparticles were associated with endocytosis, bio-energetics and oxidative stress-mediated neurodegenerative (SLCA21) diseases. Future experiments will examine the relationship of nanoparticle's physical properties and genomic events in brain cells (e.g., microglia, neurons) that are potential targets. (This abstract has been reviewed by NIEHS and does not necessarily reflect EPA policy.)

GOLD NANOPARTICLES DISRUPT ZEBRAFISH EYE DEVELOPMENT AND PIGMENTATION.

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The concerns regarding the potential toxicity of engineered nanoparticles (NPs) is gaining greater attention. The zebrafish embryos exposed to citicomic-functionalized, N,N,N trimethylammoniumethanethiol (TMAT), gold nanoparticles (AuNPs) with 1.5nm core size develop smaller and pale grey eyes (i.e., microphthalia). To determine the apoptosis resulted from AuNPs exposure, acidine orange staining, TUNEL and flow cytometry analysis of DNA content were conducted using embryonic BeadChips. PCA analysis indicated that the number and types of transcripts activated by 10 nm PVP were significantly distinct from the other experimental treatment. Bioinformatic analysis (KEGG analysis, IPA) indicated that pathways activated by 10 nm nanoparticles were associated with endocytosis, bioenergetics and oxidative stress-mediated neurodegenerative (SLCA21) diseases. Future experiments will examine the relationship of nanoparticle's physical properties and genomic events in brain cells (e.g., microglia, neurons) that are potential targets. (This abstract has been reviewed by NIEHS and does not necessarily reflect EPA policy.)

USE OF AN INTESTINAL CO-CULTURE MODEL TO STUDY THE EFFECTS OF Ag AND TiO2 NANOPARTICLES: MUCUS PROTECTIVE ROLE AND SIZE DEPENDENT EFFECTS.

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Nanoparticles (NPs) have hundreds of applications, from medicine to personal care products. The increasing production and use of NPs will unavoidably lead to their release in the environment with the potential of unpredictable effects. The aim of this study was to establish a more relevant intestinal co-culture model with the use of Caco-2 and HT29-MTX (mucus secreting) cell lines and employ the model for the evaluation of the effects of Ag (20 and 200 nm) and TiO2 (21 nm) NPs and Ag ions. In addition, their uptake was evaluated with Nanoscale Secondary Ion Mass Spectrometry (NanoSIMS 50). After 14 days the mucus layer completely covered the monolayer of differentiated cells seeded at a ratio of 90:10 (Caco-2:HT29-MTX). An overall higher metabolic activity compared to the untreated control was observed during this period; however the particles showed a continuous increase in adsorbed protein beginning after approximately 2 hours of incubation. PEG coated particles were also administered via tail-vein injection to ICR mice and the total gold content in the blood, liver, and spleen were monitored over 48h after the dose. Approximately 2h after dosing, the concentration of gold in the bloodstream began to decrease while the tissue concentrations began to increase, paralleling the protein binding behavior of the particles. Taken together, these results suggest that after approximately 2h, the PEG coating has degraded to a point at which it is no longer effective at resisting protein adsorption. Although still well dispersed, once this layer of protection has been lost, the particles can be readily recognized by the body and removed from circulation by the liver and spleen.

IN VITRO BIODISTRIBUTION OF SILVER NANOPARTICLES IN ISOLATED PERFUSED PORCINE SKIN FLAPS.

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Nanomaterials are increasingly playing a role in society for uses ranging from bio-medicine to microelectronics; however, pharmacokinetic studies, which will be necessary for human health risk assessments are limited. Currently, the most widely used nanoparticle in consumer products is silver (Ag). The objective of this study was to quantify the biodistribution of two types of Ag nanoparticles, Ag-citrate and Ag-silica, in the ex vivo tissue perfusion system, the isolated perfused porcine skin flap (IPPSF). IPPSFs were perfused for 4hr with 0.84μg/ml Ag-citrate or 0.48μg/ml Ag-silica followed by a 4hr perfusion with media only during a washout phase. Arterial and venous concentrations of Ag were measured in the media by ICP-MS. The normalized volumes of distribution estimated from the noncompartmental analysis of the venous concentrations indicated distribution of Ag greater than the vascular space, however since total Ag was measured, the extravascular distribution could be due to Ag ions diffusing across the media. The estimated clearance for both types of Ag nanoparticles was 1 ml/min, which was equal to the flow perfusion rate indicating that there was no detectable elimination of Ag from the system. Venous concentrations of Ag for both types of nanoparticles were best fit with a two-compartment model in which estimated rates of distribution between the central and peripheral compartment were similar but slower than clearance from the central compartment due to the media flow. By 4 hrs following infusion of the Ag nanoparticles, the recovery of Ag in the venous effluent was 90 ± 5.0% and 87 ± 2.2% of the infused Ag for Ag-citrate and Ag-silica, respectively. (Supported by NIH RO1 ES016138)

PROTEIN ADSORPTION BEHAVIOR OF PEG-COATED GOLD NANOPARTICLES IN HUMAN PLASMA.

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Colloidal gold has generated a significant amount of interest as a vehicle for in vivo therapeutic and diagnostic applications. Like most nanomaterials, gold particles must be stabilized prior to parenteral administration to prevent rapid agglomeration and adsorption of opsonins. Suspension stability and surface passivation are typically achieved via polymer coatings, most often involving polyelectrolyte glycol (PEG). Stability of the polymer coating is a critical determinant of the efficacy and fate of the particles as a therapeutic or diagnostic agent. In this study, the stability of the PEG coating on colloidal gold particles and its relationship to protein binding, particle stability, and particle uptake is examined. It was hypothesized that clearance of these particles was mediated by agglomeration and/or degradation of the protective PEG coating, allowing for opsonization and uptake from the bloodstream. PEG coated particles were incubated in human plasma over a 48h period and analyzed for total adsorbed protein and particle size. No agglomeration was observed during this period; however the particles showed a continuous increase in adsorbed protein beginning after approximately 2 hours of incubation. PEG coated particles were also administered via tail-vein injection to ICR mice and the total gold content in the blood, liver, and spleen were monitored over 48h after the dose. Approximately 2h after dosing, the concentration of gold in the bloodstream began to decrease while the tissue concentrations began to increase, paralleling the protein binding behavior of the particles. Taken together, these results suggest that after approximately 2h, the PEG coating has degraded to a point at which it is no longer effective at resisting protein adsorption. Although still well dispersed, once this layer of protection has been lost, the particles can be readily recognized by the body and removed from circulation by the liver and spleen.
found in the co-culture for all particles. The co-culture system showed a significantly lower response to reactive oxygen species (ROS) formation upon exposure to Ag 200 nm. In conclusion, the co-culture model could be more relevant as the mucus layer potentially traps and prevents NPs from reaching the epithelium. An overall protective effect against oxidative stress and metabolic activity decrease was observed. Ag NPs induced size-dependent effects on IL 8 release that could not be attributed solely to ions. Furthermore, the NanoSIMS 50 could be a useful tool for NP uptake evaluation and intracellular localisation.

ACUTE VASCULAR EFFECTS OF NANOPARTICLE INFUSION IN ISOLATED PERFUSED SKIN.

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Engineered nanomaterials are widely used in various biological and industrial applications. Their safety profile has largely been defined by in vitro inhalational exposures or using various in vitro cell-based techniques. Some concern has arisen to their potential adverse effects in the vascular system as evidenced by endothelial cell toxicity or deposition of particles in endothelial cell walls. In the process of conducting biodistribution studies in the ex vivo isolated perfused porcine skin flap (IPPSF) with arterial infusion of nC60, quantum dots (QD), citrate or silica coated spicules, biodistribution studies in the vasculature were performed. Biodistribution profiles during perfusion and skin flap weights prior to and following perfusion have been collected on all 3,600 IPPSFs conducted over the past few decades, providing a robust database on which to assess vascular changes. The most pronounced and earliest effect of nanomaterial was an increase in arterial vascular resistance during perfusion. This was accompanied by increases in perfused skin flap weight, possibly indicative of edema or vascular congestion. These studies suggest that systemic exposure to a number of diverse nanomaterials may have adverse effects on the vascular physiology that merits further attention when conducting in vivo studies.

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91 BIOLOGICAL EQUIVALENT-BASED RISK ASSESSMENT—THE CASE OF BPA AND HCHO.

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The work deals with the refinement of Risk Characterization Ratio (RCR) calculation for Bisphenol-A and HCHO by employing the concept of Biological Equivalents (BEs). To utilize the concept of BEs, the appropriate internal dose modeling was employed. The models developed were a multi-compartmental mother-fetus PBPK model for BPA, while the corresponding PBPK model for HCHO describes the formation of DPH (an HCHO-DNA adduct) in the nasopharyngeal cavity. The BE for BPA was estimated (equal to 0.16 μg/L) by calculating the steady-state concentration of free-plasma BPA for a typical adult, receiving orally an amount equal to the one suggested by EFSA TDI (~82% of the overall variance). Cluster analysis with the principal components indicated that 744 and 502 duodenal and 1021 and 167 jejunal genes exhibiting sigmoidal dose-response profiles at 8 and 91 days, respectively. At 8 days, the interquartile EC50 range overlapped between duodenum and jejunum, but the median EC50 was -10 times lower in duodenum (5 μg/L) compared to jejunum (52 μg/L). At 91 days, the median EC50 values were comparable between duodenum (49 μg/L SDD) and jejunum (59 μg/L SDD). Functional annotation identified differential expression associated with oxidative stress, cell cycle, cell death and immune response, consistent with changes in oxidative stress and inflammatory markers. QRT-PCR confirmed induction of oxidative stress genes, including Gpx2 and Gclc. Genes involved in dietary iron absorption/transport were also repressed, suggesting SDD may interfere with iron homeostasis. These results indicate that, like the mouse, the rat intestinal epithelium is responsive to Cr(VI). Comparisons of gene expression changes in rats and mice should inform the mode of action underlying the different tumor outcomes in the small intestine. Funded by the ACC Cr(VI) Panel.

94 ASSESSMENT OF GENOTOXIC POTENTIAL OF CR(VI) IN THE MOUSE DUODENUM VIA TOXICOGENOMIC PROFILING.

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Chronic exposure to hexavalent chromium [Cr(VI)] as sodium dichromate dihydrate (SDD) leads to intestinal tumors in mice, and oral cancers and mild hypoperfusia in rats. In this study, the dose-dependent gene expression effects of SDD (0.3-520 mg/L) were evaluated in female Fischer rat duodenal and jejunal epithelia following 7 and 90 days of exposure in drinking water. Agilent 4x44K whole-genome microarrays identified 3269 and 1815 duodenal, and 4557 and 1534 jejunal differentially expressed genes (fold change>1.5, P<0.009) at days 8 and 91, respectively. Comparisons at day 8 showed significant overlap (2312 genes) between duodenum and jejunal gene expression, with the former exhibiting greater ef-ficacy (i.e. maximum fold change). The overlap was ~50% lower at day 91 with comparable fold inductions in both tissues. Automated analysis identified 744 and 310 duodenal and 1021 and 167 jejunal genes exhibiting sigmoidal dose-response profiles at 8 and 91 days, respectively. At 8 days, the interquartile EC50 range overlapped between duodenum and jejunum, but the median EC50 was -10 times lower in duodenum (5 μg/L) compared to jejunum (52 μg/L). At 91 days, the median EC50 values were comparable between duodenum (49 μg/L SDD) and jejunum (59 μg/L SDD). Functional annotation identified differential expression associated with oxidative stress, cell cycle, cell death and immune response, consistent with changes in oxidative stress and inflammatory markers. QRT-PCR confirmed induction of oxidative stress genes, including Gpx2 and Gclc. Genes involved in dietary iron absorption/transport were also repressed, suggesting SDD may interfere with iron homeostasis. These results indicate that, like the mouse, the rat intestinal epithelium is responsive to Cr(VI). Comparisons of gene expression changes in rats and mice should inform the mode of action underlying the different tumor outcomes in the small intestine. Funded by the ACC Cr(VI) Panel.

95 COMPARATIVE TOXICOGENOMIC ANALYSIS OF CR(VI) EFFECTS IN RAT AND MOUSE SMALL INTESTINE.

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Continuous drinking water exposure to hexavalent chromium [Cr(VI)] as sodium dichromate dihydrate (SDD) is linked to intestinal tumor development in mice but not rats. In this study, the dose-dependent gene expression effects of SDD (0.3-
520 mg/L) were evaluated in female Fischer rat and B6C3F1 mouse duodenal (Duod) and jejunal (Jej) epithelia following 7 and 90 days of drinking water exposure. Using HolomoGeneID, 13899 orthologs were identified as represented across the mouse and rat whole-genome 4x44K Agilent microarrays. At day 8, comparative analysis (fold change > 1.5, P < 0.099) identified 5013 differentially expressed mouse orthologs compared to 2790 rat orthologs in the Duod, with comparable numbers of orthologs (rat 3782, mouse 3334) in the Jej. Ortholog comparisons at day 91 suggest mouse Duo (3484) and Jej (3620) are ~2.5 times more responsive to Cr(VI) compared to rat Duo (1504) and Jej (1305). Automated dose-response modeling suggests the mouse had more orthologs exhibiting a sigmoidal dose-response compared to the rat, although the median EC50s were over comparable (39-55 mg/L SDD). Functional annotation identified differentially expressed orthologs associated with oxidative stress, cell cycle, cell death and immune response, consistent with changes in GSH/GSSG levels, hyperplasia, apoptosis and inflammation. Several differentially expressed orthologs exhibited species-specific expression that was verified by QRT-PCR. For example, genes associated with inflammation (C2l2q: Mouse, C3: Rat, T. g. house, transport (S.25a2: T. r. mouse) and growth (Arg1: T. mouse, I. r. rat) exhibited divergent expression. Implications of these analyses for the mode of action for Cr(VI)-induced intestinal carcinogenesis will be discussed. Funded by the ACC Cr(VI) Panel.

96 MODE OF ACTION FOR INTESTINAL CARCINOGENESIS OF INGESTED HEXAVALENT CHROMIUM IN MICE.


Chronic ingestion of hexavalent chromium (Cr(VI)) has been shown to cause small intestinal tumors in mice at concentrations ≥ 60 mg/L of sodium dichromate dihydrate (SDD). To assess the mode of action (MOA) for carcinogenesis in rodents and relevance to humans, we conducted 90-day drinking water studies in both rats and mice, with collection of histopathological, biochemical, toxicogenomic, and pharmacokinetic data, as well as measures of DNA modification at concentrations of 0.3-520 mg/L SDD. These studies were designed to assess whether intestinal tumors are initiated through a mutagenic or non-mutagenic MOA. After 7 days of exposure, change in redox status (decreased GSH/GSSG) occurred ≥60 mg/L in the mouse duodenum; cytotoxicity in the villi and apoptosis in the crypt were observed at ≥ 170 mg/L and crypt hyperplasia was observed at 520 mg/L. At day 91, these effects progressed, each occurring at lower doses, with changes in redox status starting at 14 mg/L. Cytogenetic examinations of intestinal crypts revealed apoptosis at the highest two concentrations, but no increase in micronuclei. Toxicogenomic studies of intestinal crypts revealed apoptosis at day 8 at the highest two concentrations, but no increase in micronuclei. Several differentially expressed orthologs exhibited species-specific expression that was verified by QRT-PCR. For example, genes associated with inflammation (C2l2q: Mouse, C3: Rat, T. g. house, transport (S.25a2: T. r. mouse) and growth (Arg1: T. mouse, I. r. rat) exhibited divergent expression. Implications of these analyses for the mode of action for Cr(VI)-induced intestinal carcinogenesis will be discussed. Funded by the ACC Cr(VI) Panel.

98 DEVELOPMENT OF A UNIT RISK FACTOR FOR NICKEL AND INORGANIC NICKEL COMPOUNDS BASED ON AN UPDATED CARCINOGENIC TOXICITY ASSESSMENT.


It is important for the TCEQ to conduct up-to-date assessments of all carcinogens (including nickel) emitted in Texas based on the latest scientific data and analyses. For nickel, because there are industrial emission sources in Texas which may increase air concentrations in neighboring communities and the expected nickel species exposure profile from Texas sources is different than that of nickel refinery workers, it was important to use studies with more environmentally-relevant exposures to help ensure generalizability to the Texas public (i.e., the most predictive toxicity factor possible). Consequently, the TCEQ has developed an inhalation unit risk factor (URF) for nickel based on excess lung cancer in two epidemiological studies of nickel refinery workers with nickel species exposure profiles most similar to emissions expected in Texas (i.e., low in sulfide nickel). One of the studies (Enterline and Marsh 1982) was used in the 1986 USEPA assessment, while the other (Grimsrud et al. 2003) is an update to an earlier study (Magnus et al. 1982) used by USEPA but not known to have been used previously to derive URF estimates. The linear multiplicative relative risk model with Poisson regression modeling was used to obtain maximum likelihood estimates and asymptotic variances for cancer potency factors (β) using cumulative nickel exposure levels versus observed and expected lung cancer mortality (Enterline and Marsh 1982) or lung cancer incidence cases (Grimsrud et al. 2003). Life-table analyses were used to develop URFs for inhalation exposure to the two studies, which were then combined using weighting factors relevant to confidence to derive the final URF for nickel of 1.7E-04 per μg/m3. The de minimis air concentration corresponding to a 1 in 100,000 extra lung cancer risk level is 0.059 μg/m3. The TCEQ will use this conservative value to protect the general public in Texas against the potential carcinogenic effects from chronic exposure to nickel.

99 THE USE OF SURROGATE DATA AND CHEMICAL ESTIMATION METHODS IN THE DEVELOPMENT OF A UNIT RISK VALUE FOR CUMENE (1-METHYLBENZENE).


Chemune (1-methylnaphthalene) has demonstrated clear carcinogenicity in mice and rats (NTD 2009) and is based on kidney lesions, body weight, stunting, xenyle and benzene. Like other alkylbenzenes cumene may undergo side chain or ring oxidation via cytochrome P450. Likely metabolites would include 4-isopropylphenol and the corresponding catechol, dimethylphenylcarbinol, quinone methide, and 4-isopropylquinone. Although no PBPK model for cumene was identified in the published literature, models for ethylbenzene and related aromatics (α-xylene, toluene, benzene) have been published. In order to assess internal dosimetry of cumene in the rodent bioassays our approach was to use ethylbenzene metabolic parameters as a surrogate for cumene in a PBPK model and derive chemical parameters for cumene with chemical estimation methods. We then compared PBPK-derived internal dose metrics with applied dose to fit the quantal tumor incidence data. The metabolic parameters for ethylbenzene were Vmax = 7.6 mg/hr/kg/74 and Km = 0.1mg/L. Four sets of cumene partition coefficients were developed using different estimation methods and evaluated in mouse and rat PBPK models. The modeled dosimetry (metabolized dose) gave superior fits to the most sensitive site tumor data for female mice (lung) compared to the applied dose. For alveolar and bronchiolar adenomas and carcinomas combined the best estimate benchmark dose and fit statistics were BMD10 = 11.2, BMDL10 = 7.2 mg/kg-d; X2 = 0.25, and P = 0.62. The slope factors for mice and humans were 0.014 and 0.084 mg/kg-d, respectively. The latter value would correspond to a unit risk value of approximately 0.012/mg/m3 assuming a default low dose uptake of 50%. Potency values obtained through PBPK modeling were about twice the values based on applied doses. Several of the parameter sets gave similar values indicating the robustness of the approach.
100 CARCINOGENIC RISK ASSESSMENT FOR THE USE OF METHYLENE BLUE IN DAIRY COWS.

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Methylene blue (MB), though not approved as an animal drug by FDA, is used as a food-animal antidote for nitrate poisoning because there is no viable alternative. CVM recommended in 1990s a 180-day slaughter withdrawal period in ruminants. Since then, new information has emerged including total residue data and a 2008 NTP study report. Therefore, the purpose of this evaluation was to determine if the previously recommended 180-day withdrawal time for both edible tissue and milk could be reduced. To achieve such a goal, we followed a step-by-step approach. First, the NTP report (NTP TR 540) was evaluated and we concurred with the finding that MB is genotoxic and carcinogenic. Second, we calculated the concentration of total residue of carcinogenic concern of the test compound in the total diet of test animals that corresponds to a maximum lifetime risk of cancer in the test animals of 1 in 1 million. We allocated 70% and 30% of the So to tissue and milk, respectively. Third, we used the allocated So to derive Sm, the permitted concentration of residues of carcinogenic concern in a specific edible product. Fourth, we reviewed the available residue data for MB in tissues and milk, and found that neither the depletion rate of MB in tissues down to Sm concentration nor the depletion rate of MB in milk could be established. Based on the above assessment of the carcinogenic concern of MB and residue information, we conclude that (1) the available residue data are not sufficient to allow a shorter than 180-day withdrawal time for both tissue and milk, (2) a depletion study for total residues (typically 14C-radiolabel) with adequate sampling times and number of animals is needed in order to determine the depletion profile of MB in tissues and milk, and (3) the Food Animal Residue Avoidance & Depletion Program (FARAD) recommended 14-day withdrawal period for edible tissues and 4-day milk discard time for MB is not supported by the information available to CVM.

101 CONSIDERING MODE OF ACTION IN ESTIMATING A NSRL FOR 3-MCPD

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Pursuant to California’s Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65), 3-monochloronorpropene-1,2-diol (3-MCPD) was listed as a carcinogen on October 8, 2010. As neither California EPA nor U.S. EPA has developed a cancer potency estimate (i.e., slope factor [SF*]) for 3-MCPD that could serve as a basis for a non-significance-risk level (NSRL), an independent risk assessment was conducted. SF* for 3-MCPD were estimated using available experimental bioassay data for male and female Fischer 344 and Sprague-Dawley rats administered 3-MCPD for 2 years via drinking water. A multistage cancer risk extrapolation model was implemented by using a bootstrap Monte Carlo procedure to the 14C-radiolabeled) with adequate sampling times and number of animals is needed in order to determine the depletion profile of MB in tissues and milk, and (3) the Food Animal Residue Avoidance & Depletion Program (FARAD) recom mendation of 14-day withdrawal period for edible tissues and 4-day milk discard time for MB is not supported by the information available to CVM.

103 A QUANTITATIVE RISK ASSESSMENT OF 2, 3-PENTANEDIONE, BASED ON PRELIMINARY DATA.

D. A. Dankovic1 and D. L. Morgan. 1Risk Evaluation Branch, CDC/NIOSH, Cincinnati, OH and 2Respiratory Toxicology, NIEHS, Research Triangle Park, NC.

Only preliminary (pilot study) toxicity data are currently available for the food flavoring 2,3-pentanedione (PD). However, it is possible to compare the pilot study data for PD to similar data for diacetyl and derive an estimate of the relative toxicities of PD and diacetyl in mice. PD data were taken from a study described by Morgan et al. [2010] (Toxicologist 114(1):316) and compared to a diacetyl pilot study, Morgan et al. [2008] (Toxicologist 103 A QUANTITATIVE RISK ASSESSMENT OF 2, 3-PENTANEDIONE, BASED ON PRELIMINARY DATA.

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104 US EPA’S PROPOSED TOXICITY VALUES FOR TCDD: IMPLICATIONS FOR DECISION-MAKING REGARDING THE SAFETY OF FOODS IN THE UNITED STATES.

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USEPA recently released a draft RfD (0.7 pg/kg-d) and oral CSF (0.001 pg/kg-d) for 2,3,7,8-TCDD. The CSF equates to intakes of 0.1–0.001 pg TEQ/kg-d1 at 10-4-10-6 risk levels, respectively. These intakes are substantially lower than tolerable daily intakes (TDIs) developed by other regulatory and public health agencies (eg, JECFA has established a provisional tolerable intake equivalent to 2.3 pg/kg-d), as well as considerably below estimated breastmilk intakes (242 pg/kg-d). Our objective was to compare intakes of dioxin-like compounds (DLCs) from US foods to intakes associated with the USEPA draft toxicity values as a means of assessing the implications of these values. Daily intakes were based on USDA estimates of average total dietary intakes of DLCs, which were calculated using data from the Total Diet Study and consumption data from USDA. Risk and hazard were estimated using standard equations and exposure factors for child, adolescent, adult and age-adjusted scenarios. Daily intakes from food greatly exceeded the intakes associated with the CSF at all risk levels for all age groups. Daily intakes also exceeded the RfD for a number of infant, child and adolescent age groups. The cancer risk associated with lifetime dietary DLC exposure (3x10-4 to 9x10-4) was above USEPA’s acceptable risk range. The noncancer hazard estimates were found to exceed the USEPA’s target HQ of 1 for infants (1.4-4.9), children (2.5-12.4), and adults (3.4). The 1.3 when the full value of the detection limit was imputed for non-detects (NDs). In contrast, when intakes were compared to the JECFA value, which is protective of
both cancer and noncancer, results indicated that daily intakes are below the level of concern for all age groups regardless of the value imputed for NDs. Collectively, these data indicate that different conclusions about the toxicity of TCDD lead to substantially different conclusions regarding the safety of foods, and demonstrate the importance of using best available science rather than simply relying on precautionary approaches.

105 ASSESSMENT OF RISKS TO CHILDREN EXPOSED TO HYDROCARBON CONSTITUENTS FROM THE GULF OF MEXICO OIL SPILL.

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On April 20, 2010, an oil exploration platform, DeepWater Horizon, located approximately 41 miles off the Louisiana coast, failed causing a release of crude oil at the depth of 5,000 feet. This event has been defined as the largest offshore oil spill in US history. By July 4, 2010, surface oil came onto populated shorelines of Louisiana, Mississippi, Alabama, and Florida. On July 15, 2010, the leak was stopped and the well was officially sealed on September 19, 2010. However, approximately 185 million gallons (MG) of crude oil was released into the Gulf of Mexico. Moreover, a total of 1.8 MG of dispersant was sprayed onto or injected into the water column as part of the spill response activities. Questions about the toxicity of spilled oil/dispersant to human health and the environment linger given the state of current knowledge. The presentation focuses on the assessment of health risks to children who may be exposed to the spill constituents while playing/recreational activities. Extensive data collection activities were undertaken by various agencies resulting in a vast explosion media database on air, water, and sand concentrations of hydrocarbons. These data, after screening for adequacy and applicability to the beach exposure scenario, were adopted in the current study to populate an exposure model for a young child directly exposed to all three media. The results of exposure modeling and toxicity assessment for a range of tested constituents on GOM beaches of Louisiana, Mississippi, Alabama, and Florida are tabulated and discussed relative to the magnitude of worst-case, average, and most likely risks.

106 RISK-BENEFIT ASSESSMENT OF FLAME-RETARDANT CHEMICALS.

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As a basis for regulation of flame retardant (FR) chemicals, both potential health risks and fire safety benefits should be evaluated. Using an integrated risk assessment, we evaluate the use of organohalogen FRs to comply with California furniture flammability standard Technical Bulletin 117 (TB117). Pentabrominated diphenylether was the main FR chemical used to comply with this standard from the 1980s until 2004, when it was banned and production ceased. Major replacements include tris(1,3-dichloro-2-propyl) phosphate, removed from children’s sleepwear in 1978 due to mutagenicity concerns, and Firemaster 550, a proprietary mixture containing brominated retardants. Our fire safety evaluation, based on the fire science literature, found that (1) TB117 foam compared to non-FR foam covered in identical fire retardant, (2) differences observed between identically-constructed furniture with non-FR foam and furniture made with foam complying with TB117 are within the normal data scatter. Our human health evaluation, based on the toxicological and epidemiological literature, found that (1) previous and current FRs used for TB117 compliance are found in human body fluids and (2) carcinogenic and/or neurological, reproductive, endocrine, and developmental harm are associated with exposure to some flame retardants.

Disclaimer: The research described in this article has been reviewed by the National Institutes of Health, and approved for publication. Approval does not signify that the contents necessarily reflect the views of the Agency, nor does the mention of trade names or commercial products constitute endorsement or recommendation for use.

107 ANALYSIS OF HEALTH RISKS TO SURGICAL PATIENTS FROM INSTRUMENTS CONTAMINATED WITH USED HYDRAULIC FLUID.

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Over the course of 1-2 months in 2004, certain processing errors led to the inadvertent use of used hydraulic fluid (HF) in place of detergent in one step of a multi-step cleaning and sterilization process of surgical instruments at a US hospital system. Several thousand patients may have had surgery or minor bedside procedures performed using the instruments in question. We evaluated the presence of and amounts of residual fluid and individual constituents potentially remaining on instruments after they were exposed to HF, and conducted a screening-level risk analysis to address the potential hazards posed by their use during surgical procedures. Residual fluid was extracted from HF-exposed instruments in both saline- and solvent-extracted experiments. Of 40 organic analytes, as well as numerous polycyclic aromatic hydrocarbon (PAH) and total petroleum hydrocarbon (TPH) analytes, chemicals of interest (COIs) were identified if they were detected in either experiment. To estimate acute and chronic exposure doses, we used conservative assumptions regarding the number of instruments used during surgery and the amount of transfer of residual HF on instruments to the patient during surgery. We examined toxicity using both whole-mixture and individual-constituent approaches, and we assessed cancer and non-cancer risks using a margin-of-exposure (MOE) approach comparing acute and chronic toxicity effect levels and dietary intake levels to estimates of exposure. We also made an adjustment to oral toxic equivalents (OETs) to ensure the additional potential of greater non-cancer risks of ester exposures. MOEs for acute and chronic non-cancer effects were greater than 100 for all COIs, indicative of negligible toxicological concern. MOEs for cancer effects were greater than 1 for all carcinogenic COIs, indicative of less than 1 in a million cancer-risk. We conclude that there were no appreciable non-cancer or cancer risks to patients who underwent surgery using the HF-exposed instruments.

108 NATURAL GAS EXPLORATION AND PRODUCTION IN THE BARNETT SHALE: ASSESSMENT OF EXPOSURES TO VOLATILE ORGANIC COMPOUNDS (VOCs).

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There are an estimated 13,500 producing natural gas wells in the Barnett Shale (BS) formation, which spans approximately 24 counties in north central Texas (TX). Since 2002, natural gas production in the BS region has substantially increased, and the region contains the largest onshore gas fields in the US. The Texas Commission on Environmental Quality (TCEQ) has over 20 monitors in the region, 8 of which are in relatively close proximity to natural gas exploration and production operations (six AutoGCs analyzing 46-64 VOCs and two canister monitors analyzing 105 VOCs). Maximum 1-hour AutoGC and 24-hour canister data were compared to available federal and state acute health-based toxicity values. Annual average concentrations were compared to available federal and state chronic health-based toxicity values. The measured concentrations were also compared to typical US and TX-specific background concentrations, as well as to TCEQ odor-based values. While there were a few acute exceedances of odor-based values, our analyses showed that none of the 1-hour or 24-hour concentrations exceeded any of the acute health-based toxicity values. For the annual average concentrations, there were exceedances of some federal chronic health-based values for benzene and 1,3-butadiene at all six AutoGCs, but these levels were all below TCEQ chronic health-based values and typical US or TX-specific background concentrations. In terms of the canister monitors, the annual average concentrations of 16 chemicals exceeded some federal health-based toxicity values. However, with the exception of 1,2-dibromoethane and methyl butyl ketone, all of the annual average concentrations were below the TCEQ chronic health-based values. Importantly, all measurements for 1,2-dibromoethane and methyl butyl ketone were non-detected, and the exceedances are solely the result of conservatively assuming that these chemicals were present at a concentration equal to ½ DL. Collectively, these data do not indicate any concern regarding potential exposures to VOCs associated with these types of operations.

109 INHALATION TOXICITY OF PARTICULATE MATTER FROM MIDDLE EASTERN BASES IN RATS EXPOSED FOR TWO WEEKS.

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Personnel deployed in the Middle East are exposed to particulate matter (PM) that may have suspected toxicological properties from various sources. Deployment locations in the Middle East have been examined in some in vitro cell culture screening studies that have indicated differences in the potential toxicity between locations. For example, PM from Iraq (Talil), as compared with PM from other locations (Camp Victory), showed increased lactate dehydrogenase (LDH) and decreased production of MTT, indicators of cellular toxicity. An in vivo inhalation study was conducted by exposing male and female Sprague-Dawley rats via whole body inhalation to an aerosol of PM from Afghanistan or Iraq at 1 mg/m3 or clean air for 20 h/d, 7 d/wk for 2 weeks. After exposures, one set of animals from each exposure
group was necropsied immediately while a second set was held for a 3-week recovery period. At necropsy, bronchial alveolar lavage (BAL) fluid was collected and clinical chemistry and hematology were conducted on blood samples. Group average body weights were not significantly different for the two PM. There were some statistically significant differences in clinical chemistry and hematology results; however, differences were small and there was no overall pattern to indicate organ damage or other biochemical dysfunction. There was no significant difference in the LDH or total protein concentrations in BAL fluid. Also in BAL fluid, concentrations of inflammatory cytokines, MIP-1?, IL-1?, IL-6, and TNF-? were below limits of detection. Finally, histopathology did not identify any PM in the lung tissue and there were no findings that were considered to be induced by the test material. While in vitro studies are useful for initial screening and identified PM with potential toxicity, this two week in vivo inhalation study under conditions more relevant to military personnel deployment showed no toxicity under the conditions of this study.

110 HEALTH IMPLICATIONS OF COAL TAR AND BITUMEN COATINGS IN CAST IRON WATER MAINS.

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1KWR Watercycle Research Institute, Utrecht, the Netherlands and 2National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands. Sponsor: M. Heringa.

Between 1900 and 1990 cast iron water mains have been laid which are coated on the inside by coal tar and bitumen. Although the purpose of this coating is minimizing corrosion, the linings themselves can deteriorate over time. In 2009 one of the Dutch water companies received odor and taste complaints after maintenance in a cast iron distribution network. The complaints could be traced back to the presence of poly cyclic aromatic hydrocarbons (PAHs) in the drinking water. PAHs were presumed to originate from the coal tar or bitumen coating in the cast iron mains. As in the Netherlands normally hardly any PAHs are found in drinking water, the question arose how often and under which circumstances increased PAH levels can occur and whether human health effects may be expected as PAHs have known or suspected genotoxic potential. In a nationwide study, the Dutch water companies took samples in cast iron distribution networks at 120 locations under various conditions such as undisturbed operation, during flushing and after a mains repair. The samples were analyzed for PAHs and the health risk associated with an exposure scenario over a lifetime period was estimated. During flushing high PAH levels may occur in drinking water, sometimes even above the drinking water guideline levels, but after flushing these levels drop rapidly. After the removal of part of the coated cast iron main, increased PAH levels were found in drinking water up to 40 days after repair. Risk assessment showed that despite of exceeding drinking water guideline values, the cumulative excess cancer risk of exposure to PAHs through drinking water remained below the negligible risk of 1 in a million lifetime exposed individuals under the test conditions at the 120 measurement locations. Although no human health effects are expected, presence of PAHs in drinking water is undesired as this may lead to taste and odor problems. The drinking water companies are advised to monitor PAH levels in drinking water after interventions on cast iron mains.

111 MOE-BASED RISK ASSESSMENT OF GENOTOXIC AND CARCINOGENIC INGREDIENTS IN PLANT FOOD SUPPLEMENTS.

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PAHs are a class of chemicals common in the environment. Benzo(a)pyrene (BaP) is a well-studied PAH and a known human carcinogen. Current regulatory risk assessment practices do not account for the risk of in utero chemical exposures, thus regulatory guidelines are missing a susceptible timeframe for exposure. Here, we utilized umbilical cord blood measurements of BaP-DNA adducts to generate an estimate for cancer risk posed by prenatal exposure to BaP. Cord blood was collected at delivery from a cohort of mothers and newborns in New York City. DNA was isolated from white blood cells and BaP-DNA adducts were measured in 380 newborns. Traditional quantitative cancer risk assessment calculations were adapted to generate a cancer risk due to prenatal exposure of BaP. We used a slope factor relating BaP-DNA adducts to tumor incidence from a previously published toxicology study. Some simplifications and assumptions were made in our cancer risk estimation related to extrapolating the conditions of the toxicology study to those in humans, the pharmacokinetics of BaP-DNA adducts, and susceptibility during developmental stages. Based on our analysis, we estimate that prenatal exposure to BaP results in an average excess lifetime cancer risk of 9.3x10^-3; this is unmodified by any age-dependent adjustment factor. These results will be compared to excess lifetime cancer risk estimations in two additional cohorts: where environmental PAH exposure is typically higher. Prenatal exposures should be considered in regulatory risk assessments. We provide here an estimate of excess cancer risk posed by prenatal BaP exposure as an example of how molecular epidemiologic data may be used in risk assessments. There are gaps in this field that need to be addressed through additional research that examines the pharmacokinetics of DNA adduction and cancer risk in a prenatal exposure model, but doing so would allow utilization of cord blood-based biomarkers in risk assessments for prenatal exposures.

112 QUANTITATIVE CANCER RISK POSED BY PRENATAL EXPOSURE TO BA P.

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The frontline drug for tuberculosis treatment, isoniazid (INH), is known to cause mild elevations in alanine aminotransferase (ALT). A subset of patients (<1%) will experience rare, life-threatening liver toxicity. Mechanistic understanding of the INH hepatotoxic reaction has been limited due to a lack of animal models. In this study, a genetically diverse population of mice, or mouse diversity panel (MDP), was utilized to better understand the toxicity and to identify targets of effect in susceptible individuals. Thirty-four mouse strains were exposed for three days to isoniazid (100 mg/kg/day, i.g.) or vehicle. The primary histological finding was microvesicular steatosis in the liver which was strain-dependent in incidence and severity. To uncover the molecular mechanisms of the response, the transcriptome of the liver was evaluated using Affymetrix microarrays. Not unexpectedly, the majority of transcript changes (15,483) were significant for the mouse strain tested and not affected by treatment, a consideration that is important when selecting mouse strains for toxicity assessment. Analysis was focused instead on the 5,371 major transcript changes (15,483) that were different between the MDP and the strain tested (i.e. genotype of the individual) as these changes are the most informative for assessing the mechanisms of toxicity susceptibility. Consistent with the finding of microvesicular steatosis, the primary pathways affected were the methyleneol, safrole or ?-asarone might raise a potential concern for human health and would be of high priority for risk management. ©2011 Scientific Research Publishing, Inc. The research leading to these results has received funding from the European Community’s 7th Framework Programme (FP7/2007-2013) under grant agreement n° 245199. It has been carried out within the PlantLIBRA project (www.plantlibra.eu). This report does not necessarily reflect the Commission’s views or its future policy on this areas.


113 POPULATION-BASED TOXICITY ASSESSMENT IMPLICATES MITOCHONDRIAL DYSFUNCTION AS AN EARLY EVENT IN ISONIAZID-INDUCED LIVER INJURY.

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The frontline drug for tuberculosis treatment, isoniazid (INH), is known to cause mild elevations in alanine aminotransferase (ALT). A subset of patients (<1%) will experience rare, life-threatening liver toxicity. Mechanistic understanding of the INH hepatotoxic reaction has been limited due to a lack of animal models. In this study, a genetically diverse population of mice, or mouse diversity panel (MDP), was utilized to better understand the toxicity and to identify targets of effect in susceptible individuals. Thirty-four mouse strains were exposed for three days to isoniazid (100 mg/kg/day, i.g.) or vehicle. The primary histological finding was microvesicular steatosis in the liver which was strain-dependent in incidence and severity. To uncover the molecular mechanisms of the response, the transcriptome of the liver was evaluated using Affymetrix microarrays. Not unexpectedly, the majority of transcript changes (15,483) were significant for the mouse strain tested and not affected by treatment, a consideration that is important when selecting mouse strains for toxicity assessment. Analysis was focused instead on the 5,371 transcripts for which expression changed with treatment, and the level of change differed by the strain tested (i.e. genotype of the individual) as these changes are the most informative for assessing the mechanisms of toxicity susceptibility. Consistent with the finding of microvesicular steatosis, the primary pathways affected were the
mitochondrial dysfunction and oxidative phosphorylation pathways. These data support the hypothesis that the steatosis observed in these studies has a mitochondrial basis, as transcript changes occurred in genes that are an integral part of many components of the electron transport chain, including genes from Complexes I to V and all subunits of Complex II. Taken together, these data suggest broad disruption of mitochondrial gene expression due to INH treatment, and highlight the value of using a MDP to investigate mechanisms of toxicity.

114 HEALTH ASSESSMENT OF METHANOL USED IN HYDRAULIC FRACTURING FLUIDS.


Hydraulic fracturing (“fracking”) is a method of inducing manmade fractures in low-permeability rocks, so that trapped natural gas can flow from the rock, into the fractures, and into a natural gas well. The volume of fluids used in a fracking job can be as much as 2 to 8 million gallons per well, with water and sand typically constituting about 99.5% of the fracturing fluids. Methanol is used as a chemical additive in fracturing fluids for corrosion resistance, friction reduction, and flowback enhancement. Concerns have been raised about the use of methanol in fracking operations and the potential for impacts on human health. This study evaluated the potential health risks associated with exposure to methanol through consumption of groundwater impacted by methanol-containing fracturing fluids, and incidental ingestion of river and stream waters that received treated flowback. Modeled concentrations of methanol in groundwater and surface water were evaluated in the context of health-based screening levels developed specifically for these exposure scenarios, background dietary intake of methanol, and endogenous methanol production. Based on a high-end methanol concentration in groundwater of 0.6 mg/L, the estimated methanol intake from consumption of groundwater is 0.017 mg/kg body weight per day, corresponding to a hazard index of 0.03, and is more than 40 times less than the estimated dietary intake from fruit juice and wine. A high-end modeled methanol surface water concentration of 0.0000047 mg/L is 9 orders of magnitude below the health-based level derived for protection of incidental water ingestion during recreational activities. The results of this study indicate that, based on current practices, there is little risk to human health from the release of methanol into water as a result of hydraulic fracturing operations.

115 HEALTH RISK ASSESSMENT ON OCCUPATIONAL HAZARDS EXPOSURE AMONG WORKERS INVOLVED IN JASMINE’S AGRICULTURE.

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Many previous studies reported that the agricultural worksite is one source of health hazards to farmers. This survey study aimed to determine the potential health risk of exposure to occupational hazards among workers in a jasmine’s agricultural area, Sila sub-district, Khon Kaen, Thailand. Data were collected by an in-depth interview, observations and personal air samplings. Farmers almost used methyl carbamate and carbendazim to control the pests in the jasmine’s field. Three types of work-related jasmine’s field were jasmine farmers (n=16), jasmine collectors (n=16), and villagers who made garlands (n=22). The qualitative health risk assessment on exposure to occupational hazards was performed by considering of working procedures of those workers and possible types of hazards. Risk characterization identified that jasmine farmers had the highest risk of pesticide exposure through skin contact (40.0%), followed by inhalation exposure (26.7%). Both jasmine collectors and villagers who made garlands had the highest risk of awkward postures and repetitive works, followed by pesticide exposure through skin contact. The maximum concentration of methomyl in the breathing zone of workers (n=24) was 1.12 mg/m3 (range 0.1-12.1 mg/m3), however, carbendazim was not found. Comparison to the standard regulation on the ambient concentration of methomyl, i.e. TLV-TWA of 2.5 mg/m3 (ACGIH) or PEL-TWA of 2.5 mg/m3 (NIOSH), the results of this study did not exceed the regulation. The estimation of inhalation exposure to methomyl among workers was 0.08 mg/kg-d. The result of hazard quotient (HQ=3.24) indicated the potential health risk of jasmine farmers to the long term exposure to methomyl. Therefore, farmers in a jasmine’s agriculture and people should be aware of occupational hazards, particularly using the pesticide by better protection, continuous monitoring, and occupational health surveillance for safe working and living.

116 PROVISIONAL ADVISORY LEVEL (PAL) DEVELOPMENT FOR METHACRYLONITRILE.

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PAL values developed for hazardous materials by the US 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, and 2-yr durations. PAL 1 represents the threshold for mild effects; PAL 2 represents the threshold for serious, irreversible or escape-impairing effects; PAL 3 represents the threshold for lethal effects. PALs have not been promulgated nor have they been formally issued as regulatory guidance. They are intended to be used at the discretion of risk managers in emergency situations when site specific risk assessments are not available. Application of PAL protocols has been performed for methacrylonitrile to estimate oral and inhalation exposure limits. Following oral and inhalation exposure, methacrylonitrile is readily absorbed and widely distributed. Toxicity is due to formation of epoxide intermediates, depletion of tissue glutathione, and metabolic release of cyanide. Oral and inhalation PALs were based on clinical signs consistent with cyanide poisoning or lethality. Oral PAL 1, 2, and 3 values are NR, 0.88, and 20 mg/L for 24-hr; NR, 0.63, and 15 mg/L for 30-d/90-d and 2-yr. Inhalation PAL 1 is 0.077 ppm for 24-hr, 0.063 ppm for 30-d/90-d and NR for 2-yr. Inhalation PAL 2 is 0.20 ppm for 24-hr/30-d/90-d and NR for 2-yr. Inhalation PAL 3 is 1.1 ppm for 24-hr, 0.31 ppm for 30-d/90-d and NR for 2-yr.

117 ORAL RISK ASSESSMENT OF DIACETONE ALCOHOL: USE OF METHYL ISOBUTYL KETONE DATA TO ADDRESS DATA GAPS.


The general population may be orally exposed to diacetone alcohol (DAA) through food and drinking water, since it is used as an indirect food additive and as a solvent in applications with potable water contact. Exposure to DAA may also occur through the various industrial applications for methyl isobutyl ketone (MIBK), since DAA is the primary metabolite of MIBK. Although the longest-term oral study for DAA was a 54-day reproductive/developmental toxicity screen by gavage, the database for MIBK is robust and the spectrum of toxicity is very similar between the two substances when evaluated in single or repeated-dose oral or inhalation studies and in genotoxicity assays. Since toxicokinetic studies confirm rapid metabolism and clearance of MIBK to the more persistent DAA, the majority of the internal exposure to DAA in both oral and inhalation studies with MIBK. The kidney is a target organ after oral exposure to DAA based on hyaline droplet nephropathy in male rats and renal tubular lesions in female rats. Slight changes in reproductive performance and pup viability at high gavage DAA doses may have been secondary to maternal toxicity. The point of departure for risk assessment of DAA was considered exacerbated chronic progressive nephropathy (CPN) after chronic inhalation of MIBK in female F344 rats. Based on 59% systemic absorption inferred from studies in human volunteers and a 20 m3/day inhalation rate, the benchmark concentration (BMCL10) of 25 mg/m3 for exacerbated CPN corresponded to an internal MIBK dose of 0.042 mmol/kg/day. The internal MIBK dose was adjusted to an internal DAA dose based on 79% of the area under the plasma concentration curve (AUC) being associated with DAA and 20% with the parent compound. The internal DAA dose corresponds to an ingested DAA dose of 3.9 mg/kg/day assuming 100% gastrointestinal absorption. Using a 30x uncertainty factor to account for intraspecies variability as well as limited data to compare the AUC after MIBK inhalation to that after oral DAA intake, a Reference Dose (RfD) of 0.1 mg/kg-day was determined for DAA.

118 THE WEIGHT OF EVIDENCE DOES NOT SUPPORT ENDOCRINE DISRUPTION AS THE CRITICAL EFFECT OF BENZOPHENONE.


The general population may be exposed to benzophenone through food and drinking water due to its use as a direct and indirect food additive and as an UV stabilizer in some coatings with water contact. Although some benzophenone derivatives are suspected endocrine disruptors, the weight of evidence does not support a hormonally-mediated mode of action for benzophenone. Decreases in mammary tumors
and thyroid hyperplasia in female rats chronically fed benzophenone were likely secondary to reduced body weight rather than ‘weak endocrine’ activity of the major metabolite, p-hydroxybenzophenone. In the uterotrophic assay, the benzophenone doses achieving comparable responses to 17-β-estradiol were more than three orders of magnitude greater. Putative estrogenic responses achieved in vivo suggested a potency ranging from one to four orders of magnitude weaker than the positive control depending on the assay. Results from the reproduction research study also do not support endocrine disruption or adverse reproductive effects. The modest, non-dose-related decrease in anogenital distance in F1 female rats occurred without other evidence of an androgenic mode of action. The critical effect for human health risk assessment is considered bile duct hyperplasia in female rats. A cytotoxicity-mediated mode of action is plausible in the liver and bile duct due to enterohepatic recirculation, altered bile acid transport and/or cholestasis. The toxic moiety is unclear and there were insufficient data to construct a model describing the disposition of benzophenone or its metabolites in blood or target tissue for use as an internal dose metric. Since a reliable benchmark dose (BMD) for bile duct hyperplasia could not be estimated, the Reference Dose (RfD) of 0.04 mg/kg-day was based on the human equivalent LOAEL of 4 mg/kg-day, which corresponds to a Total Allowable Concentration of 0.3 mg/L in drinking water. A short-term exposure level (STEL) of 2 mg/L was derived based on a human equivalent BMDL_{1,2} of 5 mg/kg-day for renal tubule dilation in F1 adult female rats from the two-generation study.

119 IS SULFUR DIOXIDE A REPRODUCTIVE AND DEVELOPMENTAL TOXICANT?
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Sulfur dioxide (SO2) is regulated as a criteria air pollutant by the US Environmental Protection Agency on the basis of respiratory effects, such as asthma. In addition to respiratory effects, effects on developmental and reproductive endpoints have been observed in some studies. Based on these effects, California’s Office of Environmental Health Hazard Assessment (OEHHA) recently added SO2 as a Proposition 65 developmental toxicant, specifically citing evidence regarding reduced fecundity in males and both pre-term birth and fetal growth effects in women. We evaluated the weight of evidence regarding these effects, focusing primarily on results from epidemiology studies, along with supporting evidence from animal and in vitro studies. OEHHA’s conclusion regarding reduced fecundity is based primarily on results from a single study conducted in a highly industrialized district of the Czech Republic, while related studies do not provide robust evidence to support this association. For both pre-term delivery and fetal growth restriction, there is inconsistency among studies with respect to the critical exposure window associated with these outcomes that cannot be fully explained biologically; some studies show associations for exposure throughout pregnancy, some show associations only in either early or late pregnancy, and others show no association at all. For all three adverse outcomes, there is uncertainty regarding whether the observed associations were due to SO2 or other factors that are also associated with adverse birth outcomes, such as differences in socioeconomic status and birth season. Inadequate control of other factors associated with adverse birth outcomes may confound the observed associations with SO2, particularly if these factors are also associated with SO2 levels. In addition, OEHHA proposed biological plausibility assessments, which SO2 might cause these effects are oxidant by existing data. We conclude that the weight of evidence does not support listing SO2 as a developmental toxicant.

120 ACUTE EFFECTS OF EXPOSURE TO VAPORS OF HYDROGEN PEROXIDE IN HUMANS.
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Hydrogen peroxide (H2O2) is a colorless, reaction unstable chemical with pungent odor. It is mainly used in chemical industrial processes, in paper industry for bleaching, as a disinfectant, for water treatment and bleaching hair. The use of H2O2 has increased in Sweden. The critical effect of H2O2 is considered to be irritation of mucous membranes and airways, however, scarce data available makes it difficult to assess effect levels and thus to set safe exposure limits. The aim of the study was to investigate effects in humans of inhaled H2O2 vapors. Six female and five male healthy volunteers were exposed blinded in a balanced order to clean air, 0.4 ppm and 2 ppm H2O2 for 2 h at rest. Symptoms related to irritation and effects on the central nervous system were rated on Visual Analogue Scales (VAS). The ratings were analysed with a nonparametric test (Friedman) whereas the objective effect measurements were analyzed with repeated measures ANOVA and Students t-test. The VAS ratings were generally low although they varied considerably. The sensory irritation scores were all statistically significant (p=0.09) and nasal and throat irritation (p=0.06) showed borderline tendencies to increase during exposure to 2 ppm H2O2 compared to 0.4 ppm and control exposure. Nasal airway resistance increased after exposure to 2 ppm H2O2 (p=0.04) but not after 0.4 ppm or clean air. No significant exposure-related effects on pulmonary function, nasal swelling, breathing frequency and blinking frequency were found. Furthermore, no clear effects were seen on markers of inflammation and coagulation in blood (C-reactive protein, interleukin-6, serum amyloid A, fibrinogen, factor VIII, von Willebrand-factor and Clara cell protein), although there were some scattered tendencies to increase after exposure to H2O2 compared to clean air. In conclusion, our study suggests that short-term exposure to H2O2 is slightly irritating at 2 ppm, but not at 0.4 ppm. These data should be considered when setting occupational exposure limits.

121 DRAFT REFERENCE EXPOSURE LEVELS FOR CARBONYL SULFIDE—A NERVOUS SYSTEM TOXICANT.
J. F. Collins and A. G. Salmon. OEHHA, Cal/EPA, Oakland, CA.

Carbonyl sulfide (COS), a chemical intermediate and a byproduct of oil refining, has been proposed as a grain fumigant to replace methyl bromide, which is being phased out due to its ozone depleting ability, and to supplement phosphogas, which is experiencing increased insect resistance. Morgan et al. (Toxicol Appl Pharmacol 200:131–145, 2004) exposed groups of male F344 rats to 0, 75, 150, 300, or 600 ppm COS for 6 h once and then held them for 2 weeks. Adverse effects were noted at 600 ppm in the respiratory system and the central nervous system. Thus 600 ppm was a LOAEL and 300 ppm was a NOAEL for acute effects. Morgan also exposed F344 rats to 0, 75, 150, 200, 300, 400, 500, or 600 ppm COS 6 h per day, 5 days per week for up to 12 weeks. After 12 weeks at 400 ppm, the predominant lesions were necrosis in parietal cortex area 1 and neuronal loss, microgliosis, and hemorrhage in the posterior colliculus; occasional necrosis was seen in the putamen, thalamus, and anterior olivary nucleus. Thus 400 ppm was a LOAEL and 300 ppm a NOAEL for subchronic effects. Based on these reports we have estimated a draft acute (1-hour) Reference Exposure Level (REL) of 2.7 ppm and a draft chronic REL of 90 ppb for COS based on effects on the rat nervous system. Although derived by approved methodology, the RELs for carbonyl sulfide have not undergone external peer review.

122 DERIVATION OF DRAFT CHRONIC REFERENCE EXPOSURE LEVELS FOR BOTH DIOXIN-LIKE AND NONDIOXIN-LIKE PCB CONGENERS.
J. Yang, A. G. Salmon and M. A. Marty. OEHHA, Cal/EPA, Oakland, CA.

Polychlorinated biphenyls (PCBs) are ubiquitous global contaminants with important toxic effects, although their production was banned nearly 3 decades ago. Health risk assessment for this class of chemicals is complex, since exposure occurs to mixtures of many PCB congeners in differing proportions. Therefore, toxicological and exposure data for individual PCB congeners are most relevant to health risk assessment. Five PCB congeners were selected based on toxicity and exposure information, which cover both dioxin-like (DL) and non-dioxin-like (NDL) PCBs as previously described in our publication of TEF_{v} values. Although extensive experimental data are available on PCB toxicities, investigators have used different methods, and measured different biomarkers, with variable results, complicating study-to-study comparison. To overcome this difficulty, we compared several similar subchronic studies that investigated health effects of dietary exposure to these congeners in rats. Among several biomarkers, we find that thyroid hormone is one of the most consistently sensitive biomarkers for these PCB congeners. We then determined the no-observed-adverse-effect levels (NOAELs) for PCBs 28, 77, 118, 126, and 153; these are 36, 8.7, 17, 0.01, and 34 μg/kg-day, respectively. By setting occupational exposure limits.
123 PROVISIONAL ADVISORY LEVELS (PALS) FOR CARBON DISULFIDE.
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PAL values developed for hazardous materials by the US 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, 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, but are intended for use at the discretion of risk managers in emergency situations when site-specific risk assessments are not available. PALS are developed using standard protocols with appropriate human and animal data. Carbon disulfide (CS2) is a colorless liquid with a high vapor pressure that has been used in manufacturing cellulose rayon by the viscose process since the early 1900s, and is also used to produce carbon tetrachloride, cellophane, and other sulfur compounds. Large amounts are made commercially (>10^7 tons in 2002). Carbon disulfide is fat-soluble and concentrates in lipid-rich tissues, especially the nervous system, which is the primary target organ (CNS and PNS) in humans and animals. Chronic occupational exposure has also been associated with blood lipid changes and heart disease. Oral PALS were derived using a rabbit developmental study: PAL 2 and PAL 3 values were 29 and 88 mg/L, respectively, for 24 hours and for 30 days. Oral PAL values were not derived due to insufficient data. Inhalation PALS were derived using human and animal data. The PAL 1, 2, and 3 were, respectively, 1.7, 5.3, and 7.5 ppm for 24 hours; 0.89, 4.1, and 5.4 ppm for 30 days; 0.71, 4.1, and 5.4 ppm for 90 days, and 0.38, 0.79, and 3.2 ppm for 2 years. These values were approved by the Expert Consultation Panel for Provisional Advisory Levels in July 2011.

124 INTERSPECIES ADJUSTMENTS TO RODENT-DERIVED DIOXIN TOXIC EQUIVALENCY FACTORS ARE NECESSARY FOR PCBs.
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The World Health Organization Toxic Equivalency Factor (TEF) scheme is used to estimate the combined human health risk of complex mixtures containing dioxins and “dioxin-like” compounds. Individual TEF values are primarily based upon toxicity data from rodent feeding studies. The literature now abounds with human-specific relative potency (REP) estimates, derived from in vitro system studies, which question the relevancy of rodent-derived TEFs for human health risk assessment. For instance, human-derived REP estimates for the most potent “dioxin-like” poly-chlorinated biphenyl (PCB) congener, PCB 126, are approximately 40-fold lower than its assigned TEF of 0.1. This species difference is robust, repeatable, and significant. Since both PCB 126 and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; the reference compound) exhibit similar in vitro pharmacokinetics and rodent-derived PCB126 REPs are invariant between in vitro and in vivo studies, the human-specific in vitro REP estimate for PCB 126 can serve as a direct replacement to the current TEF. Although pharmacokinetic issues may cloud the direct applicability of in vitro REP estimates for PCB congeners other than PCB 126 in adjusting TEFs representing oral intake, studies using human cells have determined that these congeners exhibit either very weak (i.e., partial agonism) or undetectable “dioxin-like” activity. Overall, from this information we have developed interspecies adjustments to the current TEF values for PCBs. Following a weight-of-evidence approach, in vitro human data indicate that most PCB congeners should be removed from the TEF scheme entirely. In addition, the in vitro sensitivities of human cells to “dioxin-like” PCBs also calls into question current Aroclor risk values, where the critical effects are mainly due to the “dioxin-like” toxicity of PCB congeners observed in ultra-sensitive animal models (i.e., rats and rheas monkeys).

125 SUBCHRONIC TOXICITY OF BENZYL ACETONE IN RATS.
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The subchronic toxicity of benzyl acetone, a widely used fragrance ingredient, was evaluated in a 13-week oral toxicity study in Sprague Dawley rats with a 4-week recovery period. Male and female rats (10/sex/dose) were dosed via gavage at dose levels of 0 (control), 55, 165, 250 or 500 mg/kg/day. Corticosterone in urine was monitored with the vehicle, corn oil. An additional 5 rats/sex were treated with 0 and 500 mg/kg/day and were maintained for a 4-week recovery period. Observations included clinical observations, food consumption, body weights, functional observa- tional liver histopathology, hematology, plasma chemistry, urinalysis, estrous cycling, sperm analysis, gross necropsy and histopathology. One female at 55 mg/kg, 1 male at 165 mg/kg and 1 female at 500 mg/kg were found dead. Reasons for these deaths could not be established. Mean hemoglobin values were re- duced in females during week 7 and in males and females during week 14. Kidney weights were significantly increased in males at 500 mg/kg, but this finding had re- solved with recovery. Liver weights were significantly increased in both sexes at 165, 250 and 500 mg/kg; this effect was also reversible with recovery. Histopathological findings included reversible hepatocellular hypertrophy in livers from males at 250 mg/kg and in males and females at 500 mg/kg, reversible thyroid follicular cell hyper- trophy in males at 500 mg/kg and tubular lesions in male kidneys at 250 and 500 mg/kg which were associated with an increase in hyaline droplet formation. This finding was compatible with the diagnosis of alpha-2-micro-globulin nephropathy and was not considered relevant. Based on these results, the NOAEL was concluded to be 165 mg/kg.

126 A RAPID PROCESS FOR SETTING TOXICOLOGICAL LIMITS IN SUPPORT OF EQUIPMENT CLEANING IN A CONTRACT DRUG MANUFACTURING FACILITY.
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In drug manufacture, production equipment is cleaned to assure that there is minimal carryover of residual chemicals from one drug product run to the next. Validation of these cleaning methods is a critical step in assuring the safety and quality of the drug products, and includes setting Toxicological Limits (TLs), amounts of residual chemicals that can safely be carried over to the next product. Industry practices to set TLs are evolving away from default X-fold reduction in drug substance, to chemical-specific, health-based risk assessments. Also, assessment has expanded to include intentional and unintentional additives as well as the drug substance. In a contract manufacturing plant new drug products are constantly added, demanding a rapid method to set TLs. We report a tiered process to efficiently set TLs. Whenever available, published health-based human exposure limits or minimum recommended therapeutic doses, are used as the bases for setting TLs. In the ab- 2

127 CUMULATIVE RISK ASSESSMENT OF THREE ALDEHYDES PRESENT IN TOBACCO SMOKE: USING MARGIN-OF-EXPOSURE (MOE) AND MODE-OF-ACTION (MOA) EVALUATIONS.
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There has been significant interest in further characterising tobacco smoke toxicants both from the perspective of future regulatory frameworks aimed at monitoring or lowering toxicant levels and from the perspective of tobacco product development focused on selective toxicant reduction as part of broader harm reduction initiatives. We have previously described the use of the Margin of Exposure (MOE) model as part of a quantitative risk assessment paradigm for tobacco smoke toxicants, in conjunction with the preparation of mode of action (MOA) reviews. To generate a combined MOE assessment two assumptions are made: 1) the compounds are structurally similar and 2) that they share similar toxicological proper- ties. The generation of MOAs reduces the number of assumptions made in combined MOE assessments by ensuring similar toxicological properties and lesion types are seen. In this example, three structurally similar aldehydes found in tobacco smoke have had MOE and MOA assessments completed. The aldehydes investigated were acetaldehyde, formaldehyde and propionaldehyde. Multiple MOEs for each toxicant were generated from data available in the literature. The MOA reviews identified the same four key events associated with the carcinogenic potential of all three of these aldehydes. For each of the four key events, the lowest generated MOEs have been combined, using the following formula: MOEcombined = 1 / [1/MOE1 + 1/MOE2 + 1/MOE3 + 1/MOE4]. The cumulative MOEs generated were: Genotoxicity – 0.13; Cytotoxicity – 80.55; Hyperplasia / Metaplasia – 7.42; Tumours – 74.38. In all instances, the MOEs are lower than 100 suggesting that these toxicants are of a very high prior- ity for risk reduction research (Cunningham et al., Food Chem Tox, doi:10.1016/j.fct.2011.07.019). This is the first attempt at generating a combined MOE assessment for a group of toxicants found in tobacco smoke, based on the
However, screening (HTS) assays in the determination of chemical testing priorities. Momentum has been growing in Toxicology to assess the utility of high-throughput assays. This daily dose was then compared to human exposure estimates to aid in prioritization. Unlike the Phase I set, comprised primarily of food-use, data-rich pesticides, the -700 chemicals in Phase II are much more diverse structurally and in their usage and exposures. In vitro hepatic clearance and plasma protein binding assay data generated for Phase II chemicals indicate similar distributions to those observed with the Phase I set. The majority of chemicals are highly bound to plasma proteins, with median and upper quartile values at -3 and -20% unbound, respectively, for both sets. In vitro hepatic metabolic clearance values are also similar, with median and upper quartile values at -8 and -18 μL/min/106 cells, respectively, for both. In contrast to the Phase I chemicals, review of USEPA and CDC documents revealed that human exposure estimates are available for <10% of the Phase II chemicals. Our data indicate that the PK behavior of Phase II chemicals is similar to that of the Phase I set. Strategies to provide human exposure estimations, in part through EPA's ExpoCast project, are being explored to compensate for the limited information available for these chemicals. This abstract does not necessarily reflect EPA policy.

A modified Joint Director Laboratories (JDL) data fusion (DF) framework was developed to integrate data from disparate sources to estimate risks associated with potential exposure to a petroleum hydrocarbon mixture. The framework was used to detect patterns and integrate various toxicological datasets from the F1 group of hydrocarbons. F1 toxicological data were fused where available. The main objective of our case study was to demonstrate the applicability of the DF-based approach in risk assessment and management projects. For chemical mixture risk assessment, the problem formulation was defined using an illustrative example of a contaminated site. Traditionally, health risk assessments of mixtures are evaluated using a surrogate of chemical mixture data (e.g., current practice of F1 hydrocarbons assessment) or through component data. For neurotoxicity response analysis, neurotoxic metabolites toxicological data were fused with predictive toxicological data and then probability-boxes (p boxes) were developed to represent the toxicity of each compound. The neurotoxic response was given a rating of "low," "medium" or "high". These responses were then weighted by the percent composition in the illustrative F1 hydrocarbon mixture. The resulting p-boxes were fused according to Dempster-Shafer Mixture rule of combination. The fused p-boxes were fused again with toxicity data for n-hexane. We analyzed system biology curated n-hexane datasets. These types of dataset integration exercises from various disparate sources may help contaminated sites risk assessment and risk management project where a key risk management decision may be required based on site specific exposure analysis, operable exposure pathways and toxicity assessment. Part of the work was presented at the Alliance for Risk Assessment workshops. Currently we are exploring data fusion applications in remediation risk management and predictive toxicology tools.

The NRC (2009) Science and Decisions report discusses methods for assessing noncancer risks using linear extrapolation procedures similar to those used to assess risks posed by exposures to carcinogens. As part of the Alliance for Risk Assessment...
workshop series, this study was conducted to demonstrate four potential approaches to extrapolating risks below the point of departure, identified using an extension of the Benchmark Dose (BMD) method that allows the development of risk values at doses above the Reference Dose (RfD). The use of an internal measure of dose (blood methylmercury concentration), rather than external concentrations, was also considered in the risk evaluation by incorporating data from the National Health and Nutrition Examination Survey (NHANES). Methylmercury provides an unusual case in which the available BMD/BMDLs (USEPA 2001) are not only based on human responses, but were also estimated based on biomarkers (i.e., levels in hair and cord blood) measured in individuals as part of the evaluation for potential health effects. Both simple and more complex PBPK models are also available that allow the risk assessor to estimate external exposure rates associated with internal biomarkers. The dose-response conducted here addressed multiple issues raised by the NRC (2009) report including the impact of human variability, sensitive subpopulations, and method of extrapolation above and below the BMD/BMDL and RfD. The results demonstrate that depending on the assumptions used to estimate risks at exposures above the RfD, very different fractions of the population would be expected to have adverse events. This could have significant impacts on decision making in the risk assessment process. This case study exhibits how risk assessments using biomonitoring data for both dose-response and exposure helps to reduce uncertainty in the risk assessment process.

133 APPLYING THE “SCIENCE AND DECISIONS” CONCEPTUAL MODELS (CMS) TO DIOXIN.
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The Alliance for Risk Assessment provided the opportunity to conduct this case study and compare the three CMSs for dose-response from the NRC 2009 report Science and Decisions: Advancing Risk Assessment. This exercise profits our understanding the strengths and weaknesses of applying these models. Dose-response assessments for TCDD were conducted with all three CMSs for the endpoints of hepatic and bile duct tumors in rats. Recently, an expert panel considering the aryl hydrocarbon receptor (AHR) concluded that sustained AHR activation was a pivotal key event in the mode of action (MOA) of rodent liver tumorigenesis. We use a biomarker of AHR activation as a dose metric for species extrapolation. The median dose at 10% risk from linear CM1 was 1 ppm/kg-d, that from nonlinear CM2 was 300 ppm/kg-d, and that from linear CM3 was 6 ppm/kg-d. These results show the differences and potential uncertainty for decisions using risk estimates based on policy versus those based on scientific evidence.

134 ASSESSING EXPOSURE DOSE EQUIVALENT OF VINYL CHLORIDE IN DRINKING WATER FROM SHOWERING AND BATHING ACTIVITIES USING PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING.
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Historically, safety evaluation of chemicals in drinking water accounted only for exposures via the ingestion route. Recently, however, performing chemical risk assessments for drinking water have begun to account for the different routes of exposure where daily intake are calculated as equivalent dose using chemical data and average daily exposure assumptions. With more available information such as pharmacokinetic and toxicity data, the multiroute dose closely related to the target response can be estimated using a physiologically-based pharmacokinetic (PBPK) model. The objective of this study was to investigate these methods in a proposed tiered analysis within a drinking water exposure assessment framework. A PBPK model was derived to determine the human dose equivalent of vinyl chloride in drinking water following a shower exposure scenario. For an average shower exposure, a dermal and inhaled point of departure in humans was extrapolated from a rodent PBPK model and target liver metabolism dose-response relationship. The resulting model dose estimates were transformed to an oral dose equivalent (L-eq) for vinyl chloride in drinking water, and compared with values calculated using the tiered approach. The modeled dose estimates (inhaled 0.5 and dermal 2.0 L-eq) were as conservative as the L-eq values that were generated using chemical exposure data (inhaled 1.0 and dermal 3.0 L-eq). A tiered analysis framework based on available data using the different computational techniques is a scientifically meaningful approach supporting risk assessment practices of chemicals and contaminants in drinking water.

135 DISCREPANCIES AMONG ACUTE GUIDELINE VALUES FOR EMERGENCY RESPONSE.

Acute guideline values are tools for public health risk assessment and management during planning, preparedness and response related to sudden airborne release of hazardous chemicals, for example after an industrial accident or terrorist attack. A comparison of the two most frequently used set of values, i.e. the Acute Exposure Guideline Levels (AEGL) and the Emergency Response Planning Guidelines (ERPG), reveals that the individual values diverge by a factor of 3 or more for almost 40% of the 88 investigated substances, including many of high production volume. These deviations can be explained by differences in selection of critical effect or critical study and in a few cases differences in interpretation of the same critical study. The issue of astatistics was investigated in more detail, as this as a potentially susceptible subpopulation of considerable size. It is stated in the AEGL Standing Operating Procedures that astatistics are considered as a susceptible group, still 67% of the AEGL chemicals do not include any data or comments on astatistics. Moreover, only 6% of the AEGL chemicals include references to experimental data from human volunteers with astatistics and many of these references do not appear in nine other sets of acute reference values that we examined (covering guidelines for emergency response as well as short-term occupational exposure limit). Thus, there seems to be a significant lack of data related to the susceptibility of astatistics and where data are present they are frequently unknown or disregarded. In conclusion, there is a need for international harmonization in the area of acute guideline values. Key factors for broad international acceptance include transparency in the key documentation and the decision process as well as agreement on definitions of toxicological tiers and susceptible target populations such as astatistics. Lack of harmonization and transparency may interfere with trufstful and efficient communication and management during preparedness and response to sudden chemical releases.

136 COMPARATIVE STUDY ON THE UTILITIES OF ANIMAL EXPERIMENTAL DATA FOR RISK ASSESSMENT OF INHALATION EXPOSURE TO CHEMICALS.
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Health risk assessment of chemicals in ambient air should be evaluated using the information obtained from epidemiological studies first in Japan, but toxicity data derived from epidemiological studies are often limited to the cases of occupational exposure. As preventive measures against exposure have been progressed in recent years, the cases toxicity expression due to high dose will be expected as quite rare. In future health risk will be assessed mainly on the basis of animal study instead of epidemiological study. However, there are species differences between human and animals. We intended to confirm whether the extrapolation of species difference using uncertainty factor and other is appropriate. Already, air quality standards or guideline values for air are established in Japan on the basis of epidemiological study. We re-evaluated cancer risk of vinyl chloride (VC) and 1,3-butadiene (1,3-BD) on the basis of animal study and compared with the result of assessment based on epidemiological study. Guideline values for air of VC and 1,3-BD in Japan was already established as 10 and 2.5 mg/cubic meter, respectively, which was evaluated from cancer risk level at 1 in 100,000 based on epidemiological studies. Cancer risk level of VC and 1,3-BD at 1 in 100,000 evaluated on the basis of animal inhalation studies was 1.7 - 19 and 0.6 - 7.2 mg/cubic meter, respectively; the guideline value for air of these chemicals were in the ranges of risk levels evaluated from animal studies. Usefulness of animal experiment data for health risk assessment will be discussed.

137 EFFECTS OF ACUTE EXPOSURE OF HUMAN PRECISION-CUT LUNG SLICES TO CHEMICALS.
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Introduction: A number of low-molecular weight (LMW) chemicals at workplaces are involved in the development of occupational asthma, one of the most common lung diseases in developed countries. Risk assessment is performed in animal experiments, however, in the context of REACH and the principle of 3Rs there is an increasing public demand for alternative methods. Human precision cut
lung slices (PCLS) is an *ex vivo* approach in which all relevant cell types of lung tissue are present in their natural position. The aim of the study was to establish PCLS as a relevant toxicity model for LMW chemicals.

Methods: Human PCLS were prepared from peripheral tumor-free tissue from resected lung lobes of cancer patients. PCLS were incubated with 20 industrial chemicals in serum-free DMEM under standard submerged cell culture conditions. PCLS without test substances were incubated as controls. After 24 hours incubation, induced toxicity was assessed by vitality staining and determination of enzymatic activity using WST-1 assay. The concentrations of chemicals resulting in toxicity very closely and also correlates with LD50 values from in vivo studies. Thus, conclusions can be made.

Results: Concentration dependent toxicity could be shown for all tested chemicals with EC25 ranging from 0.051 μg/mL to 1895 μg/mL. Individual EC25 values correlated significantly with data published for in vitro approaches with THP-1 and NCTC cell lines (r = 0.87 and 0.83, respectively). Furthermore EC25 of human PCLS correlated with LD50 data published for in vivo rat inhalation toxicity with r of 0.53 (p value of 0.08).

Conclusion: The toxicity of chemicals in human PCLS resembles the in vitro situation very closely and also correlates with LD50 values from in vivo studies. Thus, PCLS can be regarded as an *ex vivo* primary tissue toxicity model, displaying toxicity on all relevant cell types of lung tissue and excluding abnormal characteristics of cell lines.

138 ARE OELS AND DNELS THE SAME THING? A QUANTITATIVE COMPARISON OF THE EUROPEAN INDICATIVE OCCUPATIONAL EXPOSURE LIMITS AND THE DERIVED NO-EFFECT LEVELS FOR WORKERS UNDER REACH.

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The new European Union (EU) REACH legislation requires Derived No-Effect Levels (DNEL) to be calculated for substances produced in quantities >10 tonnes/year. Meanwhile, the setting of occupational exposure limits (OEL) continues both at the member state and the EU level. Most OELs established at the EU level are not binding for the Member States (Innovative OEL Values, IOELV). The IOELVs are implemented by the European Commission as an EU Directive following proposals from the Scientific Committee for Occupational Exposure Limits (SCOEL). Although the DNELs and IOELVs thus belong to different sets of legislation and serve different purposes, the latter may, according to REACH, under certain circumstances be used as worker-DNELs. On the other hand, worker-DNELs will be derived for several thousand substances, far more than the roughly 100 substances for which IOELVs have been established. Thus, the procedure to set health-based OELs may become influential on that of DNELs and vice versa. In this study, we compare the safety margins of 88 SCOEL recommendations with those of the corresponding worker-DNELs. The latter were derived according to the interpretation of the REACH guidance document. Overall, the REACH safety margins were approximately six times higher than those derived from the SCOEL documentation but varied widely with REACH/SCOEL safety margin ratios ranging by two orders of magnitude, from 0.5 to 58 (n=88). The discrepancies may create confusion in terms of legal compliance, risk management and risk communication. We also found that the REACH guidance document, although encompassing detailed advice on many issues, including default assessment factors for species and route extrapolation, gives no quantitative guidance on when and how to depart from defaults.

139 DERIVATION OF SURROGATE ACUTE EMERGENCY GUIDANCE LEVELS (AEGLS) BY STATISTICAL CROSS-EXTRAPOLATION WITHIN AND ACROSS SEVERITY THRESHOLDS.


AEGLS are comprehensively peer-reviewed protective action criteria for assessing the risk of hazardous chemical inhalation exposures in emergency response situations. They are developed for three severity thresholds (AEGL-1: discomfort, AEGL-2: disabling, AEGL-3: lethal) at five exposure durations (1/6, 1/2, 1, 4, and 8 hours). Currently, only 66 compounds have finalised AEGLS, 192 interim, and 12 proposed. And of those, 12% are missing AEGLs for certain exposure durations and threshold levels, with 10% found in the AEGL-1 threshold (n=420). Statistical analysis of AEGL pairs across different thresholds and exposure durations showed high linear correlations between them (0.62 < R² < 1.0, log-scale). Within a threshold, AEGLS were most similar to their adjacent upper exposure duration value (i.e. 8-hr, R > 0.85). The threshold analysis of AEGLs-1 showed highest correlations with the 8-hr AEGLs of the other thresholds (0.91 < R² < 0.98). Interestingly, cross-threshold analysis of AEGLs-2 (and also AEGLs-3) showed that they were most correlated with the 1/6-hr AEGLs of the other thresholds (0.81 < R² < 0.97, respectively). From these analyses two high-correlation and time-specific extrapolation methods for estimating missing AEGLs were deduced. Since most missing AEGL data were from AEGL-1 threshold, the analysis suggested that for each compound, these values can be confidently cross-extrapolated within and across thresholds using scaling coefficients derived by the orthogonal least-squares regression. AEGL data at adjacent upper exposure duration were observed to be lower than LD50, perhaps due to a lesser time-scaling factor applied. In the future, structure-activity relationships, time-scaling relationships, and biological relevance of AEGL compounds will be investigated, which may explain the high correlations across exposure durations and severity threshold levels established in the present study.

140 STATISTICAL ANALYSIS OF THE ATSDR DATABASE OF CHEMICAL HEALTH GUIDANCE VALUES (HGVS).

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Minimal risk levels are developed by ATSDR to be used as HGVs for populations exposed to hazardous chemical substances. However, HGVs are currently available for only a small number of substances. A goal of this work is to develop QSAR (Quantitative Structure Activity Relationship) models using toxicological information that has been rigorously peer-reviewed by responsible agencies, and then apply these models to estimate provisional HGVs (pHGVs) for other toxic chemicals that have not been yet reviewed. The development of a robust database of trustworthy toxicological information is the key component on this path; at present, the ATSDR oral toxicity database contains 678 data records for 548 unique pure chemicals. The data were collected with an emphasis on organic compounds. For each substance, the database record contains the following information: HGV, uncertainty factors for its derivation, LOAEL (lowest-observed-adverse-effect-level), NOAEL (no-observed-adverse-effect-level), POD (point-of-departure), exposure duration, and other experimental details about toxicity studies. Descriptive statistics analyses were performed for each toxicity category. Multivariate relationships were explored using the principle component analysis. Bivariate analysis was applied to relate NOAELs at intermediate to chronic exposure durations, and the NOAELs to LOAELs. The fitted ratios were 2 ± 0.5 (95% confidence level) and 4.5 ± 1.1, for intermediate-to-chronic LOAEL and LOAEL-to-NOAEL extrapolations, respectively. Both were within the 10-fold range, which is commonly used as a default uncertainty factor in HGV derivation. Using the new knowledge extracted from the ATSDR database, more chemicals within the feature domain of the database will be collected to expand the coverage of the chemical space available for a QSAR model. A QSAR model will be used to enhance the database coverage. A robust QSAR pHGV model will benefit public health by improving the assessment of toxicity risks caused by individual chemicals and mixtures.

141 MODELING OF FUROSEMIDE IN DILISYM™ REVEALS TESTABLE HYPOTHESES ABOUT HEPATOTOXICITY MECHANISMS.

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A predictive, quantitative, mathematical model (DILISym™) is under development as a public-private initiative based on the physiological processes involved in drug-induced liver injury. The model includes multiple scales, ranging from molecular/cellular interactions to organ-tissue level considerations. Simulated mice, rats, and humans accurately reproduce the hepatotoxic responses to acetaminophen and methotrexate, providing validation of the DILISym™ model for reactive metabolite mediated toxicity. Furosemide (FS) hepatotoxicity is also thought to be reactive metabolite mediated, but with some mechanistic differences. FS was also recently included in the DILISym™ model, simulating the quantitative aspects of drug metabolism, covalent binding, glutathione, and ATP levels that are described in the literature and from unpublished experiments in mice dosed with 400 mg/kg. ALT levels were accurately predicted based on these inputs, and multiple hypotheses for the ATP reductions were tested with additional modeling and simulations. Dose-dependent necrosis and associated increases in ALT levels do not appear to be de-
dependent upon mitochondrial toxicity. Rather, simulations that included substantial (4x) increases in cellular energy expenditure or decreases (75%) in mitochondrial ATP production due to substrate limitations were more consistent with the experimental hepatotoxic responses. Specific laboratory experiments that will test these hypotheses were identified and are currently underway, including providing glycolytic substrate to sustain ATP production and levels in mice treated with FS. Incorporating FS into the DILIsym™ model provided an increased understanding of the mechanisms associated with FS hepatotoxicity, identified gaps in knowledge, and suggested multiple testable hypotheses to close these gaps.

A chloracne-like response in rhesus monkeys (Macaca mulatta) serves as the critical endpoint for the Aroclor 1254 reference dose (RfD). An interspecies uncertainty factor of 3 was used in the derivation of the Aroclor 1254 RfD, despite the fact that rhesus display an ultra-sensitive response to polychlorinated biphenyls (PCBs), including lethality. Since the establishment of this RfD in the mid-1990s, much has been learned regarding the mechanisms behind “dioxin-like” responses such as lethality. Since the establishment of this RfD in the mid-1990s, much has been learned regarding the mechanisms behind “dioxin-like” responses such as lethality. Furthermore, our laboratory and others have demonstrated that human cells are relatively insensitive to the “dioxin-like” effects of PCBs. Building upon our earlier studies using fresh hepatocytes in vitro, we have examined the potency of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the “dioxin-like” congener PCB 126, and Aroclor 1254 to induce Cytochrome P450 1A1 (CYP1A1) gene expression in keratinocytes derived from human donors and rhesus monkey. Both PCB 126 and Aroclor 1254 were at least 2 orders of magnitude more potent inducers of CYP1A1 in rhesus keratinocytes compared to human keratinocytes, similar to that observed previously for hepatocytes. Interestingly, Aroclor 1254 failed to induce CYP1A1 beyond 1% of the maximal response level achieved by TCDD in human keratinocytes. The current observation of a clear species difference in sensitivity to PCBs between human and laboratory animal cells has been independently replicated in numerous studies and involves species differences in the arylhydrocarbon receptor pathway. From our quantitative data, we have calculated a data-derived extrapolation factor (DDEF) to account for interspecies differences in toxicodynamics and applied this DDEF to estimate a range of possible Aroclor 1254 RfDs. The proposed RfD estimates, utilizing the best available science, may explain why real-world exposure to PCBs have not resulted in “dioxin-like” toxicity in humans.

PS 142 THE AROCLOL 1254 REFERENCE DOSE AS A CASE STUDY FOR THE DEVELOPMENT OF DATA-DERIVED EXTRAPOLATION FACTORS TO REPLACE THE INTERSPECIES UNCERTAINTY FACTOR.
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A chloracne-like response in rhesus monkeys (Macaca mulatta) serves as the critical endpoint for the Aroclor 1254 reference dose (RfD). An interspecies uncertainty factor of 3 was used in the derivation of the Aroclor 1254 RfD, despite the fact that rhesus display an ultra-sensitive response to polychlorinated biphenyls (PCBs), including lethality. Since the establishment of this RfD in the mid-1990s, much has been learned regarding the mechanisms behind “dioxin-like” responses such as lethality. Furthermore, our laboratory and others have demonstrated that human cells are relatively insensitive to the “dioxin-like” effects of PCBs. Building upon our earlier studies using fresh hepatocytes in vitro, we have examined the potency of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the “dioxin-like” congener PCB 126, and Aroclor 1254 to induce Cytochrome P450 1A1 (CYP1A1) gene expression in keratinocytes derived from human donors and rhesus monkey. Both PCB 126 and Aroclor 1254 were at least 2 orders of magnitude more potent inducers of CYP1A1 in rhesus keratinocytes compared to human keratinocytes, similar to that observed previously for hepatocytes. Interestingly, Aroclor 1254 failed to induce CYP1A1 beyond 1% of the maximal response level achieved by TCDD in human keratinocytes. The current observation of a clear species difference in sensitivity to PCBs between human and laboratory animal cells has been independently replicated in numerous studies and involves species differences in the arylhydrocarbon receptor pathway. From our quantitative data, we have calculated a data-derived extrapolation factor (DDEF) to account for interspecies differences in toxicodynamics and applied this DDEF to estimate a range of possible Aroclor 1254 RfDs. The proposed RfD estimates, utilizing the best available science, may explain why real-world exposure to PCBs have not resulted in “dioxin-like” toxicity in humans.

PS 143 DIFFERENTIATING ALCOHOL SOURCES IN A DRAM SHOPPE CASE—A NOVEL MASS-BALANCE MODELING APPROACH.
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Multiple alcohol sources in Dram Shoppe cases are commonplace and it is difficult to scientifically differentiate between law-abiding establishments and other uncontrolled alcohol sources. This can lead to unfortunate liability as usually only a single Blood Alcohol Concentration (BAC) is collected after an accident and many times it is not uncommon for all alcohol sources to be considered contributory. To address just such a problem, a time-based, mass-balance model, based on the standard Widmark Equation combined with Monte Carlo simulation, was developed. The model requires only five input parameters: amount of ethanol consumed per hour at each source, body weight; Widmark “r” factor; Widmark “β” factors and the total time from beginning of the drinking episode to the accident. At least one BAC, taken after the accident, is also required to validate the input variables selected for the model. The mass balance of each alcohol source is calculated at hourly intervals. The alcohol mass-balance calculation is applied to the input parameters “r” and “β”. The full range of Widmark “r” and “β” values from the literature were utilized in the calculations. The mass balance calculations are used to determine the relative contribution of the alcohol remaining in the body for each hour. Remaining alcohol is added to the next hour’s alcohol consumption. The model calculates each source’s contribution to the hourly BAC, over time and defines when a particular source’s contribution to the total BAC is eliminated. While this situation only evaluates two-time consuming cases, the model can easily accommodate three to four alcohol sources if the drinking scenario lasts for at least 12 to 14 hours. The type of alcohol is irrelevant to the model and the drinking episode can be discontinuous as long as a measurable BAC is still present. This model was able to determine when Source A’s alcohol liability ended while confirming that Source B’s alcohol was solely contributory to the accident.

PS 144 ASSESSING THE IMPACT OF CHILD/ADULT DIFFERENCE IN PRESYSTEMIC CLEARANCE ON THE HUMAN KINETIC ADJUSTMENT FACTOR (HKAF) FOR INGESTED TOXICANTS.
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HKAF, as developed by IPCS/WHO, is aimed at replacing the 3.2-fold default value used for human variability in noncancer risk assessment. The value of chemical-specific HKAF has been reported by several authors based on child/adult differences in systemic clearance, without accounting for the additional impact of the presystemic clearance or first-pass effect (FPE) which is critically important for the oral route. The objective of this study was to evaluate the impact of child/adult differences in FPE on the magnitude of HKAF for ingested toxicants. Steady-state equations (SSE) that account for presystemic and systemic clearance were validated by PBPK modeling and were used to compute two dose metrics (arterial blood concentrations (Cass) and rates of metabolism (RAMs)) for oral exposures to 1 μg/kg/d in neonates (0-30 d), infants (1-12 mo), toddlers (1-3 y), and adults. The required physiological parameters were computed on the basis of body weight (BW) and height (H), whereas distributions of BW, H and hepatic enzyme content were derived from the literature. Simulations were compared with Monte Carlo simulations in each subpopulation for hypothetical CYP2E1 and CYP1A2 substrates exhibiting a range of blood:air partition coefficients (Pb: 1–10,000) and hepatic extraction ratios for the average adult (E: 0.01–0.99). Adjustment of the catalytic turnover as a function of the hepatic enzyme ontogeny could, in some cases, reduce significantly the magnitude of the FPE in children. HKAF was computed as the ratio of the 95th percentile value of dose metrics in children over the 50th percentile value in adults. HKAF > 3.2 were observed only based on Cass for various Pb values, in neonates when E ≥ 0.3 for CYP2E1 substrates (max.: 6.3) and in both neonates and infants when E ≥ 0.1 and 0.7 respectively, for CYP1A2 substrates (max.: 28.3).

Overall, this study showed that child/adult differences in presystemic clearance influence the variability of Cass and the resulting HKAF.

PS 145 A SYSTEMS BIOLOGY ANALYSIS TO INFORM HUMAN HEALTH RISK ASSESSMENT OF BENZENE.

Benzene is a ubiquitous environmental hematotoxicant. Benzene causes acute myeloid leukemia (AML) and myelodysplastic syndromes and has been associated with lymphoproliferative disorders including childhood lymphoblastic leukemia. Through its metabolites, benzene induces multiple alterations that likely contribute to the leukemogenic process. Biological plausibility for a causal role of benzene in these diseases comes from benzene’s genotoxic effects and toxicity to hematopoietic stem cells or progenitor cells, from which leukemias arise. This is manifested as lowered blood counts (hematotoxicity), even in individuals occupationally exposed to low levels of benzene. This effort explores a systems biology approach, encompassing endpoints that are relevant to the leukemogenic process and observed at low workplace benzene exposures. The analyses utilized samples from low occupational exposures to examine the dose-response relationship for blood cell counts, biochemical pathways, and expression of genes in the AML pathway. Multiple statistical analyses, including both parametric and non-parametric models that make minimal assumptions about model structure, were explored. The findings revealed dose-dependent responses below 1 ppm for the AML pathway genes, at both the pathway and gene expression level. In addition, the pattern of response appears to change around the 1 ppm region. These responses are considered in view of hypothesized pathways of metabolism that may only be operative below this level. In all, these analyses aid in elucidating: 1) the adverse effects of benzene at low exposures on pathways and mechanisms relevant to hematotoxicity and leukemia; 2) susceptible populations; and 3) quantitative approaches to estimate the low-dose human health risks of benzene exposure. Disclaimer: The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the US EPA.
from a component. Monte Carlo analysis was used to characterize the uncertainty in HI and MHQ that arises from the uncertainties in the components’ human health standards. Probabilistic models of the uncertainty in HI and MHQ values were created for 3,099 mixtures of 81 plant protection products (PPPs), previously analyzed in Price and Han (2011). Different sets of MCR values were obtained based on different levels of conservativeness (50th, 95th, and 97.5th percentiles) in estimating HI and MHQ. These HI and MCR values were compared to values set using actual (deterministic) PPP standards. The models treat the concentration data and Points of Departure in the standards as point estimates while sampling values for the uncertainty factors (e.g., animal-to-human extrapolation) from probability distributions taken from the literature. The deterministic standards on average fell at the 6th percentile of the standards’ uncertainty distributions while the deterministic values of HI and MHQ on average fell between the 95th and 97.5th percentiles of the uncertainty distributions. The averages of MCR values of the mixtures based on the 50th (MLE), 95th and 97.5th percentiles of HI and MHQ were 3.0, 1.5 and 1.4 respectively. The average MCR value based on the deterministic standards was 1.8. These indicated that compared to treating the uncertainty factors deterministically (i.e., point estimation) for setting the PPP standards, using MLE of PPP toxicity can generate larger values of MCR. However, increased conservativeness (95th and 97.5th percentiles of HI and MHQ) results in smaller MCR values and implies less need for CRAs.

There are a vast number of possible combinations of chemicals to which individuals are exposed and risk assessment of these exposures are of resource-intensive nature. A decision tree for the assessment of cumulative chemical risks has been developed by Cefic based on WHO and EU guidance and industry-sponsored research on the Maximum Cumulative Ratio MCR. The MCR is a tool to determine whether risk assessment of a mixture and the MCR is a practical, conservative and robust tool. Its application to the cumulative assessment of the risks from constituents in this formulation demonstrates the potential value of the MCR for characterizing the need for cumulative risk assessments.

There is considerable experimental support for the hypothesis that interactive effects are not likely to occur among components of a mixture when the constituents are present at concentrations below their respective NOAEL values. Nevertheless, greater-than-additive effects can occur when the components of the mixture are present at their NOAELs, especially for compounds with the same mode of action. This study evaluated the potential for interactions to occur between components of a binary mixture (saline extracts of BUNA rubber and nitrile rubber) that are toxicologically similar, but do not necessarily have the same mode or mechanism of action. Interactive effects of the mixture components were evaluated at the respective NOAEL of each component of the binary mixture, and at concentrations just below (1/3 of NOAEL), or well below (1/10 of NOAEL) their NOAEL values. Interactive effects were evaluated using in vitro hemolysis of bovine erythrocytes as the endpoint. Greater-than-additive interactive effects were observed when the mixture components were present at the NOAEL, but not when one of the mixture constituents was present at 1/3 or 1/10 the NOAEL and the other was present at the NOAEL, or when both were below the NOAEL. These results lend support to...
the hypothesis that interactive toxicological effects can occur when the components of a toxicological mixture are present at their respective NOAELs, but are not likely at concentrations below their NOAELs, at least in this test system. From a regulatory perspective, such findings are important for assessing the potential for toxicological interactions to occur in low-dose exposure scenarios.

151 THE IMPACT OF MIXTURE COMPOSITION, MIXING RATIO AND DOSE ON THE INTERACTIONS AMONG THE FOUR TRICHLOROMETHANES (THMs) REGULATED IN DRINKING WATER.

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Oxidizing disinfectants reduce microbial contamination but react with organic and inorganic materials in water forming disinfection byproducts (DBPs). The U.S. EPA regulates 4 THM DBPs (chloroform, CHCl3; bromodichloromethane, BDCM; chlorodibromomethane, CDBM; bromoform, CHBr3) as a group. Our objective was assessment, under an assumption of dose addition, of hepatotoxicity (serum sorbitol dehydrogenase, alanine aminotransferase, aspartate aminotransferase) of the 6 binary combinations of the 4 THMs. Individual THMs and mixtures were tested in female CD-1 mice (14 days of daily gavage) across a dose range that included no/low and overt response (0.1 – 3.0 mmol/kg/day). DBPs were compared at equimolar ratios (EQ) and at ratios representing disinfection by chlorine (Cl). Dose additivity models consistent with Benzbrium’s definition of additivity were developed with individual THM data. Toxicity increased significantly with dose for each individual THM and endpoint. The overall test for departure from dose additivity was significant (p<0.02 – p<0.001) for each mixture containing CHBr3 (the least prevalent THM in chlorinated water); with Hochberg’s correction applied (alpha=0.05), less-than-additive (antagonistic) toxicity was observed at the highest dose level of the EQ (CHCl3:CHBr3, BDCM:CHBr3) or the Cl (CDBM:CHBr3) mixtures. Deviation from additivity was not detected at the lower dose levels of the CHBr3 containing mixtures. Toxicity of the CHCl3:BDCM, CHCl3:CDBM and BDCM:DBCM mixtures was generally consistent with dose additivity. In summary, these data emphasize the importance of careful consideration of mixture composition and mixing ratio as well as the need for dose-response assessment that includes both the low dose and the overt response region when evaluating the health risks from exposure to mixtures present in the environment at low levels. (This abstract may not reflect EPA policy.)

152 IN VIVO TOXICOKINETIC INTERACTIONS BETWEEN THREE MEDICINAL DRUGS AND TRICHLOROETHYLENE IN RAT.

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We recently demonstrated that some pharmaceuticals can modulate the in vivo metabolism of trichloroethylene (TCE) in rats and humans. The objective of this study was to assess the in vivo significance of observed interactions between TCE and three selected drugs, i.e., valproic acid (VA), salicylic acid (SA) and naproxen (NAP). All groups of rats were exposed by inhalation to TCE (50 ppm; 6 h) in inhalation chambers. Groups co-treated with one of the drug were also given an oral dose (10 times the recommended daily dose, mg/kg) just an hour before of TCE exposure. Blood, urine and various tissue samples were collected at different time points and analyzed by headspace-GC coupled to an electron capture detector to determine the levels of TCE and metabolites (i.e., trichloroethanol [TCOH] and trichloroacetate [TCA]). Results show that NAP significantly increases free and total TCOH levels in the blood (i.e., up to 50%). This modulation was found to be significant up to 90 minutes after the end of exposure (p<0.05 for free TCOH, p<0.001 for total TCOH). This increase in blood TCOH can be explained by an inhibition of glucuronidation. VA and SA coexposures, revealed a significant increase (i.e., up to 50%) in blood levels of TCE (p<0.05). For VA, the peak interaction occurs at 30 and 60 minutes, while for SA this occurs at 120 minutes after the end of exposure. It can also be observed that these two drugs behave differently on TCOH levels. VA has no significant effect on blood TCOH levels, but is responsible of a significant increase in urine TCOH (p<0.05). SA, on the other hand, induced an increase of total TCOH levels in blood at 30 and 60 minutes after the end of exposure (p<0.05). Only NAP had a significant (p<0.05) impact on TCA levels in the liver (increase). The results of this study confirm that these drugs may interfere with TCE kinetics in vivo. Future efforts should be directed at evaluating how chronically medicated populations have greater health risks related to TCE exposure.

153 THE OXIDATIVE METABOLITES OF MEHP INHIBIT TESTOSTERONE PRODUCTION IN A RAT TUMOR CELL LINE ASSAY.

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Phthalate esters have been shown to disrupt sexual development in the male rat after gestational exposure, which is at least partially due to reduced testosterone synthesis in the fetal Leydig cell. While human exposure is primarily to the diester, rapid hydrolysis to the monoester occurs in the gut, blood and liver via side chain cleavage from the phthalic acid backbone. We previously developed a rat Leydig cell (R2C) assay to test antiantiandrogenic activity of the PE's in vitro. In agreement with in vivo studies, T synthesis in R2C cells was inhibited by monoethoxyl (MEHP) and monobutyl (MBP), but not monochlor phthalate. In vivo, MBP is primarily cleared by glutathione conjugation and excretion in urine and feces. However, MEHP undergoes extensive oxidative metabolism resulting in hydroxy, oxo and carboxyl metabolites. The oxidative metabolism occurs primarily on carbons 2 and 5 of the side chain. In this study, we have exposed R2C cells in monolayer culture to four metabolites of MEHP (5-hydroxy, 5-oxo, 5-carboxy and 2-carboxy MEHP) to ascertain whether or not these metabolites may contribute to the overall in vivo potency of MEHP. Compared to MEHP, the 5-oxo metabolite was approximately 5 times more potent at reducing testosterone. The remaining metabolites exhibited potencies below that of MEHP; however, all were able to reduce testosterone production in R2C cells. The order of potency was 5-hydroxy>MEHP>5-oxo>5-carboxy-2-carboxy. The toxic equivalents for the four metabolites compared to MEHP were approximately 5, 0.8, 0.6 and 0.35 for 5-hydroxy, 5-oxo, 5-carboxy and 2-carboxy, respectively. Thus, these oxidative products should be considered when determining the contribution of DEHP to cumulative risk from phthalate exposures.

154 IMPLEMENTATION OF THE HAZARD INDEX APPROACH IN FIELD ASSESSMENT OF CHEMICAL MIXTURES.

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The issue “Do chemical mixtures pose added risk?” has been a long time concern of health assessors. Three basic data driven approaches with some variations are part of various federal agency guidance. Of these, when exposure data are available, the hazard index (HI) approach is used most often. A recent review of 80 record of decision documents (ROD) revealed the following breakdown for the HI values: 0 to <1 (19%); 1 to <10 (36%); 10 to <100 (30%), and100 to 2,523 (15%). In the RODs, HI values can be found for various age, gender and life stages of a human population. A HI > 135 for neurological effects and HI > 52 for hepatic effects was calculated in an ATSDR health assessment of the Conrail site, where high level exposures occurred to trichloroethylene and carbon tetrachloride. Thus, when data are available effect specific HIs are calculated that can help in decision making. In this case, portable water was provided to the population as an alternative source. Because HIs are based on the principle of dose or response addition chemical interactions such as synergism and antagonism should also be considered when using HI values. Palmerton, a zinc smelter site, had high soil levels of zinc, lead, and substantial levels of other metals. These pollutants can potentially cause neurological, hematological, hepatic and testicular toxicities. The issue at this site was can the soil cleanup-goals for lead be relaxed because of zinc inhibition of other toxicities. The recommendation for this site was that additional joint toxicity data for testicular toxicity were needed and until then the clean-up levels could not be lowered. It is important to understand the advantages and limitations of a method before its implementation. Even though this is the most often used approach, HI values have several uncertainties associated with them. They can be fine-tuned for specific use and can provide insight to the risk characterization and risk communication.

155 MIXTURES OF ENDOCRINE DISRUPTORS: HOW SIMILAR MUST MECHANISMS BE FOR CONCENTRATION ADDITION TO APPLY?

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Rationale: Must molecular mechanisms be the same for concentration addition or is it sufficient to affect similar pathways and common outcomes? Does concentration addition provide a closer approximation to observations than alternative models such as effect summation? Suppose effects are mediated by hormone A through several uncertainties associated with them. They can be fine-tuned for specific use and can provide insight to the risk characterization and risk communication.
Results: Under concentration addition, the isolates must be negatively sloped straight lines. We show that linearity of the isolobes depends crucially on $g(B)$. The mixture is concentration additive if $g'(B)$, the second derivative of $g(B)$ with respect to $B$, is zero. It is supra-additive if $g'(B)$ is positive and sub-additive if $g'(B)$ is negative. We describe functions $g(B)$ that lead to all three cases as well as one that is supra-additive in some regions and sub-additive in others.

Discussion: At least in this simple model, concentration addition cannot be assumed based on action on a common hormone: mixtures of competitive antagonists and compounds that alter hormone synthesis can lead to results that are concentration additive, supra-additive or sub-addative. Nevertheless, concentration addition provides a closer approximation to the mechanistic model than effect summation. Care needs to be taken in extrapolating from the model used here to other situations, but analysis of simple mechanistic models appears to be a useful strategy.

156 DISCRIMINATORY POWER OF STANDARD TOXICITY ASSAYS USED TO EVALUATE CIGARETTES.

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Toxicological acceptability of materials or products are typically based upon results from a battery of in vitro and in vivo assays. In most cases decisions are based on absence or presence of biological activity. In some cases, however, the tested product is biologically active in toxicity assays (e.g., cigaretes). In these cases it is difficult to a-priori determine the assays statistical discriminatory power. A large experimental dataset from a multi-year program of cigarette ingredient testing performed at two separate laboratories using similar methods was used to determine discriminatory power for chemical-analytical testing. Salmonella mutagenicity, cytotoxicity and 90-day rodent inhalation assays. Discriminatory power was measured using the minimum detectable difference (MDD) statistical method, which is the smallest difference that could reliably be detected assuming a defined sample size, significance level (p<0.05), power (80%) and variability. The MDD of 37 different cigarette mainstream smoke constituents ranged from 6-29% of the average yield. MDDs for the in vitro Salmonella mutagenicity and cytotoxicity tests ranged from 20-81% and 18-49% of the average activity, respectively. In the 90-day nose-only inhalation studies MDDs were: 30-40% for body weight; 6-43% for organ weights; 20-81% and 18-49% of the average activity, respectively. In the 90-day nose-only inhalation studies MDDs were: 30-40% for body weight; 6-43% for organ weights; 20-81% and 18-49% of the average activity, respectively. In the 90-day nose-only inhalation studies MDDs were: 30-40% for body weight; 6-43% for organ weights; 20-81% and 18-49% of the average activity, respectively.

157 THE PHOSPHORYLATION PATTERN OF HSP27 REVEALS THAT THE TOXICITY PATHWAY OF OKADIC ACID PREDOMINATES OVER THAT OF PALTOTOXIN IN HUMAN CELLS EXPOSED TO THE TOXIN MIXTURE.

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Toxicity pathways (TP) and their interactions in biological systems have been studied by analysis of proteomes of cultured human cells exposed to toxins and toxin mixtures. When MCF-7 cells were exposed to either okadac acid (OA) or paltotoxin (PTX), two algal toxins possessing different molecular mechanisms of action, concomitant modifications of stress response proteins marked their cytotoxic effect (Sala et al., Chem. Res. Toxicol; 22, 2009, 1077-1085 and 2009-2016). The phosphorylation of the Hsp27 protein, in particular, was affected, and isoforms phosphorylated in Ser82 were found in cells exposed to either OA or PTX. Based on these observations, we have analyzed the effects mixtures of OA and PTX on the cellular protein phosphorylation patterns in MCF-7 cells, to probe possible crosstalks between the TP's of these toxins. Immunoblotting analyses of cell extracts using antibodies recognizing the total Hsp27 protein pool and several residue-specific phosphorylations confirmed our previous data on Ser82, and showed that a prominent increase in phosphorylation at Ser15 of Hsp27 in response to OA but via different specific mechanisms. Are combinations of B and C concentration additive? Methods: We used simple mechanistic, pharmacodynamic models, deriving mathematical models using equilibrium binding and mass balance. Assume A binds the receptor at one site R with effects proportion of AR. Let C act as a competitive antagonist via the Gaddum equation. Let B affect synthesis of A via a function describing the joint response surface of B and C, and a function describing its isolobes.

Discussion: At least in this simple model, concentration addition cannot be assumed based on action on a common hormone: mixtures of competitive antagonists and compounds that alter hormone synthesis can lead to results that are concentration additive, supra-additive or sub-addative. Nevertheless, concentration addition provides a closer approximation to the mechanistic model than effect summation. Care needs to be taken in extrapolating from the model used here to other situations, but analysis of simple mechanistic models appears to be a useful strategy.

158 EVALUATION OF MIXTURE EFFECTS AT DOES RELEVANT FOR HUMAN SAFETY.

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Mixture toxicity has been a topic of interest for many years, with thousands of studies being conducted. Currently, the European Commission is looking at ways to include mixture toxicity in existing legislation. The focus should however remain on effects of mixtures which reflect real-life exposures, where each chemical is likely to be present below its threshold of toxicity. An ECETOC Taskforce has reviewed the evidence for unexpected effects, when each component in a mixture is at a dose below its threshold of toxicity. A survey of literature revealed that in over 500 papers was conducted with strict criteria for papers to be accepted as relevant for the review. Considered relevant were studies where the combinations of chemicals were the) tested at doses close to (but below) the NOAEL of each component, b) tested at doses well below the NOAEL of each component, or c) real or simulated environmental mixtures. The task force aimed to evaluate a) whether the evidence on interactions at environmentally or human-relevant doses demonstrates toxicologically relevant effects and determine whether there are any associations with specific modes of action, e.g. endocrine disruption, b) whether it is appropriate to assume dose additivity for substances with common modes of action and c) the adequacy of current human safety risk assessment practice in light of the conclusions of the above. From the high number of included studies, a relatively low number discussed effects at or well below NOAEL. The majority of these described additivity while only a limited number of studies reported deviation from additivity (both antagonism and synergy); the majority of which was observed in in vitro studies. Occasionally, the nature of the interaction could not be determined, whilst a small number of studies showed no effects at all. These findings indicate that current legislation (based on single chemicals) with a modification to accommodate for additivity when appropriate, is sufficiently protective against mixture effects on human health.

159 MOTORCYCLE EXHAUST INDUCES BEHAVIORAL TOXICITY IN FEMALE RATS AND OFFSPRING.

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Emissions from two-stroke motorcycles create a major environmental burden in urban areas where motorcycles are a popular means of transportation. Motorcycle exhaust (ME) has higher levels of benzene and butadiene than exhausts from diesel and gasoline passenger cars. Particulate matters of ME contain carcinogenic aromatic hydrocarbons including benz(a)pyrene and benz(a)anthracene. The major purpose of the present study was to determine the behavior effects of ME exposure on female rats and maternal ME exposure on rat offspring. Female rats were exposed to 1:10 diluted ME by inhalation one hour each in the morning and afternoon daily and Monday through Friday for eight weeks. Control rats were exposed to clean air. The results of forced swim, light-dark transition, and tail-flick tests showed that ME produced depression-, anxiety-, and antinociception-like behavioral effects in female rats, respectively. At twelve weeks after cessation of ME exposure, depression-like behavioral effect persisted in female rats exposed to ME; in contrast, anxiety- and antinociception-like effects were not detected. At the end of ME exposure, control and ME-exposed female rats were mated with untreated male rats for two weeks. Behavior tests were done using offspring rats on postnatal day 56. Maternal ME exposure produced depression-like behavior, but not anxiety-like behavior, in male and female offspring. The maternal exposure produced antinociception-like effect in male offspring, but not in females. These results show that ME inhalation exposure induces depression-, anxiety-, and antinociception-like behavior in female rats and maternal ME exposure produces a depression-like effect in offspring. The underlying basis for the variable reversibility and developmental effect in ME behavior toxicity remains to be investigated.
Motor gasoline is manufactured by blending naphtha, i.e., refinery feedstocks composed of hydrocarbon constituents suitable for use in gasoline. The types of hydrocarbons found in naphtha are normal-, iso- and cyclo-paraffins as well as aromatics with carbon numbers in the range of approximately C4-C10. Heavy straight run naphtha (HSRN, CAS number 64741-41-9) has higher levels of cyclic-paraffins than other naphthas, and was tested as a “worst case” example. HSRN was tested in a combined repeated dose/reproductive toxicity test (OECD 422) to assess the potential for systemic toxicity, neurotoxicity, and for reproductive and developmental effects. Rats were exposed by inhalation at levels of 100, 500 or 3000 ppm, 6 h/day. Exposures started 2 weeks prior to mating and then through a 2 week habitation period. Rats taken for assessment of subchronic toxicity were sacrificed after approximately 30 days of exposure. In the reproductive toxicity study, exposures continued until gestational day 19; the rats were then held without exposure to final sacrifice on post-natal day 4. All animals survived the treatment period.

Principal systemic effects were increased liver weights in males and females, attributed to increased metabolic demands; increased kidney weights in males, attributed to an α2u-globulin-mediated process; and histological changes in the thyroid, judged by the pathologist to be secondary to liver enzyme induction. None of these were considered to be toxicologically important. There were no treatment-related effects in functional observation tests or in the assessment of motor activity. There were no significant reductions in fertility, no significant changes in other reproductive parameters, and no evidence of developmental toxicity. The overall no observed adverse effect concentration was 3000 ppm.

### PS 160 TOXICOLOGICAL ASSESSMENT OF HEAVY STRAIGHT RUN NAPHTHA

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We have previously demonstrated using A549 cells that exposures to ozone in a photochemically aged air mixture induces greater toxicity than exposure to ozone alone. This study is an extension of these studies, aimed at comparing adverse effects in different human-derived cell cultures to understand potential differences. Commerically available EpAirway 3-D human-derived tracheal/bronchial epithelial cells from MatTek Corporation and A549 human alveolar epithelial cells were used. In vitro cultures were exposed to clean chamber air (sham), 400ppb of ozone, 100ppb of NOx, 400ppb of NOx and 100ppb of ozone, 400ppb of NOx and 500ppb of ozone, 100ppb of NOx and 900ppb of ozone, or 400ppb of ozone and 900ppb of ozone. After 24 hours of exposure, the cell cultures were fixed, stained, and observed using an inverted fluorescence microscope. Cell viability was determined using the MTT assay. Cell morphology was assessed using phase-contrast microscopy. The results of this study suggest that ozone and NOx have a synergistic effect on cell viability, and that the combination of ozone and NOx is more toxic than either gas alone.
Basolateral supernatants, apical washes, and RNA were collected at 0, 9, and 24 hours post-exposure. Cytotoxicity was assessed via lactate dehydrogenase (LDH), IL-6 and IL-8 were measured to determine the inflammatory response, and heme oxygenase-1 (HO-1) levels were used to determine oxidative stress. Analysis of A549 LDH levels demonstrates that a photochemically aged urban mixture does induce greater toxicity than exposure to ozone alone. However, this effect is not observed in the EpiAirway LDH levels detected. Similarly, IL-6 trends were observed in the A549 cells, but not the EpiAirway cells. Although EpiAirway cells produced much higher levels of IL-6 compared to A549, no exposure trends can be observed due to the high variability in expression. These findings indicate that type and magnitude of responses induced by air pollution mixtures may be cell culture model dependent. Therefore, it is important to understand the limitations of each in vitro model and critical to identify appropriate endpoints for each model.

Petroleum coke (CAS number 67471-79-3), the material remaining after thermal decomposition of petroleum streams, is primarily inorganic carbon but also contains some entrained volatile hydrocarbon material, sulfur, and trace metals. Previous studies have shown that in rats, repeated exposure to petroleum coke dust by inhalation results in dust accumulation in the lungs and inflammatory changes but does not cause tumors or produce other systemic effects. As part of the petroleum industry response to the USEPA High Production Volume (HPV) challenge program, the potential reproductive effects of respirable coke dust (3.3 micron MMAD) were assessed in a subchronic toxicity/reproductive toxicity screening test in rats (OECD 421). The repeated-dose portion of the study in which male rats were exposed 6/day, 7 days/week for at least 4 weeks, provided further evidence for petroleum coke dust accumulation and inflammatory responses in rats exposed to 100 mg/m3 and 300 mg/m3. But there was no evidence of systemic toxicity. The low exposure level (30 mg/m3) was a no observed effect concentration for all endpoints. Therefore, it is important to understand the limitations of each in vitro model and critical to identify appropriate endpoints for each model.

The study aims at the development of a computational framework for assessing mechanistically the risks imposed by major components of ETS, by assimilating biomonitoring data, internal dose modeling and Biology Based Dose Response (BBDR) modeling for three organic contaminants, namely 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone, benzene and formaldehyde, related to lung cancer, leukemia and nasopharyngeal cancer respectively. A nicotine-cotinine PBPK model was developed, allowing the use of biomarkers such as urinary cotinine and nail nicotine for short and long term exposure assessment through reverse modeling. Reconstructed exposure scenarios are fed forward to BBDR models coupling PBPK/D with mechanistic pathology models for each carcinogenic contaminant. Genetic polymorphisms linking variation of human susceptibility to xenobiotics were explicitly accounted for. Extrapolation to the wider population through hierarchical population modeling incorporated all variabilities and uncertainties governing exposure and response to xenobiotics from ETS. Individual risks estimated for lung cancer, leukemia and nasopharyngeal cancer ranged within values of order 10-5, 10-7 and 10-9 respectively under the typical urinary cotinine-driven exposure scenarios. Exposure duration had a non-linear effect on lung cancer, while the limited variability of formaldehyde exposure was compensated by the strongly non-linear mechanism governing formaldehyde-DNA adduct formation. Considering that benzene toxicity relies upon the presence of its toxic metabolites, polymorphism variability among gene variants of CYP2E1 and NQO1 was found to be determinant for overall risk assessment. A multi-tiered approach is proposed here as a valid alternative to classic epidemiological approaches, since cancer risk is estimated using models with strong biological underpinning.
CRUDE OIL FROM VARIOUS SOURCES YIELDS DIFFERENT ACUTE TOXICITY OUTCOMES IN RATS: CORRELATION WITH ‘H-NMR AND EPR SPECTRA.

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Crude oil is a mixture of alkanes, simple and polyyclic aromatics, nitrogen, sulfur and condensed PAH asphaltene with traces of porphyrin nickel and vanadium that varies with source. We previously identified several adverse effects with acute, oral exposure of rats to Louisiana sweet crude oil and here extend those observations to Nigerian (Qua Iboe) sweet and Iraq high sulfur oils (ONTA, Inc., Toronto, Canada). Chemical fingerprinting of the oils by 'H-NMR and EPR has been used to correlate constituent contents with toxicological effects. Female Sprague-Dawley rats were orally gavaged with 2 daily doses of 2.5 and 5 mg/kg of oil, 0.5 ml/kg benzene or vehicle (0.5% DMSO in corn oil). After 48 h, rats were euthanized, blood was taken for hematology and clinical chemistry and femur bone marrow cells assayed for myeloid progenitor cells (CFU-GMs). All oils elevated serum alkaline phosphatase (ALP) with LA oil being the least potent and effective. BUN was increased 40% by high dose of Iraqi oil. Granulocytes were increased 1.5-2 fold by high dose of LA and Iraqi oil. Liver weights increased 25-75% with both doses of all oils with LA oil being least effective. Spleen weights decreased 30% with high doses of Iraqi and Nigerian oil. CFU-GMs were decreased only with high dose of all oils with LA oil being least effective. Spleen weights decreased 30% with high doses of Iraqi and Nigerian oil. CFU-GMs were decreased only with high dose of LA oil (40%). Benzene decreased spleen weight by 20%, but was without effect on other endpoints. 'H-NMR spectra exhibited qualitatively similar alkane peaks for all oils, but some differing aromatic peaks. Benzene peak (7.38 ppm) of Iraqi oil was less intense than that of the other oils. EPR spectra of all oils exhibited the asphaltene free radical peak with intensity greatest for Iraqi oil. Vanadium porphyrin was detected in Iraqi oil, but not the others. Summarizing, LA sweet crude oil was uniquely myelosuppressive and with no or lesser effect on spleen and liver weights and BUN. Elevated BUN was uniquely associated with Iraqi oil, the source richest in asphaltene free radical and vanadium porphyrin and lowest in benzene. (LA Board of Regents)

CYTOTOXICITY PROFILING: A NOVEL METHOD FOR ENVIRONMENTAL CONTAMINANT ASSESSMENT.

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The traditional method of assessing the toxicity of environmental contaminants is to use animal studies to determine the toxicity, but concerns over the use of animals has driven the search for alternative in vitro methods. We have developed an alternative system for measuring in vitro toxicity utilizing a real time impedance based cytotoxicity system. Using an array of cell lines derived from major organs, we are able to produce a cytotoxicity profile, or toxicological fingerprint, for a number of environmental samples. Unique profiles were derived for the chemicals that potentially can be used to identify the mechanism of action for the chemical in question and estimate the toxicity. In order to apply this technique to a “real world” samples we conducted experiments to determine any matrix effect of natural water. Using cell lines from different organ origins (CNS, liver, lung, kidney), we tested water samples from ground water (well), and surface water (lake) and compared these results to the chemical testing. The current work demonstrates 1) the feasibility of using the impedance based cytotoxicity assay to test environmental water samples without sample cleanup and concentration, 2) the agreement between the impedance based cytotoxicity testing and chemical results, and 3) the proof of concept for cytotoxicity profiling using impedance based cytotoxicity testing.

ARE IN VITRO EFFECTS OF METAL MIXTURES PREDICTABLE FROM THE RESPONSES TO THE SINGLE COMPONENTS?

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At occupational settings people are often exposed to mixtures of metals, which increases complexity of toxicological risk assessment. For example in battery manufacturing or recycling of electronic devices workers can simultaneously be exposed to Cd, Co and Pb. To gain more insight into the effects of Cd(II), Co(II) and Pb(II) in single or combined application we conducted experiments with the human lung tumor cell line H322 and primary cultures of normal human bronchial epithelial cells (NHBEBC). Incubation of H322 with Cd (2.5 μM) for 24h reduced viability to 87%, as measured by MTT-assay. Whereas treatment of H322 with Co (≥ 20 μM) or Pb (≥ 30 μM) was not cytotoxic, simultaneous treatment with Cd, Co and Pb was not more toxic than Cd alone. When induction of ROS was analysed by DCF-assay, significant inductions where detected at following concentrations: Cd (0.13 μM; 134%), Co (0.5 μM; 129%), Pb (2.65 μM; 159%). If metals where applied in these concentrations simultaneously, ROS-induction was 150%. To understand cellular responses to low doses of Cd (0.13 μM), Co (0.42 μM) and Pb (2.65 μM), a gene array study was conducted with NHBEBC. Different algorithms for the analysis of the raw data were compared in order to find the procedure most suitable to detect differentially expressed genes. Moreover applying a newly developed processing setup with pre-processing by GCRMA and data analysis by shrinkage generalized interaction t-test, it was possible to detect genes reacting in a cooperative manner to combined metal exposure. The top five of these genes are: GOS1, TOR1AIP1, ARL1, SDF2L1 and DHCR7. These experiments demonstrate that a thorough analysis of the mechanism of action - beyond tests on cytotoxicity or induction of ROS - could be useful for predicting the effects of metals on human lung cells.

HIGHLY SENSITIVE AND RESPONSIVE SPECIES-SPECIFIC AH RECEPTOR-BASED CALUX CELL LINES FOR HIGH-THROUGHPUT CHEMICAL SCREENING AND SPECIES COMPARISONS.

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The CALUX (chemically-activated luciferase expression) cell bioassay is the most widely used Ah receptor (AhR)-based technique for screening of environmental, biological and food samples for the presence of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related chemicals. Recently, the AhR-CALUX bioassay has been used for high-throughput screening of chemical libraries in order to identify AhR agonists/antagonists for use in therapeutic applications. We previously developed several CALUX cell lines for large scale high-throughput screening applications, but these cell lines are not sensitive or responsive enough for such purposes. Accordingly, stable transfection of mouse, rat, human and guinea pig cell lines with a luciferase reporter plasmid in which the number of AhR-responsive DNA binding sites has been increased to 20, produced a series of species-specific recombinant cell lines with significantly improved sensitivity (minimum detection limits for TCDD in these cell lines range from 10 fM to 30 pM TCDD) and dramatically increased induction of luciferase activity. The significantly enhanced induction response in the cells has allowed the bioassay to be utilized in a 1500-well microplate format for high-throughput screening of chemical libraries for AhR agonists. Comparison of a series of natural products and extracts in these cell lines has revealed dramatic species specific differences in AhR response, with indirubin being selectively more potent in human cells than that of other species and extracts of Polygonum seeds activating the AhR in rat, guinea pig and human cells, but antagonizing that in mouse cells. These more sensitive and responsive cell lines not only improve use of the CALUX bioassay for large scale screening purposes, but provides novel systems to study species differences in AhR response. (ES00469, ES007685,ES012498)

STABLE REPORTER CELL LINES FOR THE IDENTIFICATION AND CHARACTERIZATION OF SUBTYPE-SELECTIVE ESTROGENIC LIGAND.

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Estrogen signaling is mediated by two estrogen receptors (ERs), ERα and ERβ, which have unique roles in the regulation of breast cancer cell proliferation. ERα mediates proliferation in response to estrogen and ERβ inhibits proliferation in breast cancer cells, suggesting ERβ selective ligands may be beneficial for promoting the anti-proliferative action of ERα. Identification and characterization of subtype selective ligands can be achieved using transcriptional assays, but cell lines in which ERα or ERβ are independently expressed are required. Of the available reporter cell lines, none have been generated in breast cancer cells. Here we describe the generation of two isogenic breast cancer cell lines, Hs578T/ERELuc and...
H578T-ERβLuc, with stable integration of an estrogen responsive luciferase reporter gene and characterization the subtype selectivity of two natural phytoestrogens, cosminin and liriquitin. H578T-ERαLuc and H578T-ERβLuc cell lines are highly sensitive to estrogenic chemicals and ER subtype selective ligands, providing a tool to characterize the transcriptional potencies and subtype selectivity of estrogenic ligands in the context of breast cancer cells. In addition to measuring reporter activity, ERβ target gene expression and growth inhibitory effects of ERβ selective ligands were determined as biological endpoints. These cell lines are valuable tools for characterizing estrogenic ligands, and the data support the anti-proliferative role for ERβ in breast cancer.

RECEPTOR AND LIGAND-DEPENDENT FUNCTIONS OF PPARα/β and PPARγ ON CELL PROLIFERATION IN THE A431 HUMAN CARCINOMA CELL LINE.

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The peroxisome proliferator-activated receptor (PPAR) isoforms (PPARα, PPARβ/δ, and PPARγ) are known to modulate proliferation in skin tumorigenesis models. To examine the functional role of PPARα/β and PPARγ in skin cells with greater detail, the Migr1 retinoviral system was used to generate stable expression of each receptor in the A431 human carcinoma cell line. As compared to controls, the expression of a known PPAR target gene was greatly enhanced in response to PPARα/β or PPARγ ligands in A431 cells over-expressing these receptors. Over-expression of either PPARα/β or PPARγ did not alter A431 cell growth under normal cell culture conditions. Administration of PPARγ ligands in A431 cells over-expressing PPARγ reduced cell growth; however, similar alterations in cell growth were not observed with ligand activation of PPARα/β. In contrast, anchorage-independent cell growth was not influenced by either over-expression or ligand activation of PPARα/β or PPARγ. Over-expression of PPARα/β modestly inhibited clonogenicity, but ligand activation of PPARα/β markedly inhibited clonogenicity as compared to controls. Over-expression of PPARα/β did not influence clonogenicity, but ligand activation of PPARα/β in A431 cells over-expressing PPARα/β resulted in reduced clonogenicity. Over-expression of PPARα/β or PPARγ markedly reduced tumor volume in ectopic xenografts, but PPAR ligands had no further influence on tumor volume. Collectively, these studies demonstrate that stable over-expression of PPARα/β or PPARγ in A431 cells increased the efficacy of ligand activation and reduced A431 cell growth. Importantly, over-expression of receptor did not modulate normal cell growth, but increased PPAR expression or ligand activation had major impacts on clonogenicity and/or tumor volume. This model will be a valuable tool to dissect the functions of PPARα/β and PPARγ in human skin tumorigenesis.

FUNCTION AND EXPRESSION OF TRPA1 IN RESPONSE TO ENVIRONMENTAL IRITANT EXPOSURE IN VITRO.

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Headaches are the most common symptom reported after exposure to both indoor and outdoor air pollution. Many of these headaches are attributed to Multiple Chemical Sensitivity (MCS), an acquired chronic disorder estimated to affect 15% of the US population. The induction of MCS is often due to low level exposure to air pollution and industrial chemicals such as acrolein. Acrolein and other environmental irritants have been shown to activate transient receptor potential A1 (TRPA1) channels in trigeminal neurons. Trigeminal neurons are the sensory neurons of the head and are activated in migraine headaches. TRPA1 channels are excitatory calcium generating ion channels expressed in a subset of sensory neurons. Activated TRPA1 channels have been shown to cause release of neuropeptides such as calcitonin gene-related peptide (CGRP). We have shown that 30μM acrolein application results in a 6-fold increase over baseline of CGRP release in trigeminal neurons in vitro. CGRP release is associated with pain, inflammation, and headache. Our work uses primary cultures of trigeminal neurons as well as transfected cell lines incubated with low levels (1-10μM) of environmental irritants for multiple days in vitro to simulate chronic exposure. We examine changes in expression using quantitative PCR. Function of TRPA1 channels is examined using Fluoro-4 AM calcium imaging and radioimmunoassays to measure CGRP release from neurons. As MCS has emerged as a global public health concern, understanding the mechanism of MCS is a very important and relevant question with regard to public health and workplace safety. The objective of these studies is to describe the impact of low levels of environmental irritants on TRPA1 expression and function.

ACTIVATION OF THE AH RECEPTOR SIGNALING PATHWAY BY SHEAR STRESS: GENERATION OF ENDGENOUS LIGANDS AND/OR EXPERIMENTAL ARTIFACT?

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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). AhR regulates the expression of hundreds of genes including TCDD-inducible poly(ADP-ribose) polymerase (TiPARP, PARP7). TiPARP is a member of the PARP superfamily, which is an enzyme family that mediates poly(ADP-ribose)ylation of protein targets. Poly(ADP-ribose)lation is post-translational modification associated with a number of biological functions including DNA repair, transcription, and apoptosis. TiPARP contains a C-terminal PARP catalytic domain, a conserved WWE (tryptophan-tryptophan-glutamate) domain and a zinc-finger domain. However, the biological role of TiPARP and whether TiPARP modulates AhR action is unknown. The aim of the present study was to investigate modulation of TCDD-induced AhR activity by TiPARP. We investigated the effects of loss and overexpression of TiPARP on TCDD-induced cytochrome P450 1A1 (CYP1A1) and CYP1B1 gene expression in T47D human breast carcinoma and HuH7 hepatoma cell lines. RNA-mediated knockdown of TiPARP significantly increased TCDD-induced CYP1A1 and CYP1B1 expression. TiPARP knockdown also reduced TCDD-induced AhR protein degradation following 24 h treatment. TiPARP overexpression decreased TCDD-induced CYP1A1- and CYP1B1-regulated reporter activity in a dose-dependent manner. Interaction studies demonstrated that overexpressed GFP-tagged TiPARP co-localized and co-immunoprecipitated with AhR. TiPARP truncations and point mutants revealed TCDD-induced inhibition required the zinc-finger and catalytic function. Catalytic assays demonstrated TiPARP possesses mono-ADP-ribosyltransferase (mART) activity rather than poly(ADP-ribose)lation activity. Auto-mART activity required both the catalytic domain and central portion (residues 275-328). Collectively, these results demonstrate TiPARP as a mART and a negative regulator of AhR signalling.

ACTIVATION OF THE Ah RECEPTOR SIGNALING PATHWAY BY SHEAR STRESS: GENERATION OF ENDGENOUS LIGANDS AND/OR EXPERIMENTAL ARTIFACT?

M. S. Deisenroth1, A. Gojova1, J. E. Bohonowycz1, J. Morgan1, G. He1, A. Baraket2 and B. Zhao3. 1Department of Environmental Toxicology, University of California Davis, Davis, CA, 2Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China and 3Department of Mechanical and Aerospace Engineering, University of California Davis, Davis, CA.

The Ah receptor (AhR) is a ligand-dependent transcription factor that can be activated by structurally diverse synthetic and natural chemicals. Endogenous activators of the AhR have been proposed, but few have been identified that are active at physiological concentrations. The role of AhR in cardiovascular and vascular development and disease and AhR activation in endothelial and hematopoietic cells suggests the existence of endogenous AhR activators. Incubation of endothelial cells or cell lines in fluid-flow, arterial shear-stress conditions activates the AhR-dependent gene expression, suggesting that vascular shear-stress produces an AhR agonist(s). While production of this AhR activator was serum-dependent and suggested to be a shear-stress modified low density lipoprotein, the actual source of the shear-stress dependent AhR ligand is unclear. Using a closed-loop laminar flow shear-stress system we demonstrated a rapid and flow-dependent transient activation of AhR-dependent gene expression in stably transfected mouse hepatoma cells; there was no induction in the absence of serum. This suggests that shear stress generates AhR agonist from serum, but subjecting serum to comparable shear forces by other methods did not generate AhR agonists. Since AhR agonists are present in rubber and plastic products (components of the flow chambers and tubing), we examined whether these might be a source of AhR agonists. Serum, but not media, could...
readily extract AhR agonist activity from rubber tubing and gaskets used in the flow cell system. Our studies demonstrate that the binding of the AhR in flow cell systems is predominantly an artifact derived from the experimental system rather than shear stress generation of endogenous AhR agonists from serum. (ES004699;ES007685)

PS 178 CAN THE BINDING BY STRUCTURALLY DIVERSE AGONISTS ALTER THE NUCLEOTIDE SPECIFICITY OF AH RECEPTOR DNA BINDING?
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The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor belonging to the basic-helix-loop-helix-PAS family of regulatory proteins. It is best characterized for its role in mediating the toxic and biological responses resulting from exposures to halogenated aromatic hydrocarbons, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. The AhR resides in the cytosol until ligand binding promotes nuclear translocation and heterodimerization with the aryl hydrocarbon receptor nuclear translocator protein (ARNT) after which binding of the ligand:AhR:ARNT complex to its specific DNA binding site (the dioxin responsive element (DRE)) stimulates transcription of downstream genes. While the sequence of the DRE is well defined, several reports have suggested that the nucleotide specificity of AhR DNA binding may be ligand-dependent. To examine whether ligand-dependent nucleotide specificity of AhR DNA binding can occur we have used PCR-assisted binding site selection analysis to examine DNA binding of the guinea pig hepatic AhR complex activated by structurally diverse agonists. Ligand activated AhR complexes were incubated with oligonucleotides containing a 15-base variable region (all nucleotides represented at each position), ligand:AhR:ARNT:oligonucleotide complexes were isolated by immunoprecipitation, and the DNA was PCR amplified and used in the next round of selection. Following four rounds of selection, sequence analysis of the isolated oligonucleotides did not reveal any major differences in the nucleotide specificity of the DRE for the compounds tested. While some minor single base variations were observed in the established DRE consensus sequence, these sequence variations did not alter the DNA binding of AhR complexes activated by any tested agonist. Our results are consistent with the established role of the canonical DRE sequence as the only AhR DNA binding site and that the binding of structurally diverse agonists to the AhR does not alter its nucleotide specificity of DNA binding. (R01ES012498)

PS 179 FUNCTIONAL ANALYSIS OF THE SV23, SV24, AND SV25 SPICE VARIANTS OF HUMAN CONSTITUTIVE ANDROSTANE RECEPTOR (CAR): COMPARISON OF GINKGO BILOBA EXTRACT WITH OTHER KNOWN ACTIVATORS OF CAR.
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Ginkgo biloba extract has several biological activities, including protection against neurotoxicity and oxidative stress. Constitutive androstanoid receptor (CAR) controls the transcription of genes involved in a broad array of biological functions, including bioactivation and detoxification of toxicants. Naturally-occurring splice variants of human CAR (hCAR) exist, including hCAR-SV23 (insertion of amino acids SPTV), hCAR-SV24 (APYLT), and hCAR-SV25 (SPTV and APYLT). G. biloba extract was reported to activate hCAR-SV24 and the wild-type (hCAR-WT). However, it is not known whether it selectively affects hCAR splice variants and how it activates hCAR isoforms. We evaluated the impact of G. biloba extract on the functionality of CAR-SV23, hCAR-SV24, hCAR-SV25, and hCAR-WT, and compared it to that of phenobarbital, di-2-ethylhexyl phthalate (DEHP), 6-(4-chlorophenyl)-imidazo[2,1-b][1,3]thiazole-5-carboxaldehyde O-(3,4-dichlorobenzyl) oxime (CITCO), and 1,4-bis-[3,5-dichloropyridyl]oxo]benzene (TCPPOB) in cell-based reporter gene assays. Among the hCAR splice variants investigated, only hCAR-SV25 was activated by G. biloba extract and this in vivo-activated form required co-transfection of a retinoid X receptor-β (RXRβ) expression plasmid. The extract activated CAR-SV23 to a lesser extent than hCAR-WT. CITCO activated hCAR-SV3, hCAR-SV24, and hCAR-WT, whereas phenobarbital activated hCAR-WT, and DEHP activated hCAR-SV23, hCAR-SV24, and hCAR-WT. TCPPOB did not affect the activity of any of the isoforms. G. biloba extract and phenobarbital did not bind or recruit coactivators to the ligand-binding domains of hCAR-WT and hCAR-SV23, whereas positive results were obtained with the controls (CITCO for hCAR-WT and DEHP for hCAR-SV23). In conclusion, G. biloba extract activates hCAR in an isoform-selective manner, and hCAR-SV23, hCAR-SV24, and hCAR-WT have overlapping but distinct sets of ligands. (Supported by Canadian Institutes of Health Research)

PS 180 ANALYSIS OF THE MECHANISMS OF LIGAND-DEPENDENT TRANSFORMATION OF THE AH RECEPTOR INTO ITS DNA BINDING FORM.
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The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor that mediates toxic and biological effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. AhR-mediated biological responses demonstrate dramatic interspecies differences which may occur in part during ligand-dependent conversion of the AhR into its high affinity DNA binding form. This process is termed AhR transformation and its mechanism is not fully understood. Instances of decreased transformation efficiency (measured as the ratio of total AhR (determined by ligand binding) to the amount that binds to DNA in a ligand-dependent manner) were analyzed and the underlying mechanisms determined. Unlike guinea pig, rat or CH mouse hepatic cytosolic AhR, mouse C57BL cytosolic AhR demonstrated decreased transformation efficiency and this appeared to result from insufficient levels of ARNT in the cytosolic extract. Similarly, while TCDD binding to the C57BL mouse AhR was reversible (i.e., TCDD could dissociate from the AhR), TCDD binding to other cytosolic AhRs was irreversible. Further analysis of in vitro synthesized C57BL mouse AhR revealed that a decrease in transformation efficiency occurred in the presence of sodium molybdate (an agent that stabilizes the association of the AhR with Hsp90) as well as with insertion of selected point mutations within the AhR. Of these mutations, D371A was found to negatively affect the initiation of AhR dimerization with ARNT, while that of R217A affected the ligand-dependent conformational change of the AhR. Additionally, evidence to support the hypothesis that the binding of ARNT results in a progressive displacement of Hsp90 from the AhR was obtained from the detection of a transient molybdate-stabilized hsp90:AhR:ARNT complex. Together, these findings provide further insights in the mechanisms of ligand-dependent transformation of the AhR into its DNA binding form and demonstrate that AhR transformation is a multi-step progressive process with several defined intermediate steps. (ES007685; ES012498)

PS 181 TRICLORCARBAN MEDIATES INDUCTION OF XENOBIOTIC METABOLISM THROUGH ACTIVATION OF NUCLEAR RECEPTORS CAR AND ER-ALPHA.
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Triclocarban (3,4,4’-trichlorocarbanilide, TCC) is used as a broad-based antimicrobial agent that is commonly added to personal hygiene products. Because of its extensive use in the health care industry and resistance to degradation in sewage treatment processes, TCC has become a significant waste product that is found in numerous environmental compartments. While TCC has been linked to a range of negative health and environmental issues, few studies have been conducted to examine the impact of exposure to TCC and the underlying mechanisms linked to the negative responses. In this study, experiments were initiated to investigate the role of TCC exposure towards activation of xenobiotic metabolism through regulation by the nuclear xenobiotic receptors (XRs). TCC treatment to humanized CYP2B6 (α) mice resulted in the induction of the liver Cyp2b10 gene. These observations demonstrated that TCC be classified as an endocrine disruptor, we also demonstrated that it activates the estrogen receptor-alpha (ERα) leading to induction of Cyp1b1 gene in female ovaries. Activation of ERα by TCC in receptor-based assays also promotes induction of the human UGT1A1 gene. Through the use of reverse genetics, this induction was shown to be dependent upon the constitutive androstane receptor (CAR) since no induction occurred in hUGT1A1/cArγ mice. Induction of the UGT1A genes by TCC also corresponded with induction of Gsp2b10, another CAR target gene. TCC was demonstrated to be a phenobarbital-like agonist of CAR in receptor-based assays. While it has been suggested that TCC be classified as an endocrine disruptor, we also demonstrated that it activates the estrogen receptor-alpha (ERα) leading to induction of Cyp1b1 gene in female ovaries. Activation of ERα by TCC in receptor-based assays also promotes induction of the human UGT1A1 gene. These observations demonstrate that TCC activates XRs CAR and ERα both in vivo and in vitro. Activation of these xenobiotic-sensing receptors amplifies gene expression profiles that might represent a mechanistic base for potential human health effects from exposure to TCC. (Supported by USPHS Grants ES010337 and ES004699).
Remarkably little is known about how TCDD causes toxicity. AHR cross-talks with multiple signaling pathways and hundreds of genes have been found to be activated by TCDD in an AHR-dependent manner. Still, a unifying mechanism of dioxin toxicity is lacking that can explain the multitude of developmental consequences from exposure during early life, tumor promotion and modulation of immune cell development. Over a long period it has been claimed that structurally diverse compounds can act as AHR agonists although they show low binding affinity. This has led to the prevailing view of a promiscuous ligand-binding specificity of the AHR and lowered the interest in finding an endogenous high affinity ligand. We claim that the physiological functions of the AHR are activated by an endogenous ligand with hormone/vitamin like properties. Our ongoing chemical and biological characterization of 6-formylindolo[3,2-b]carbazole (FICZ), which we have suggested to be the enigmatic endogenous AHR ligand, shows that this signal substance has very interesting biological properties. It has the highest affinity for binding to the AHR of all compounds tested so far and it is also an ideal substrate for the CYP1 enzymes. We have documented effects of FICZ, on cell cycle regulation at femtomolar concentrations. Also, new studies by others e.g. those reporting effects of FICZ at low concentrations on the function of various cells of the immune system support its physiological importance. FICZ seems to be ubiquitously present in bio-logic materials that contain the amino acid tryptophan, such as cell culture media. Thus, the presence of FICZ may have erroneously influenced the interpretation of earlier studies like those that describe ligand-independent activation of the AHR. We propose that the endogenous ligand for the AHR is considerably more important than previously thought. We also propose that deficiency as well as excess of FICZ may explain toxicity by TCDD and other agents that induce or inhibit its metabolic clearance and thereby disrupt FICZ-dependent homeostatic processes.

Several polyphenols have been shown to activate the aryl hydrocarbon receptor (AHR) in spite of the fact that they bind to the receptor with low affinity. The aim of this study was to investigate if quercetin, curcumin and resveratrol activate the AHR indirectly by interfering with the metabolic degradation of a high affinity AHR ligand FICZ. Especially, we wanted to test the ability of some polyphenols to interfere independently with the metabolic degradation of a high affinity AHR ligand FICZ. We propose that the endogenous ligand for the AHR is considerably more important than previously thought. We also propose that deficiency as well as excess of FICZ may explain toxicity by TCDD and other agents that induce or inhibit its metabolic clearance and thereby disrupt FICZ-dependent homeostatic processes.

Constitutive androstane receptor (CAR, NR1:3), a member of the nuclear receptor superfamily, plays an important role in the transcriptional activation of multiple metabolizing enzymes such as cytochrome P450 (CYP). CAR can also be induced in a time and dose dependent manner when added together with FICZ. Furthermore, recombinant human CYP1A1 enzyme and clearance of FICZ, recombinant human CYP1A1 enzyme and metabolism of FICZ, recombinant human CYP1A1 enzyme and CYP1A1 transcription and enzyme activity were up-regulated by all three compounds and the effects were only observed in the commercial DME medium. Quercetin, curcumin and resveratrol prolonged CYP1A1 induction in a time and dose dependent manner when added together with FICZ. To investigate the inhibitory effects of polyphenols on CYP1A1 enzyme activity and metabolic clearance of FICZ, recombinant human CYP1A1 enzyme and CYP1A1 enzyme and metabolite studies were used. The intracellular levels of FICZ in cells co-treated with FICZ and polyphenols were quantified by HPLC. The CYP1A1 enzyme activity and clearance of FICZ were inhibited in a dose-dependent manner by polyphenols. Our finding demonstrates the following: i) quercetin, curcumin and resveratrol do not activate the AHR without trace levels of FICZ being present in the culture medium, ii) polyphenols seem to induce CYP1A1 through inhibition of metabolic degradation of FICZ. The balance of differentiation of multipotent mesenchymal stromal cells (MSC) between osteogenesis and adipogenesis is a central element of bone homeostasis. Peroxisome proliferator activated receptor γ (PPARγ) sits at the crossroad, promoting adipogenesis and suppressing osteogenesis. Adipocyte differentiation in bone marrow is potentially deleterious to both bone integrity and lymphopoiesis. Because organotins are dual ligands for PPARγ and its heterodimerization partner, the retinoid X receptors (RXRs), we hypothesized that tributyltin (TBT) and triphenyltin (TPhT) would have similar yet distinct effects on MSC differentiation from either a PPARγ agonist (Rosiglitazone; Rosi) or RXR (Bexarotene; Bex) agonist. While TBT, TPhT, Rosi, and Bex all shared the ability to suppress osteogenesis (alkaline phosphatase activity, nodule number, mineralization) in primary bone marrow cultures, only TBT, TPhT and Rosi induced adipogenesis. Gene expression studies support our hypothesis that the organotin-induced transcriptional program is distinct. TBT and Rosi strongly induced the expression of PPARγ, its gene target FABP4 and a terminal adipocyte marker adipins and suppressed expression of Runx2, its gene target osteocalcin and an osteoblast maker alkaline phosphatase. Intriguingly, only TBT and Bex upregulated the expression of the liver X receptor gene target ABCA1. In vivo exposure to TBT and Rosi altered a serum marker of bone turnover (PINP), decreased cortical thickness (μCT) and increased marrow adiposity (histology). Surprisingly, only TBT increased the expression of markers of both adipogenesis (FABP4, adipin) and osteogenesis (intercal) in bone. TBT alone upregulated ABCA1 expression in bone. These data suggest that RXR and PPARγ may contribute independently to TBT-induced adipogenesis and reduction in osteogenesis and that TBT alters bone homeostasis leading to bone loss in vivo.

The Ah receptor (AHR) is rapidly degraded both in vivo and in vitro following binding to ligands typified by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The AHR is also degraded after cells are exposed to compounds such as geldanamycin.
The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that is regulated by the lipid soluble environmental toxicant 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD). TCDD activation of the AHR induces the transcription of several genes that are important for xenobiotic metabolism. Obesity is a risk factor for several types of cancers and identifying molecular mechanisms that influence the interactions between adipose tissue and cancerous epithelial cells will be important for developing new cancer therapies given the current increases in obesity in the United States. Adipocytes mediate this risk through multiple mechanisms involving several adipocyte derived signaling molecules which stimulate tumor growth directly or indirectly by promoting angiogenesis and inflammation. We have discovered that pharmaceutical AHR antagonists inhibit adipocyte-supported growth of human breast cancer cells. However, the mechanisms by which the AHR potentially mediates adipocyte regulated breast cancer cell growth are not clear. To further understand the role of the AHR in mediating the effects of adipocyte derived signaling molecules on breast cancer cell growth, short interfering RNA against the AHR, a p-dioxin (TCDD). TCDD activation of the AHR induces the transcription of several genes that are important for xenobiotic metabolism. Obesity is a risk factor for several types of cancers and identifying molecular mechanisms that influence the interactions between adipose tissue and cancerous epithelial cells will be important for developing new cancer therapies given the current increases in obesity in the United States. Adipocytes mediate this risk through multiple mechanisms involving several adipocyte derived signaling molecules which stimulate tumor growth directly or indirectly by promoting angiogenesis and inflammation. We have discovered that pharmaceutical AHR antagonists inhibit adipocyte-supported growth of human breast cancer cells. However, the mechanisms by which the AHR potentially mediates adipocyte regulated breast cancer cell growth are not clear. To further understand the role of the AHR in mediating the effects of adipocyte derived signaling molecules on breast cancer cell growth, short interfering RNA against the AHR, a
Inhalation exposure to environmental particulate air pollutants (PM) is correlated with a number of specific adverse health effects in humans. However, the molecular “sensors” that detect PM and initiate deleterious responses that may culminate as compromised health are not known. Recently, TRPA1 was shown to be activated by diesel exhaust particle (DEP), but the molecular consequences of this phenomenon have not been demonstrated. The hypothesis of this study was that activation of TRPA1 by DEP would cause pneumotoxicity. Studies in vitro indicate that the activation of TRPA1 by DEP is selective and it is the electrophilic components of DEP that activate TRPA1. Mutation of the electrophilic binding site had no effect. DEP induced calcium flux in isolated mouse sensory neurons overlapped with responsiveness to the prototype TRPA1 agonist AITC and were abolished by co-treatment with the TRPA1 antagonist HC-030031. Mice instilled with DEP exhibited a reduction in lung compliance and increased expression of a number of pro-inflammatory and ER stress-associated genes. Both changes in lung compliance and the pro-apoptotic ER stress response gene GADD153 were attenuated by HC-030031 co-treatment implicating TRPA1 as the mediator of these processes in vivo. This study identified TRPA1 as a specific molecular sensor for specific chemical components of DEP and suggests that activation of TRPA1 in airway cells may be a critical first-step in DEP-induced pneumotoxicities including irritation, inflammation, and tissue injury. This work was supported by the 2011 Colgate-Palmolive Postdoctoral Fellowship and NIH grant ES017431.

191  GNFS1 DIMINISHES ARYL HYDROCARBON RECEPTOR OCCUPANCY AT THE IL-6 PROMOTER CONTRIBUTING TO MODULATION OF INFLAMMATORY PHENOTYPE IN FHS-RA CELLS.

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Rheumatoid Arthritis (RA) is a chronic inflammatory disease of unknown etiology which manifests itself, in part, as inflamed human fibroblast-like synoviocytes (HFLS). Upon inflammation, FLS, in RA aggressively proliferate to form a pannus, leading to expression of inflammatory mediators. Epidemiological studies have identified a positive correlation between cigarette smoking, a source of agonistic aryl hydrocarbon receptor (AHR) ligands, and development of rheumatoid arthritis. To test whether AHR activation in the myocardium reproduces cardiac toxicity, we expressed it under the control of a myocardium specific promoter in zebrafish. AHR activation produced pericardial edema and altered heart looping; however, the cardiovascular defects seen following TCDD exposure. Notably, myocardial edema was abolished by co-treatment with the TRPA1 antagonist HC-030031. Mice instilled with DEP exhibited a reduction in lung compliance and increased expression of a number of pro-inflammatory and ER stress-associated genes. Both changes in lung compliance and the pro-apoptotic ER stress response gene GADD153 were attenuated by HC-030031 co-treatment implicating TRPA1 as the mediator of these processes in vivo. This study identified TRPA1 as a specific molecular sensor for specific chemical components of DEP and suggests that activation of TRPA1 in airway cells may be a critical first-step in DEP-induced pneumotoxicities including irritation, inflammation, and tissue injury. This work was supported by the 2011 Colgate-Palmolive Postdoctoral Fellowship and NIH grant ES017431.

192  CONSTITUTIVE AHR ACTIVATION IN CARDIAC MYOCYTES INDUCES HEART MALFORMATION IN ZEBRAFISH.

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Exposure of zebrafish embryos to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) activates the zebrafish aryl hydrocarbon receptor 2 (Ahr2) to produce multiple developmental defects, particularly within the cardiovascular system. These defects include un looping of the heart, pericardial and yolk sac edema, reduction of cardiac output, decreased peripheral blood flow, disruption of bulbus arteriosus formation, and impaired common cardinal vein regression. The gene targets responsible for these developmental defects following AHR activation remain unknown. A primary difficulty in unraveling the target genes is a lack of understanding regarding the site of action of TCDD and how that contributes to specific endpoints of toxicity. Cardiac toxicity could be the result of TCDD acting directly on myocardial cells or it could be secondary to AHR activation in endothelial cells or at other distal sites. To test whether AHR activation in the myocardium reproduces cardiac endpoints of TCDD toxicity we constructed a constitutively active AHR and expressed it under the control of a myocardium specific promoter in zebrafish. AHR activation within the myocardium was sufficient to reproduce some, but not all, of the cardiovascular defects seen following TCDD exposure. Notably, myocardial AHR activation produced pericardial edema and altered heart looping; however, bulbus arteriosus formation and common cardinal vein regression occurred normally. These results link specific endpoints of TCDD cardiac toxicity to AHR activation within cardiac myocytes and demonstrates that these endpoints are not secondary to AHR activation within other cell types. (Supported by NIEHS, NIH Grants RO1 ES012716 and T32 ES007015)

193  ROLE OF TRPA1 IN DIESEL EXHAUST PARTICLE-INDUCED PNEUMOTOXICITY.

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Inhalation exposure to environmental particulate air pollutants (PM) is correlated with a number of specific adverse health effects in humans. However, the molecular "sensors" that detect PM and initiate deleterious responses that may culminate as compromised health are not known. Recently, TRPA1 was shown to be activated by diesel exhaust particle (DEP), but the molecular consequences of this phenomenon have not been demonstrated. The hypothesis of this study was that activation of TRPA1 by DEP would cause pneumotoxicity. Studies in vitro indicate that the activation of TRPA1 by DEP is selective and it is the electrophilic components of DEP that activate TRPA1. Mutation of the electrophilic binding site had no effect. DEP induced calcium flux in isolated mouse sensory neurons overlapped with responsiveness to the prototype TRPA1 agonist AITC and were abolished by co-treatment with the TRPA1 antagonist HC-030031. Mice instilled with DEP exhibited a reduction in lung compliance and increased expression of a number of pro-inflammatory and ER stress-associated genes. Both changes in lung compliance and the pro-apoptotic ER stress response gene GADD153 were attenuated by HC-030031 co-treatment implicating TRPA1 as the mediator of these processes in vivo. This study identified TRPA1 as a specific molecular sensor for specific chemical components of DEP and suggests that activation of TRPA1 in airway cells may be a critical first-step in DEP-induced pneumotoxicities including irritation, inflammation, and tissue injury. This work was supported by the 2011 Colgate-Palmolive Postdoctoral Fellowship and NIH grant ES017431.
threat to human health, the molecular mechanisms through which it causes adverse effects is not well defined. A hypothesis of the laboratory is that TRPV1, V2, V4, M2, or M8. Several chemical components of WSPM were also assayed, revealing new TRPA1 agonists. Differential activation of TRPA1, as a function of particle size, demonstrated that PM<10 μm were most potent. The mechanism of TRPA1 activation by WSPM largely involved the electrophile/oxidant sensitive domain of TRPA1, but ongoing studies suggest that the menthol binding site of TRPA1 also is a target for non-electrophilic WSPM components. WSPM also potently activated TRPA1 in isolated sensory neurons which was associated with changes in the expression of mRNA for the stress marker c-fos, the pro-inflammatory neuropeptides substance P and neurokinin A, as well as TRP channels. This study identifies TRPA1 as a molecular sensor for WSPM in airway sensory neurons which is hypothesized to be a critical step in elucidating the precise mechanisms by which WSPM causes respiratory toxicities. Support: NIH ES017431 and The University of Utah Biotechnology Undergraduate (BioURP) program.

**A NOVEL ROLE OF THE CONSTITUTIVE ANDROSTANE RECEPTOR IN HUMAN Hepatic DIFFERENTIATION.**

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The constitutive androstanone receptor (CAR) is an orphan receptor that regulates the expression of genes involved in hepatic metabolism and clearance of xenogenous and endogenous substances. Recent studies have indicated detection of CAR expression in fetal livers of mouse, rat and human. Coupled with our findings demonstrating that CAR is significantly increased in hepatic-like cells derived from human embryonic stem cells (hESCs) [CBI 190: 62, 2011], we hypothesized that CAR plays an important functional role in hepatic differentiation. Over-expression of CAR in hESCs transduced by a lentiviral vector enhanced mRNA expression of hepatic-specific markers, including transcription factors (C/EBPα, HNF1α, and HNF4α), plasma proteins (α1-antitrypsin and albumin), transcriptional enhancers (CYP2B6, CYP3A4 and CYP3A7), and metabolic enzymes (HMGCS2 and PEPCK). In particular, CYP3A4 and CYP3A7 expression were 39- and 30-fold elevated, respectively, in the CAR transduced hepatic-like cells compared with non-transduced cells after 20 days of differentiation. The transduction of CAR also accelerated maturation of hepatic-like cells, with CAR-over-expressing cells exhibiting a 2.5-fold increase in albumin secretion at day 20. Inducible activity of CYP3A7 by 5μM CITCO in CAR-over-expressing cells was also significantly higher than that of non-transduced cells. Further, knockdown of CAR mRNA via siRNA attenuated the differentiation-dependent increase in hepatic marker expression, including CAR, C/EBPα, HNF1α, HNF4α, FOXA1, and e-tetraoctoate. In contrast, expression of the pregnane X receptor (PXR), another nuclear receptor, was extremely low in fetal liver tissue and not increased during the differentiation process. In addition, over-expression of PXR in hepatic hESCs did not result in enhanced expression of genes involved in hepatic differentiation/maturation. These data suggest that CAR, but not PXR, contributes importantly to hepatic lineage commitment.

**IDENTIFICATION OF STANNIOCALCIN 2 AS A NOVEL TRANSCRIPTIONAL TARGET OF THE ARYL HYDROCARBON RECEPTOR.**

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The aryl hydrocarbon receptor (AhR) is a ligand-activated member of the Per-ARNT-Sim (PAS) superfamily of transcription factors. The AhR is traditionally recognized for its role in the adaptive metabolism of polycyclic and halogenated aromatic hydrocarbons. However, current AhR research has focused on the understanding of normal AhR biology and physiological activities due to the generation of the AhR knockout mouse. Recent reports from our lab and others suggest the AhR has a role in regulating cell survival and death. These newly recognized roles along with traditional AhR activities have steered our laboratory in the direction of identifying AhR target genes in the absence of an exogenous ligand. Using primarily hepatocytes isolated from AhR−/− mice, we are able to generate AhR null hepatocytes through introduction of Cre recombinase using an adenoviral expression system. This model allows us to monitor changes in the transcriptome that occur immediately after the loss of the AhR. In order to assess AhR transcriptional control in relation to apoptosis we performed qRT-PCR using SYBR arrays containing 84 key genes involved in cell death. However, no significant changes were identified leading us to perform a comprehensive DNA microarray analysis. Stanniocalcin 2 (Stc2) was consistently down regulated with the loss of the AhR in both the microarray screen and SYBR validation. In silico analysis of the Stc2 promoter region identified 9 putative AhR binding elements (XRE) within 1 kb of the Stc2 transcriptional start site. Further analysis of AhR dependent Stc2 induction reveals Stc2 as a new AhR target gene induced in ER stress responses. As a result we are now in position to characterize the AhR-Stc2 relationship that ultimately leads to cell survival, a mechanism consistent with normal AhR biology and physiological function. [ESO12018, ES017254, ES006676]

**198 ATMOSPHERIC BEHAVIOR, DEPOSITION, AND BUDGET OF RADIOACTIVE MATERIALS FROM THE FUKUSHIMA DAIICHI NUCLEAR POWER PLANT IN MARCH 2011.**


To understand the atmospheric behavior of radioactive materials emitted from the Fukushima Daiichi nuclear power plant after the nuclear accident that accompanied the great Tohoku earthquake and tsunami on 11 March 2011, we simulated the transport and deposition of iodine-131 and cesium-137 using a chemical transport model. The model roughly reproduced the observed temporal and spatial variations of deposition rates over 15 Japanese prefectures (60-400 km from the plant), including Tokyo, although there were some discrepancies between the simulated and observed rates. These discrepancies were likely due to uncertainties in the simulation of emission, transport, and deposition processes in the model. A budget analysis indicated that approximately 13% of iodine-131 and 22% of cesium-137 were deposited over land in Japan, and the rest was deposited over the ocean or transported out of the model domain (700 × 700 km2). Radioactivity budgets are sensitive to temporal emission patterns. Accurate estimation of emissions to the air is important for estimation of the atmospheric behavior of radionuclides and their subsequent behavior in land water, soil, vegetation, and the ocean.

**199 HUMAN HEALTH RISK ASSESSMENT FOLLOWING THE FUKUSHIMA NUCLEAR POWER PLANT ACCIDENT.**

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Following the accident at the Japanese nuclear power plant in Fukushima, as consequence of the severe earthquake and tsunami, WHO has been working with other international partners to advise countries on potential health implications. WHO is now engaged in an international effort to assess the human health risk of radiation exposure due to this incident for the population globally. Different scenarios have been defined regarding exposed population within and outside Japan and different age groups (adult, 10y child, 1-2y child). As a first step, together with FAO and IAEA, WHO has established an international expert group for the initial evaluation of total radiation exposure, considering internal exposure from ingestion (food and drinking water) and inhalation, and external exposure. Total dose assessments will be available by the end of 2011, these will feed into the work of another expert panel to estimate the potential health risks. Primary input data for the oral exposure assessments are monitoring results rather than modelling results, focussing on the first four months after the accident. Inhalation doses will be estimated both for Japan and the rest of the world on the basis of atmospheric dispersion modelling. External dose estimates will be based on atmospheric dispersion modelling and on data on ground deposition levels and on gamma dose rates. The outcome of the preliminary dose assessments will feed into estimates for dose response relationships between effective doses and total cancer, effective dose and all solid cancers or leukemia; and between thyroid dose and risk of thyroid cancer.

**200 AIRBORNE RADIATION MONITORING IN CALIFORNIA FOLLOWING THE 2011 FUKUSHIMA DISASTER.**

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Shortly after the March 11, 2011 earthquake and tsunami, concerns rose about the threat of a radioactive plume reaching the US from the destabilized Fukushima Daiichi reactors. California officials tried to access the most reliable model predictions from the National Atmospheric Release Advisory Center (NARAC) and the Defense Threat Reduction Agency (DTRA). Unfortunately, those models were not available to agencies responsible for radiation monitoring in California. In their absence, models from the Comprehensive Nuclear Test Ban Treaty Organization and
the Norwegian Institute for Air Research quickly spread through the internet and media outlets. Both emphasized extremely minor consequences; however, the plume images themselves fueled fear and speculation and resulted in a deluge of public inquiries. Thankfully, California has robust radiation monitoring capabilities, including a filter-based program operated by the California Department of Public Health (CDPH) and 11 near-real time radiation monitors operated by the California Air Resources Board (ARB) and local air districts on behalf of the Environmental Protection Agency’s (EPA) RadNet Program. From March 12-20, gross beta and gamma counts were not significantly different from data prior to the Fukushima disaster. Not until the first filters were analyzed was it clear that gaseous iodine-131 (I131) and cesium-137 (Cs137) particulates — isotopes of greatest concern following a nuclear power accident — arrived in California. Concentrations were far below any public health concern. However, the fact that I131 and Cs137 were detected catapulted state and local agencies into a tremendous risk communication effort. ARB executives launched a consolidated web portal for California-specific data and joined efforts with others in a multi-agency coordination group to focus the work of technical specialists and to develop consistent messages. Airborne radiation quickly returned to background, but the “failure” resulted in many lessons learned for California’s emergency response and public health agencies.

Environmental risk assessment and risk communication were of eminent importance after meltdown of the FDNP nuclear reactors caused by the devastating tsunami attack. After release of half a million tera Bq of radionuclides, approximately 10% of that of Chernoyyl disaster, environmental radiation monitoring and risk communication by Japanese Government was failure in the early stages. The governmental spokesman, one of Cabinet member, simply announced measurement data of air space radiation and claiming safety, even though he was not reliable authority of the academy. After six months of the event, I conducted questionnaires survey among the participants after risk communication seminars about radiation and food safety in the neighboring communities from Fukushima prefecture. The result showed that announcement of environmental radiation monitoring data rather increased their anxieties and suspicion about the government’s concealment of the catastrophic damage. They have been looking for information about margin of safety and risk. As my conclusion, simple and clear messages about risk and basic knowledge of radiation toxicology shall be given to the inhabitants with environmental measurement data sets.

The health effects of low doses of radiation (LDR) are not fully understood, and few have considered the role of background radiation in gene-environment interactions. Radiation leakage at Fukushima and the concern over intentional release of radioactive materials by terrorists have heightened public awareness of this issue. The Pacific Northwest National Laboratory’s (PNNL) low dose radiation systems biology program is investigating the consequences of low dose radiation exposures in experimental human organotypic skin culture. Skin is the 4th most sensitive site in atomic bomb survivors, exhibits dose-dependent transitions for radiation carcinogenesis and is the most advanced human organotypic model system. Our approach combines genomic, proteomic and metabolomic platforms to interrogate tissue radiation responses and exploits advanced bioinformatics and computational modeling resources developed at PNNL to interpret complex data sets. Many signaling networks are activated by low doses of radiation, including p38 MAPK pathway, IGF1 pathway and cell proliferation/apoptosis. Further, advanced imaging capabilities have led to the discovery of new LDR sensitivities in molecular feedback control processes that regulate major signaling networks (e.g. ERK). Our objective is to understand the consequences of LDR on cellular homeostasis and how this impacts tissue response to subsequent challenges. We are currently developing radioresistant engineered tissue model systems as contrast to normal tissue responses to LDR. The long term goal is to determine scenarios where LDR effects might modulate human health or modify subsequent challenges induced by a second insult, (e.g. in the case of a radiological incident wound healing, physical trauma or burns.)

Biological modeling has helped improve our understanding of how radiocative contaminants distribute in the body after an intake, as well as how radiation interacts with the body to cause acute damage. For example, application of a detailed inhalation model together with physiological models for internalized Cs-137 and Am-241 has improved radiation dose estimations. Modeling of the treatment of these nuclides by Prussian Blue and DTPA further enables the evaluation of the efficacy of treatment at different times after exposure and for different treatment durations. The resulting knowledge improves the risk assessment for both acute effects, as well as long-term health risks. Physiological models for Cs-137 and Am-241 and their treatment will be presented along with practical examples of their application. Another useful application of biological modeling employs a systems biology approach to examine how radiation interacts with acute injury (e.g. burn or trauma) to provide valuable information concerning common mechanistic interactions between the two different injury types (e.g. radiation together with burn). This modeling effort provides a more accurate understanding of the pathophysiology of combined injury and can improve estimation of the outcomes anticipated after major radiological events like the Chernobyl accident. Thus, biological modeling can help better predict health consequences of radiological hazards from nuclear accidents, and therefore provide valuable information that can be used to minimize the impact of radiological incidents.

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Prolonged genomic changes after radiation exposure are stochastic and thus cannot be identified on the basis of common gene expression in hematopoietic stem/progenitor cells. Changes, if available, may be radiation-effect-related changes in stromal/niche functions; however, little evidence has been reported to date. Recent progress in functional research on hematopoietic stem/progenitor cells has suggested that their changes seem to be due to oxidative stresses caused by stromal/niche alterations. According to our collaborator’s recent report, surveying the stromal capability of lineage(-)c-kit(+)SCA1(+) (LKS) cells in lethally irradiated and repopulated mice, monitored by the Ly5.1 isotype for 270 days after transplantation (Otsuka et al. Exp Hematol, 2010), each bone marrow niche/stromal cell maintained stem/progenitor compartment, such as the LKS fraction or various colony forming units, respectively were found to be functionally different. Specifically, the LKS fraction was likely to be unable to differentiate and migrate to peripheral blood after 270 days when the recipient stroma was irradiated. Presumably, although primitive (possibly osteoblastic) niches were found to be radiosensitive, intermediate (possibly vascular) niches seemed to be moderately radiosensitive and promoted progenitor cells to proliferate and differentiate, and the general type of stromal cells was less sensitive to radiation and promoted mature blood cells to migrate to peripheral blood. Each stromal cell component or detailed regulating gene repertoire is not yet identified. The findings of this study suggest that each stromal cell compartment was functionally separated along with the hematopoietic cell differentiation from primitive stem cells via various progenitor cells to mature blood cells.

ACUTE RADIATION SYNDROME: HEMATOPOIETIC CHARACTERIZATION OF A NONRODENT MODEL.

Current regulations require evaluation of radiomimetic drug candidate efficacy and safety in a rodent and a nonrodent model prior to approval under the FDA Animal Rule. Cell blood counts were obtained from 120 Rhesus monkeys for 12 to
60 days after whole body radiation using a Cobalt-60 at radiation dose levels ranging from 400 to 1210 Gy. The blood cell counts and proportions of these blood cells were observed approximately on Day 26 at doses of 400, 600 and 634 cGy. Neutrophil counts also showed a progressive decrease after irradiation and reached earlier and lower nadirs with higher irradiation doses. Severe neutropenia (≤ 0.5 X 10^9/L) was first observed on Day 8 at 400 cGy while it was first noted on Day 5 at irradiation doses of 600 cGy and higher. The number of days of severe neutropenia was 10, 13 and 15 at radiation doses of 400, 600 and 634 cGy. Lymphocyte counts on Day 2 post-TBI were significantly decreased from baseline with a severity that was irradiation dose-dependent. Lymphocyte count recovery was initially observed approximately on Day 18 but remained lower than baseline levels throughout of monitoring period at all irradiation doses. Severe thrombocytopenia was first observed on Days 16, 11 and 10 at radiation doses of 400, 600 and 634 cGy, respectively. Platelet nadirs were observed earlier at higher irradiation doses and the recovery was comparable at irradiation doses of 400, 600 and 634 cGy. Mean platelet volume decreased parallel to platelet counts. At 634 cGy, mean platelet volume reached a minimum (≤20% from baseline) on Day 12 post-TBI. The hematology profiles obtained in this nonrodent acute radiation syndrome model were comparable to radiation exposed humans and confirm the value of this nonrodent model for drug development.

206 RADIATION EXPOSURE EXACERBATES LETHALITY AND LUNG PATHOLOGY FOLLOWING A SUBSEQUENT INFLUENZA A VIRUS INFECTION.

C. M. Manning1, C. J. Johnston1,2, B. Lawrence1, J. P. Williams1 and J. N. Finkelsten1,2,3

Since 2001, there has been increased concern with the possibility of a radiological or nuclear dispersion event. Exposed individuals that survive the acute radiation syndrome will be at risk for complications resulting from radiation injury in late-responding tissues, such as the lung. The lung’s constant contact with the outside environment also makes it susceptible to other noxious agents, such as pathogens. It is unknown whether irradiation will affect the lung’s ability to defend against a subsequent challenge. In this study, we hypothesized that irradiation will affect the lung’s response to an Influenza A virus infection. Adult, C57BL/6J mice were whole lung irradiated with 6.5, 10 or 15 Gy α-γ-radiation and infected with virus ten weeks later. Mortality, immune cell recruitment, viral titer, lung structure and lung function were assessed. Mortality after infection was increased in 10 and 15 Gy irradiated animals. Surviving 15 Gy irradiated animals failed to regain body weight lost during infection. The peak T cell response was attenuated in the 15 Gy irradiated infected animals; however, viral clearance still occurred. Irradiated infected animals also displayed persistent pulmonary edema. We further investigated the effect of irradiation on exacerbation of radiation-induced lung injury, which begins to appear at 26 weeks post-15 Gy lung irradiation. In 15 Gy irradiated infected animals, lung pathology, which included increased collagen deposition, inflammation and edema, was observed at 17 weeks post-irradiation. Consistent with pathological observations, the animals also displayed reduced pulmonary function. To determine if this contribution to the lung injury was mediated by an increase in the lung inflammatory response, the animals were treated with the anti-inflammatory drug, dexamethasone. In this study, it was concluded that irradiation can increase lethality and impair the restoration of normal lung structure and function following a virus infection. Furthermore, this effect was dependent on the lung radiation dose. Funded by NIAID U19 AI091036-01, ES-01247, ES T32 07026.

207 NEONATAL IRRADIATION SENSITIZES MICE TO ADULT PULMONARY INFLUENZA CHALLENGE.

C. J. Johnston1,2,3, C. M. Manning1,2,4, J. P. Williams1, T. D. Randall1,3, J. Rangel-Moreno1, E. Hernandy1 and J. N. Finkelsten1,2,3,4

Rationale: Significant differences exist between the physiology of the immature, neonatal lung compared to the adult, which may affect acute and late response to irradiation. Identifying these differences would be critical in developing successful mitigation strategies for this special population. Our current hypothesis suggests that radiation during this critical period is likely to alter developmental processes resulting in long-term consequences including altered susceptibility to secondary inflammatory challenge. Methods: C57BL/6J mice, 4 days of age, received total body irradiation of 5.0 Gy prior to influenza A virus challenge at 3, 6 or 11 mont post irradiation (PI). At these times mice were intra-nasally infected with 120 HAU of influenza A virus. Body weight and survival were monitored. Pulmonary response was determined by lavage and histological examination. Analysis of epithelial and inflammatory marker binding expression were done by immunohistochemical and mRNA analysis. Results: Following influenza infection, irradiated animals lost significantly more weight at 6 and 11 months post-irradiation and had reduced survival compared to the unirradiated but infected controls with a greater effect seen at 11 months post radiation. Irradiated animals had a reduced percent of lymphocytes re-cruited to the lung following infection compared to shams. Conclusions: These results demonstrate that early life radiation injury may affect the lung’s response to a subsequent challenge and further suggests that this effect may be dependent on the time post-irradiation that the challenge occurs.Funded By: NIAID U19 AI-067733-0551, U19 AI-091036-P30 ES-01247, ES T32 07026 and EPA Star PM Center R827354.

208 FETAL RADIATION EXPOSURE INDUCES TESTICULAR CANCER.

G. Sherry1, P. B. Comish1, C. C. Weng2, A. Martin3 and M. I. Meistrich4

Fetal exposures to environmental factors have been cited as the cause of the steady 3% annual increase in testicular cancer in humans. Since testicular cancers are derived from primordial germ cells (PGCs), the increase has been attributed to maternal/fetal exposures to environmental factors. We examined the effects of an estrogen (diethylstilbestrol, DES), an antiandrogen (flutamide), or radiation on the incidence of testicular germ cell tumors in a genetically predisposed mouse strain, 129.MOLF-L1 (L1) congenic mice. L1 mice were mated and vaginal-plug positive females were exposed to the agents at days 10.5 and 11.5 of pregnancy. The testes from male offspring were examined for the presence of teratoma by morphology and histology at 4 weeks of age. Neither flutamide nor DES produced noticeable increases in cancer incidence. In contrast, two doses of 0.8-Gy radiation increased the incidence of testicular teratoma from 45% to 100% in the offspring. The percentage of mice with bilateral tumors, weights of testes with teratoma, and the percentage of tumors with multiple dermal origins were higher in the irradiated mice than in controls, indicating that irradiation induced more aggressive tumors and/or more foci of initiation sites in each testis. This radiation dose did not disrupt spermatogenesis, which was qualitatively normal in tumor-free testes although they were reduced in size. Since the radiation is given at a stage when extensive DNA demethylation and chromatin remodeling is occurring as the PGCs are losing their pluripotent capacity, we suggest that the DNA repair response is interfering the epigenetic program at this critical stage. Also this is the first proof of induction of testicular cancer by an environmental agent and suggests that the male fetus of women exposed to radiation at about 5-6 weeks of pregnancy might have an increased risk of developing testicular cancer.

209 IONIZING RADIATION AND ENVIROMENTAL TOXICANTS CAN INTERACT DURING BRAIN DEVELOPMENT TO EXACERBATE COGNITIVE DEFECTS IN MICE.

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There is lack of knowledge and increasing concern about low dose exposure to ionizing radiation (IR) and induction of non-cancer effects. We are indirectly or directly exposed to persistent environmental toxicants and IR. It is therefore important to integrate different environmental agents which might act synergistically to exacerbate developmental toxic effects and thereby also change the dose-response curve. Foetuses and neonates are known to be high-risk groups for exposure to toxic agents. Epidemiological studies indicate that exposure to environmental toxicants, including IR, during early human development can have deleterious effects on cognitive development in childhood. By using the mouse as an animal model we can study the effect of a single toxic agent given directly to the neonatal animal during different stages of the rapid brain growth spurt, and also to study the combination of environmental agents in a controlled manner. In these studies we have observed that IR, and the environmental toxicants like MeHg, PCBs and PBDEs, can induce behavioural disturbances such as deranged spontaneous behaviour and habituation, learning and memory defects and reduced cognitive functions, together with altered function of the cholinergic system, when the exposure occurs during a critical phase of neonatal brain development (PND 10). In recent studies we have observed
that the effects after co-exposure to IR (0.05 – 1.5 Gy) and environmental toxicants (e.g. Methyl 0.08 – 4.0 mg/kg bw, PBDE 99 0.8 mg/kg bw) on PND 10 significantly enhance the developmental neurotoxic effects at doses where neither compound nor the IR caused any developmental defects. Furthermore, the dose of IR is down to a single exposure of just 0.1 Gy. This indicates a change in the dose-response curve of IR that can have implications for risk-estimates of non-cancerous effects.

210 LOW-DOSE IONIZING RADIATION ALTERS THE FETAL EPIGENOME OF THE AVY MOUSE.

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Low-dose (<10.0 centigray (cGy)) radiation exposure in man-made sources, such as diagnostic imaging, predominates in the US population and now comprises nearly 50% of the average individual’s yearly radiation exposure. Besides the acute effects of radiation exposure, there are concerns for long-term epigenetic aberrations. Such aberrations can disrupt normal development and are involved in the occurrence and progression of numerous diseases, including cancer. To date, analyses of high doses of radiation have shown that epigenetic disruption occurs and is necessary for the persistence of radiation-induced genomic instability; however epigenetic changes in response to low-dose ionizing radiation (LDH) in vivo have not yet been fully defined.

In order to characterize the dose-dependent epigenetic responses to low-dose ionizing radiation, we utilized the Agouti viable yellow (Avy) mouse model. This is a unique biological model that functions as a biosensor for developmental exposures that alter the epigenome.

Here we show that LDIR exposure in utero induces epigenetic changes in the Avy mouse in a dose-dependent and sex-specific manner. Acute exposure to 1.2, 2.5, 5.0 or 10.0 cGy X-ray irradiation significantly shifts offspring coat color distribution to pseudoagouti (ChiSquare: p = 0.019; p = 0.022, p = 0.0028, and p = 0.045, respectively). To assess low-dose radiation’s effect on the epigenome of Avy/a off-spring, DNA methylation levels at 11 CpG sites in and near the genetic promoter region of the Avy IAP were measured by bisulfite treatment and Sequenom analysis. Acute exposure to 2.5 or 5.0 cGy significantly increases methylation at multiple CpG sites in the Avy metastable epiallele in male offspring, but not female offspring (Repeaated Measures ANOVA, p<0.0001). Our results demonstrate that relevant, low doses of radiation can elicit epigenetic changes that lead to a persistent phenotype.

211 IS NEUROGENESIS ALTERED AFTER CHRONIC INTERNAL CONTAMINATION OF URANIUM DURING BRAIN DEVELOPMENT?


The objective of this study was to analyze the effects of depleted uranium (DU) on neurogenesis at different stages of brain development in an in utero model of internal contamination. Female rats have been exposed to DU (40 or 120mg/L) during gestation and lactation. Two time points of analysis have been chosen: embryonic day 13 and post natal day 21. In this work, we examined: 1) if contamination modified proliferation of cells in neurogenic zones by using BrDU immunostaining, 2) if cells from contaminated animals retained their multipotential properties using in vitro neurosphere and differentiation assay and 3) if neurite growth is affected by DU contamination using Golgi staining.

Preliminary results did not reveal any qualitative difference on cell proliferation in subgranular zone in contaminated rats compared to sham animals at P21. Moreover, no macroscopic morphological difference was observed on neurites of granular cells between exposed and non exposed animals. Finally, cells from the tenecephalic zone at E13 and cells from subventricular zone at P21 of exposed rats retained their capacities to form neurospheres and kept their multipotential properties as cells from non exposed rats did.

These first data suggest that chronic contamination of low dose of uranium has no effect on developmental neurogenesis at early stages. To complete these results, we have started experiments to determine if the proportion of cells implicated in neurogenesis is modified after internal contamination. We are also performing behavioral tests on animals exposed to DU to determine cognitive changes. Together, these results will provide a first view of internal contamination effects by uranium on neurogenesis during brain development.

212 URANIUM OLFACTORY ROUTE: A NASAL INSTILLATION MODEL TO STUDY BIOLOGICAL MECHANISMS INVOLVED IN THE TRANSFER TO THE BRAIN.

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The first evidence that uranium could reach the brain via the olfactory compartment has raised new concerns in terms of radioprotection of nuclear workers potentially exposed to particles inhalation. A recent study suggested that neurons located in the nasal cavity are directly involved in uranium transfer to the brain, representing a probable new contamination mode (Tournier et al., 1999). A feature of this uranium pathway seems to be a specific accumulation in the anterior part of the brain. Indeed, this is not observed when uranium is injected since its concentration is more homogenous throughout the brain.

In the light of these results, we developed a model of uranium nasal instillation to test this hypothesis, the interest being that pharmacological association with other agents and cellular localisation would be easier. A solution of uranyl nitrate at different concentrations (ranging from 0.01 to 10mg.ml-1) was instilled in the nasal cavity of 12 week old male rats. Tissue dissections: olfactory epithelium and bulbs, frontal cortex, hippocampus, cerebellum, lung-trachea and kidneys were performed 4 hours later. Uranium concentrations were measured by mass spectrometry. The results show that instilled uranium accumulates in the olfactory bulbs and is measured in the other cerebral structures but at lower concentrations. Instilled uranium was also found in lungs-trachea and kidneys. This frontal accumulation of uranium in the brain is in accordance with previous inhalation experiments (Tournier et al., 1999).

It appears that the nasal instillation model strengthen the hypothesis relying on a uranium axonal route via neurons of the nasal cavity. Future experiments will be performed to define the type of transport using lesion models or combining uranium with pharmacological agents involved in transporters, ion channels function.

213 GENETIC ALTERATIONS AND FUNCTIONAL CONSEQUENCES OF EMBRYONIC IONIZING RADIATION EXPOSURE IN ZEBRAFISH.

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The relationship and genetic mechanisms associated with ionizing radiation (IR) exposure and carcinogenesis has long been established, but recently the association between IR exposure and other diseases is starting to be recognized. Currently, there is limited information on the genetic mechanisms governing the role of IR exposure in non-cancer related adverse health effects. To begin to elucidate radiation induced genetic and functional alterations associated with non-carcinogenic diseases, zebrafish embryos were exposed to a range of IR doses (0, 1, 2, 5, and 10 Gy) at 24 hours post fertilization (hpf). No significant increase in mortality or hatching rate was observed at any dose at any developmental time point through 120 hpf. A significant decrease in body and head length and eye diameter was observed in the 10 Gy dose at 120 hpf. Genomic microarray analysis was subsequently conducted at 120 hpf to compare gene expression profiles between the control and highest IR dose at 120 hpf. No significant differences were observed in gross morphological measurements (5 Gy). In this analysis, 251 genes with a well-established function or orthology to human genes were identified as significantly altered. Gene ontology and molecular network analysis revealed enrichment of genes associated with cardiovascular and neurological development and function. As recent literature has suggested a link between IR exposure and cardiovascular alterations, expression of a subset of gene targets was assessed using qPCR with individuals exposed to 0, 1, 2, or 5 Gy to identify a potential low threshold of effects on the cardiovascular associated gene targets. Moreover, heart rate was analyzed through 120 hpf following IR dosing at 24 hpf. A significant decrease in heart rate was observed at 10 Gy, while a significant increase in heart rate was observed at 1, 2, and 5 Gy. Overall these findings indicate IR exposure at doses below those that induce gross morphological changes alters heart rate and expression of genes associated with cardiovascular and neurological functions.

214 IODIDE PROPHYLAXIS FOLLOWING NUCLEAR ACCIDENTS: PROTECTING CHILDREN.

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After the Chernobyl nuclear accident a significant increase in thyroid cancer incidence was observed in many children exposed to low doses of 131I exposure. Protecting the fetal thyroid after week 12 of gestation, the thyroid glands of new-
The possibility of a radiation disaster from a nuclear detonation or accident has existed for over 50 years and spawned much of the basic research in radiobiology in the 1950-60s. The recent Fukushima accident was yet another reminder that there remains a dire need to develop novel therapies against radiation-induced toxicities. Here we report on the development of two novel radiation countermeasure therapies: Yel001 and Yel002. These small, biologically active, drug-like molecules were uncovered in the DEL high throughput assay reducing radiation-induced cytotoxicity and genotoxicity in yeast. Radiation-modulating activity was further confirmed in yeast plate-based DEL Assay; addition of either Yel001 or Yel002 to irradiated cultures reduced cell death and genomic instability. Further, Yel compounds reduce lethality to an average of 25% in vivo following an LD100/30 dose of ionizing radiation (IR) with the first therapeutic injection administered 24 hours post exposure followed by injections at 48,72,96, and 120 hours. Additionally, treatment with Yel001 and Yel002 compounds reduces radiation-induced leukemia from 90% to 50% and 40% respectively. Of note, treatment with either Yel001 or Yel002 reduced spontaneous leukemia rate from 10% to 0%. Treatment with Yel002 following IR accelerates the recovery of the hematopoietic system after sub-lethal exposures. Toxicity has not been observed in neither in vitro or in vivo administrations. Overall, Yel compounds have much potential as stockpile therapies for radiation-induced lethality and cancer: they are highly effective when administered up to 24 hours post exposure, they reduce radiation-induced sequelae such as leukemia, and appear to have an acceptable toxicity profile.

218 ISMTR-283 PROTECTS HUMAN BONE MARROW PROGENITORS FROM THE LETHAL EFFECTS OF IONIZING RADIATION.

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Bone marrow (BM), comprising stem and progenitor cells (CFC) as well as marrow stromal/ mesenchymal cells (CFU-F), is extremely susceptible to radiation damage. BM from matched donors is often used to “rescue” patients following medical radiation for leukemia. However, in the event of accidental/environmental radiation exposure, the ability to identify appropriate donors in a timely manner is rarely possible. We initially evaluated a small molecule compound (ISMTR-283) on human BM CFC. BM cells were treated with 0.1 – 0.4 μM ISMTR-283 for 2 hours and then subjected to 2 or 4 Gy ionizing radiation using a RS 2000 Biologic X-Ray Irradiator (Rad Source). Cells were mixed with ColonyGel™ methylcellulose-based media to support CFC growth and plated in 35 mm dishes (n=5), cultured for 14 days in a humidified incubator and then scored microscopically. At 0.4 μM, ISMTR-283 demonstrated a protective effect of CFC proliferation following 2 Gy. Results (mean +/- s.d): No irradiation: 32 +/- 4 CFC, 2 Gy: 3 +/- 1 CFC; ISMTR-283 + 2 Gy: 27 +/- 4 CFC. The study was extended to evaluate the effect of ISMTR-283 on CFU-F proliferation prior to and following radiation. BM cells were treated with 0.1 – 2 μM ISMTR-283 for 2 hours and then subjected to 2 or 4 Gy. Cells were cultured for 12 days, fixed, stained and scored macroscopically. Results: No irradiation: 170 +/- 9 CFU-F; 2 Gy: 109 +/- 3 CFU-F; 4 Gy: 49 +/- 5 CFU-F; ISMTR-283 + 2 Gy: 138 +/- 4 CFU-F; ISMTR-283 + 4 Gy: 72 +/- 4 CFU-F. Additionally, BM cells were subjected to 2 or 4 Gy and 24 hours later were treated with ISMTR-283. When ISMTR-283 was added 24 hours following 4 Gy, all concentrations demonstrated a protective effect on CFU-F proliferation. Results: 4 Gy + 16 +/- 2 CFU-F; ISMTR-283 + 4 Gy: 27 +/- 4 CFU-F. The data show that ISMTR-283 can protect BM stem and progenitor cells from the lethal effects of ionizing radiation.
Antibiotic overloading into the environment and the subsequent emergence and transfer of antibiotic resistance genes poses both ecosystem and human health risks. Traditional methods to quantify resistance genes in the environment rely on PCR-based approaches that target specific resistance genes and organisms. The rapid advancement of metagenomics in tandem with next generation sequencing technologies now allows for more high-throughput genomic approaches to environmental public health monitoring and can provide community-level profiling of environmental resistance. This study is profiling the prevalence and distribution of antibiotic resistance determinants in bacterial communities in Puget Sound, WA. Metagenomic DNA was collected at six sites in Puget Sound in addition to one wastewater treatment plant (WWTP) that discharges into the Sound and pyrosequenced. A total of approximately 1.4 million sequence reads (~550 Mbp) were obtained and assembled. Bioinformatic analysis of antibiotic resistance determinants was performed at the gene, protein motif and subsystem levels. Overall, the resistance profiles across the open Sound, nearshore and WWTP samples were similar. While known and previously cultured antibiotic resistance genes had a low prevalence in all samples, there was a significant difference in the number of resistance genes across the open Sound, nearshore and WWTP samples. The protein motif and subsystem analyses showed further evidence of differential resistance determinant prevalence across the three sample types. Taxonomic differences may be responsible for the variation in resistance profiles, for example increased Actinobacteria in the WWTP sample. Variability may also be a function of environmental or anthropogenic impacts. This environmental metagenomic approach allows for a more high-throughput survey of potential resistance determinants in natural environments that can be used to guide initial public health monitoring and more targeted and functionally-based investigations.

**220 EXPOSURE TO ARSENIC AND FLUORIDE IN CHIHUAHUA, MÉXICO.**

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Combined exposure to arsenic (As) and fluoride (F) are frequent because both these minerals are known groundwater contaminants from geological sources. This study focused on evaluation of the relationship between As and F in well-water and human urine collected in Chihuahua, Mexico, where As exposure has been associated with adverse health effects. Notably, Chihuahua residents exposed to high As concentrations are also exposed to high levels of F. Here, we analyzed associations between As and F levels in urine of 600 study subjects who drink well water. The concentration of As in water was determined by hydride generation (HG)-atomic fluorescence spectrometry and As species in urine were analyzed by HG-cryotrap-atomic absorption spectrometry. The concentrations of F in water and urine were measured by potentiometry, using a selective fluoride-ion electrode. The As concentrations in water and urine ranged from 0.1 to 419 μg/L and 5 to 426 μg/L, respectively. The concentrations of F ranged from 0.01-9.9 mg/L in water and from 0.06 to 18.08 mg/L in urine. A positive significant correlation between urinary As and water As (r=0.2000; p<0.0001), and between urinary F and water F (r=0.0878; p=0.035) were obtained. Moreover, a statistically significant positive correlation was found between As and F concentrations in well water (r=0.8212; p<0.0001) but lower relationship between sum of As species and F level in urine (r=0.1675, p=0.0001), suggesting that sources other than drinking water may contribute to As or F exposure in this population. Further examination will focus on associations between the exposures to As and F and disease (diabetes and skin lesion) prevalence in the Chihuahua group.

**221 BIOLOGICAL ACTIVITY AND THE SURGING OF BERGING GLACIER.**


The Bering Glacier field program typically starts in early June and runs through the end of August. The field camp is located near the base of the glacier on Vitus Lake, on a former terminal moraine adjacent to the Glacier Syntaxis. Since 2008 we have been using this field laboratory to investigate the cause and effect relationships of glacier dynamics on the local ecosystem. The specific objective of the 2011 voyage was to obtain real time data on Bering Glacier land cover, Bering Glacier terminus characterization; and Vitus Lake turbidity mapping. The specific task for our group was to determine the degree of movement and behavior of the glaciers at Vitus Lake since our last visit in 2008. We have been mapping terminus retreat using remote sensing images and we have determined that the terminus is retreating approximately 0.38 km per year. The data obtained from the 2011 voyage will be used to provide a more precise picture of surging in this region of the Bering Glacier.

**222 COMPARATIVE INHALATION TOXICITY OF ORGANIC NAPARTICLES AND LARGER PARTICLES OF THE SAME CHEMICAL IDENTITY.**

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Several anorganic nanoparticles (NP) caused higher inhalation toxicity than the corresponding coarse particles (Oberdoerster et al. 2005). We examined an organic pigment and a polymer dispersion each as nanomaterial and as the chemical identical coarse material in short-term inhalation studies in malers.

The polymer was an anionic acrylic ester polymer containing free carboxylic groups, the different particle sizes were synthesized by varying polymerization conditions: 12, 80 or 250 nm. Although polymeric acrylic ester was reported to be irritating to the respiratory tract at 3 mg/m3, all three tested polymers - including the NP (12 and 80 nm) - did not cause any changes in lavage fluid and in histopathology at 10 mg/m3. The organic pigment was a poorly soluble pyrrol with an intense orange color. The NP (10 to 50 nm width and 30 to 400 nm length) and the coarse pigments (70 to 200 nm width and 0.3 to 3 μm length) are both needle-like. They were tested at 1, 3, 10 and 30 mg/m3 for the NP and 3, 10 and 30 mg/m3 for the coarse materials. Mild and partly reversible morphological changes were observed in lung and lymph nodes at the highest concentrations, but the more pronounced effect was found in rats exposed to the coarse material. Likewise there was an increase of lavage parameters in rats exposed to thecoarse material but not to the NP. These data demonstrate that inhalation of finer NP is not necessarily associated with higher toxicity compared to the coarse material. The results were obtained with two organic particles of rather different size and composition but are in contrast to the more severe effects seen with several anorganic NP when compared to the corresponding coarse particles.

**223 HUMAN EXPOSURE TO ROADWAY TRAFFIC PARTICLES PRODUCES RESPIRATORY OXIDATIVE STRESS.**

H. M. Kipen1, 2, R. J. Laumbach1, 2, Z. Fan1, 2, P. Ohman-Strickland3, 4, K. Kelly-McNeil1, 2, S. Ko4, C. Cepeda4 and A. Gore41 Environmental & Occupational Health Sciences Institute (EOHSI), Piscataway, NJ, 2University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, NJ, 3University of Medicine and Dentistry of New Jersey-School of Public Health, Piscataway, NJ, and 4School of Pharmacy, Rutgers University, Piscataway, NJ.

Rationale: Epidemiologic studies show that cardiorespiratory events, including MI, occur within hours to minutes of roadway traffic-related air pollution (TRAP) increases. Induction of oxidative stress is a commonly invoked mechanism, but there is little direct human evidence of acute increases in oxidative stress following TRAP
exposure. We hypothesized that acute exposure to traffic particles increases nitrite and nitrate in exhaled breath condensate (EBC) and that the effect will be dependent on the level of reactive oxygen species (ROS) and oxidative potential (OP) in the inhaled particles. Methods: 20 healthy subjects volunteered for a fasting, 2-hr, double-blind, randomized cross-over protocol to ride in rush hour on the NJ Turnpike while breathing either unfiltered or HEPA-filtered vehicle cabin air (FA) delivered by a powered air-purifying respirator. Particle count and mass concentrations were measured inside and outside of the respirator. EBC was collected before, immediately after, and 6 and 24 hours after each ride. NO2-/NO3- increased significantly by 31% and 104% respectively, immediately after the ride (p=0.02 and p=0.035), with no persistence at 6 or 24 h post exposure. Ongoing analyses will determine the levels of ROS and OP in the TRAP.

The results were compared with FA, NO2- and NO3- increased significantly by 31% and 104% respectively, immediately after the ride (p=0.02 and p=0.035), with no persistence at 6 or 24h post exposure. Ongoing analyses will determine the levels of ROS and OP in the TRAP.

Cardiovascular diseases have been associated with situations in which enhanced activity of renin-angiotensin system (RAS) overrides cardioprotective effects of kallikrein-kinin system (KKS). The lung is the target organ of exposure to airborne particulates (PM), and it is where angiotensin is activated and bradikinin deac- vated, modulating homeostasis of the cardiovascular system. By using RT-PCR we examined changes in the induction of lung and heart RAS, angiotensin type 1 (AT1R) and 2 (AT2R) receptors, angiotensin converting enzyme (ACE), bradikinin type 1 (B1R) and 2 (B2R) receptors and kallikrein (KLK1), and how they may mediate expression of heart gene reprogramming markers like t-skeletal actin (Acta1) and procollagen-III (Col3a1). Male Sprague-Dawley rats were exposed acutely - 3 days and subchronically - 8 weeks to coarse (C), fine (F) and ultrafine (UF) particulates and filtered air, with an inertial particle separator (VACES). We observed increased expression of AT1R, AT2R and KLK1 in the lung after acute exposure to F, and AT1R, B2R and ACE to UE Subchronic exposures to F and UF increased the expression of AT1R without KKS changes. Acute exposure did not induce heart gene reprogramming, although induction of B1R after C, F and UF exposures, KLK1 after F and UF exposures, AT1R after F exposures, and ACE after C and UF exposures, were observed. After subchronic exposures, heart gene reprogramming was observed, expression of Col3a1 and Acta1, in F and UF with an up-regulation of AT1R and a down-regulation of AT2R with UE, and the induction of KLK1 with F exposures. Overexpression of RAS components accompanied by a reduced expression of KKS in the lung and heart after exposure to F and UF suggests heart gene reprogramming modulated by AT1R, thus its over-expression may be an important mechanism to explain some cardiovascular system adverse effects of PM exposure. More gentle washing using pressure differential. In this study, we collected the BAL fluid (BALF) using both injection/suction (Manual) and 2) differential pressure retrieval (Pressure), from cigarette smoke-exposed rats and mice. The goals were: 1) to standardize and assess if there is qualitative/quantitative difference between the two methods and 2) to evaluate the inflammatory parameters between rats and mice following subacute cigarette smoke exposure. Animals were exposed up to 1000 μg/L WTPM, varying daily exposure duration, for 5 wk (6-10 animals/group). A total of 6 was washing performed and for both methods, the 1st and (2nd to 6th) BALF were separately collected. The supernatant from the 1st wash was analyzed for LDH, GGT, and ALP while all 6 washes were used for total cell counts and differential. In control, the BALF parameters and their variability were overall comparable between the methods. The smoke-exposed animals had increased neutrophils and lymphocytes above the control, which tended to be retrieved slightly higher by the Manual method. Interestingly, the relative retrieval of different leukocytes between 1st and (2nd to 6th) wash varied between the methods. In smoke-exposed rats, all BALF enzymes increased in a dose-related manner, while only LDH increased in mice. While both methods under the employed condition were acceptable for BAL cell and enzyme parameters, a consistent use of a selected method is recommended to minimize variability.

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soluble particle extract. Longer carbon chain fatty acids are present in the B20 while shorter carbon chains are detectable in the B0 extract. Aldehydes were identified in the B0, B20 and B100 extract. The current data suggests B0 and B3 blends, and PD extracts induce alterations in endothelial cell antioxidant responses. [This is an abstract of a proposed presentation and may not necessarily reflect official US EPA Policy.]

A NOVEL HYPOXIA STRESS TEST DEMONSTRATES CARDIOVASCULAR AND PULMONARY SUSCEPTIBILITY TO ACREOLIN GAS IN HYPERTENSIVE RATS.


High levels of air pollution increase the risk of cardiovascular morbidity and mortality, especially in susceptible populations including those with hypertension. Stress tests are useful for manifesting latent effects of exposure, particularly at low concentrations, often when no overt effects are evident. The goal of this study was to develop a hypoxia stress test to illuminate susceptibility to environmental pollu-
tants in a rodent model of hypertension. We hypothesized that acrolein exposure would increase sensitivity to hypoxia, particularly in hypertensive rats. Acreolin is an unsaturated aldehyde found in diesel exhaust and is a known environmental toxicant and potent pulmonary irritant. Spontaneously hypertensive (SH) and Wistar Kyoto (WKY; rats with normal blood pressure) rats, implanted with biopotential icant and potent pulmonary irritant. Spontaneously hypertensive (SH) and Wistar of Kentucky, Lexington, KY and 2Pharmaceutical Sciences, Wayne State University, Detroit, MI.

Ozone (O3), and tobacco smoke (TS) are pulmonary irritants and known risk factors in the development of chronic lung disease. This study was performed to de-
terminate if sequential exposures of rats to O3 and TS will produce lung injury greater than that produced by either pollutant. Adult Sprague Dawley (SD) male rats were exposed for a single 3 hour period to 1) Air (control) 2) O3 or 3) O3 followed by TS (O3/TS). At 12 hrs post-exposure, bronchoalveolar lavage (BAL) was performed, and lung tissues were isolated. These samples from the controls and exposure groups were analyzed for lung injury, airway permeability and antioxidant activity. Data revealed a significant increase in total antioxidant capacity in the BAL in the O3 group compared to that in the control, showing a development of a pro-
tective mechanism for oxidative stress damage from O3. A subsequent exposure to TS attenuated the levels of total antioxidant capacity, but the reduction was not sta-
tistically significant in comparison to that after O3-alone exposure. A significant in-
sues were processed for light microscopic examination and morphometric analysis. RESULTS: ND and HFD rats exposed to O3 or O3/CAPs, but not control ND rats exposed to filtered air alone, had an acute rhinitis with marked hyperplasia of nasal epithelium (NE). HFD rats, but not ND rats, exposed to CAPs also had an acute rhinitis but no epithelial hyperplasia. The severity of the inflammatory cell re-
sponse (numeric density of neutrophils) was not different among the rats with rhinitis. Of all the air pollutant-exposed groups, the HFD rats coexposed to O3/CAPs had the greatest numeric cellular density of NE (54, 45, 12 and 12% greater than CAPs-alone HFD, air-exposed HFD, O3/CAPs-alone ND, and O3-ex-
posed HFD rats, respectively). Though we have previously reported that repeated O3 exposure will cause rhinitis with nasal epithelial hyperplasia, this is the first re-
port of a diet-related enhancement of air pollutant-induced nasal toxicity. Underlying mechanisms responsible for HFH enhancement of O3- and/or CAPs-
induced nasal epithelial or inflammatory lesions are yet to be determined. Funded by USEPA RD5479701.

EFFECTS OF HIGH-FRUCTOSE DIET ON NASAL EPITHELIAL AND INFLAMMATORY RESPONSES TO INHALED OZONE AND AMBIENT FINE PARTICLES IN RATS.

I. P. Hotchkiss1, K. Allen2, J. G. Wagner2, M. Morishita3, R. P. Lewandowski1, L. A. Bramble1, J. T. Dvonch3 and J. R. Harkema3. 1Pathobiology, Michigan State University, East Lansing, MI, 2School of Public Health, University of Michigan, Ann Arbor, MI and 3US EPA Great Lakes Air Health, University of Michigan, Ann Arbor, MI, and 3US EPA Great Lakes Air Policy.]

Ozone (O3), and tobacco smoke (TS) are pulmonary irritants and known risk factors in the development of chronic lung disease. This study was performed to de-
terminate if sequential exposures of rats to O3 and TS will produce lung injury greater than that produced by either pollutant. Adult Sprague Dawley (SD) male rats were exposed for a single 3 hour period to 1) Air (control) 2) O3 or 3) O3 fol-
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tective mechanism for oxidative stress damage from O3. A subsequent exposure to TS attenuated the levels of total antioxidant capacity, but the reduction was not sta-
tistically significant in comparison to that after O3-alone exposure. A significant in-

ONE VERSUS FIVE DAYS OF EXPOSURE TO VARYING CONCENTRATIONS OF B100 SOYA BIODIESEL EXHAUST REVEALS A THRESHOLD CONCENTRATION FOR INCREASED SENSITIVITY TO ACONITINE-INDUCED ARRHYTHMIA.


Although biodiesel (BD) is rapidly being considered as an alternative to diesel fuel, its health effects have not been thoroughly characterized. We previously used the aconitine challenge test to demonstrate that a single exposure to petroleum diesel exhaust (DE) increases the risk of arrhythmia being triggered in hypertensive rats. Here we hypothesized that inhalation of biodiesel exhaust (BDE) increases the risk of arrhythmia, particularly with repeated exposures. Spontaneously hypertensive rats surgically implanted with radioelemelems were exposed for one or five days to 50, 150 or 500 μg/m3 of B100 soya BDE or filtered air (FA) (4 hours). Arrhythmogenesis was assessed 24hrs after the last exposure in urethane-anesthetized animals by continuous intravenous infusion of aconitine while heart rate (HR) and electrocardiogram (ECG) were monitored. Rats exposed to BDE had lower HR when compared to air-exposed animals but no ECG changes when com-
pared to controls. Sensitivity to arrhythmia was measured as the threshold dose of aconitine required to produce ventricular premature beats (VPB), ventricular tachycardia (VT), and ventricular fibrillation (VF). There was no effect of 50 μg/m3 BDE. Rats exposed to five days of 150 μg/m3 BDE successively developed VPBs, VT, and VF at significantly lower doses of aconitine than FA, however a single 
gle exposure had no effect. Both one and five days of 500 μg/m3 BDE significantly, and comparably, increased sensitivity to aconitine-induced arrhythmia when com-
pared to FA. These findings suggest that below a certain concentration, exposure to BDE does not increase arrhythmogenic sensitivity. Furthermore, given the effects of 500 μg/m3 BDE were still lower than 150 μg/m3 DE, BDE might be a safer alter-
native, particularly if exhaust levels are kept below a certain threshold concentra-
tion. (This abstract does not reflect EPA policy.)
increase in BAL albumin in O3 group compared to that in the control was observed, and a subsequent non-significant reduction was similar to that in total antioxidant levels in the O3/TS group. Lung tissue protein analysis further showed a significant reduction of extracellular superoxide dismutase (EC-SOD) and catalase in the O3 and O3/TS groups compared to the control levels, but their expression was not significantly different between O3 and O3/TS groups. None of the exposures produced any effect on the expression of manganese-SOD or copper/zinc-SOD. Overall, the results show that the increase in total antioxidants after O3 exposure does not block airway injury/penetrability. Sequenical exposures to O3 and TS decrease some antioxidant enzymes, suggesting limited cross-tolerance following such exposures. The results do not support synergistic toxic effects predicted for O3 and TS. Supported in part by funding from OVP-WSU.

232 TRANSCRIPTOMIC ANALYSIS OF LUNGS AND PERIPHERAL BLOOD OF SILICA-EXPOSED RATS.


Non-invasive or minimally invasive surrogate approaches to detect/predict target organ toxicity have significant practical applications in occupational toxicology. Presently, using a rat model, we have investigated the potential application of peripheral blood transcriptomics as a practical approach to determine silica-induced pulmonary toxicity. Rats were exposed to crystalline silica (15 mg/m³, 6-hours/day, 5 days/week). Pulmonary toxicity and global gene expression profiles of lungs and peripheral blood were determined in the control and silica exposed rats 32-weeks post-exposure period. A significant elevation in bronchoalveolar lavage fluid LDH activity and a decrease in histopathological changes in the lungs, including tumor, fibroplasia, indicated silica-induced pulmonary toxicity in the rats. Similarly, significant infiltration of neutrophils and elevated MCP1 activity in the lungs suggested silica-induced pulmonary inflammation in the rats. Microarray analysis of global gene expression profiles identified significant differential expression (>1.5 fold change and FDR <0.001) of 520 and 537 genes, respectively, in the lungs and blood of the silica exposed rats. Bioinformatics analysis of the differentially expressed genes demonstrated significant similarity in the biological processes, molecular networks, and canonical pathways enriched by silica exposure in the lungs and blood of the rats. Several genes involved in functions relevant to silica-induced pulmonary toxicity such as inflammation, cancer, oxidative stress, fibrosis, etc., were found significantly differentially expressed in the lungs and blood of the silica-exposed rats. The results of this study, in addition to providing molecular insights into the mechanisms underlying silica-induced pulmonary toxicity, further confirm the potential application of peripheral blood gene expression profiling as a toxicologically relevant, minimally invasive surrogate approach to study silica-induced pulmonary toxicity.

233 EXPOSURES TO EMISSIONS FROM COMBUSTION OF BIODIESEL FUELS (B100/B20) ELICIT DIFFERENTIAL RESPONSES IN REDOX-SENSITIVE PATHWAYS.

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Exposure to airborne particulate matter (PM) is associated with higher risk for cardiopulmonary diseases but mechanisms for the effects remain unknown. Combustion of biodiesel fuels (BD) is associated with lower emission of PM but questions remain if BD combustion is consistently better for health. To examine the potential applications of complementary research methods, we conducted a study to test the hypothesis that BD combustion has a lower impact on redox-sensitive pathways. PM samples were collected from an experimental facility that fired pure BD versus the presence of petrodiesel in the B20 blend in producing the differential responses, both of which would affect particle size and composition. (This abstract does not represent US EPA policy).

234 BIOINFORMATICS OF GENE EXPRESSION PROFILING DATA PROVIDE MECHANISTIC UNDERSTANDING OF ACUTE OZONE-INDUCED LUNG INJURY.


Acute ozone-induced pulmonary injury and inflammation are well characterized. A few studies have used gene expression profiling to determine the types of changes induced by ozone; however the pathways involved are less well understood. We presume that robust bioinformatics of the transcriptional data will allow us to better understand mechanisms of pulmonary injury caused by acute ozone exposure. Male Wistar Kyoto rats (10-12 wk) were exposed to air, or ozone (0.25, 0.5 or 1.0 ppm) and pulmonary injury and inflammation were assessed 4 and 20 h later and lung gene expression profiling was assessed 4 h later (air and 1.0 ppm ozone). At 20 h BAL fluid protein and neutrophils increased at 1 ppm ozone. Numerous genes involved in acute inflammatory response were upregulated along with changes in genes regulating processes such as cell adhesion and migration, steroid metabolism, apoptosis, cell cycle control and cell growth. The NFKB (Rela), SP1 and TP3 transcription factors were identified to be mediating activation of genes for these processes. Upstream signaling involving ErbB, toll-like receptors, Wnt, TGF-β, NOD-like receptor and others were predicted based on downstream changes in expression. Because many of these genes regulating various inflammatory and repair processes are also involved in cancer, this pathway was also identified as being significantly changed by ozone. Remarkable changes in genes regulating endocytosis and glucocorticoid receptor activities (as evident by KEGG pathway analysis) provide the insight that ozone-induced injury might be initiated from cell membrane alterations and oxidatively modified lung lining lipids and proteins. Thus, gene expression profiling data could predict mechanisms of ozone-induced lung injury. Different cellular processes such as endocytosis, cell signaling, and transcription factor activation leading to cytokines release, cell migration, apoptosis, and growth could orchestrate the inflammatory and repair processes in the lung. (Does not reflect the US EPA policy).

235 METABOLIC CHANGES FROM HIGH-FRUCTOSE FEEDING ARE ALTERED BY AIR POLLUTANT EXPOSURE.

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People with diet-induced metabolic maladies may be more susceptible to the adverse effects of air pollution (AP). The present study evaluated the effect of AP on facets of diet-induced metabolic dysfunction. Mature male Sprague Dawley rats were fed normal chow (NC) or a high fructose diet (HF) for 8 weeks prior to and during AP exposure. Inhalation exposures to ozone (O3; 0.5 ppm) and/or concentration-ambient fine particles (CAPs, 400 μg/m³) were conducted in a mobile air re- search laboratory located in an industrial area of Dearborn, MI during summer 2011. Rats were exposed for 9 days, 8 h/day (4-5 days/week). Rats were sacrificed 24h after the end of exposure. Prior to sacrifice, blood was collected for homeostatic model assessment–insulin resistance (HOMA-IR) analysis. At necropsy, hepatic tissues were collected for light microscopy and gene expression analyses using qRT-PCR and whole-genome arrays. Through HF feeding did not induce obesity, HOMA-IR was increased concurrent with peripheral steatosis. HF-induced gene expression of stearoyl-Coenzyme A desaturase (Scd1) was potentiated after exposure to O3, CAPs and O3/CAPS (~21-fold) relative to filtered air/HF group (~14-fold). Scd1 expression was repressed by O3 (~3.4-fold) and O3/CAPS (~2.5-fold) in rats fed NC. Low density lipoprotein receptor (Ldlr) was also induced in HF-fed rats that were exposed to O3 and O3/CAPS (~1.6-fold) compared to filtered air-exposed NC and HF rats. In contrast, HF feeding caused a significant decrease in phosphoepinephrine pyruvate dehydrogenase 1 (Pck1), suggesting inhibition of gluconeogenesis. Furthermore, CAP’s exposure down-regulated Pck1 in NC-fed rats (~1.7-fold) and further suppressed Pck1 on a HF diet (~6-fold). Collectively, these data suggest that acute AP exposure may exacerbate facets of the metabolic syndrome. Funded by USAEPA R83479701.
SPD is a collectin important in limiting pulmonary inflammatory responses. SPD-/− mice develop progressive lung pathology culminating in emphysema. O3 inhalation (0.8 ppm, 3 hr) resulted in increases in lung MPs were observed in old SPD-/- but not WT mice. This correlated with structural alterations suggestive of chronic inflammation and emphysema. O3 inhalation (0.8 ppm, 3 hr) resulted in increases in lung MP levels in both young and old SPD-/- mice. Activated MPs have been implicated in pulmonary disease pathogenesis; however, their role depends on their phenotype. Two groups are classically activated cytoplasmic/proinflammatory iNOS+ MPs (CAM) and alternatively activated anti-inflammatory/profibrotic YM-1+ MPs (AAM). In old WT mice, O3 inhalation increased AAM after 72 hr. Increases in AAM were also observed in lungs of air-exposed young SPD-/- mice, while in old SPD-/- mice, both CAM and AAM were present. Whereas in young SP-D-/− mice, O3 caused increases in CAM, with no change AAM, in old SPD-/- mice, AAM increased and CAM decreased. We next determined if these changes were associated with alterations in lung function. Age-related increases in resistance (Rn) and decreases in tissue damping (G) were observed in WT and SPD-/- mice, indicating effects on airways and parenchyma. In young, but not old WT and SPD-/- mice, O3 inhalation altered central airway mechanics, as shown by increased responsiveness of Rn to recruitment, but not G. Additionally, alterations in tissue mechanics were observed in old SPD-/- but not WT mice after O3, suggesting that SPD is important in maintaining lung resistive properties. These results indicate that SPD regulates MP phenotype during inflammatory responses of the elderly to inhaled irritants; moreover this leads to increased lung functional alterations. NIH ES004738, GM034310, CA152624, AR059073, HL074115, ES005022.

**Enhancement of platelet activity and plasma homocysteine level by intratracheal instillation of carbon black.**

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Carbon black is an industrial chemical with the high potential of occupational exposure. Although the relationship between exposure to particulate matters and cardiovascular diseases is well established, the cardiovascular risk of carbon black has not been characterized clearly. Here, we designed and performed this study to investigate the cardiovascular effect of carbon black and to identify the target tissues and the potential biomarker. Carbon blacks with distinct particle size, N330 (ultrafine particle) and N990 (fine particle) were intratracheally instilled to rats. After 24 hrs and 1 week, we evaluated the thrombotic activities such as platelets aggregability and plasma coagulability, and analyzed the homocysteine levels in blood plasma, along with the cardiac functions and the inflammatory response. Exposure to N330 resulted in the acceleration of platelet-dependent blood clotting at 10 mg/kg, the highest exposure tested, while N990 exhibited minimal effect, suggesting that carbon black can enhance thrombotic risk. Unexpectedly, both carbon blacks led to the prolongation of activated partial thromboplastin time (aPTT), although it was not dose-dependent. N990, but not N330, significantly elevated the plasma homocysteine, a well established etiological factor for cardiovascular diseases. Although both of carbon blacks induced mild but apparent inflammation in lungs, it did not spill over into systemic inflammation, and none of them caused cardiac symptom detectable with electrocardiography. These results suggest that carbon black exposure may induce cardiovascular risk by the induction of hyperhomocysteinemia and the exaggeration of platelet activity. Homocysteine in blood plasma may be a potential biomarker for cardiovascular toxicity following carbon black exposure.

**L-menthol inhibits respiratory irritation by cigarette smoke irritants targeting diverse chemosensory receptors.**

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Stimulation of chemosensory nerves in the respiratory tract results in irritation responses which in humans can include the sensation of tickling or pain and the urge to cough. In mice stimulation of trigeminal chemosensory nerves results in reduced breathing frequencies due to extension of the duration of braking (DB) at the onset of each expiration. L-menthol, the cooling terpene in peppermint, has analgesic properties and may inhibit respiratory irritation in smokers of mentholated cigarettes. L-menthol activates the cold/menthol receptor, Transient Receptor Potential Melastatin 8 (TRPM8) and other sensory TRP ion channels, such as TRPA1 or TRPV1. Using female C57Bl/6j mouse as the experimental model, the DB response was measured during exposure to L-menthol (6, 12, 18 or 32 ppm) in combination with either 2 ppm acrolein or 1500 ppm cyclohexanone, two cigarette smoke irritants. Whereas acrolein is an agonist of the irritant receptor, TRPA1, fluorescent Ca2+ imaging experiments in cultured murine sensory neurons identified cyclohexanone as a specific agonist of TRPV1, the capsaicin receptor. L-menthol significantly reduced the irritation response to acrolein, with an EC50 of less than 6 ppm. This effect was diminished by AMTB, an antagonist of TRPM8. The inhibitory action of L-menthol on respiratory irritation was maintained in Trpa1-/- mice. L-menthol significantly reduced the irritation response to cyclohexanone, but the EC50 was approximately 20 ppm.

These results show that L-menthol inhibits respiratory irritation responses to smoke irritants targeting diverse chemosensory receptors (TRPA1 or TRPV1) expressed in different populations of sensory neurons. The cold/menthol receptor, TRPM8, is a crucial mediator of this inhibitory effect. The potency of counter irritation by menthol may depend on the nerve population being stimulated by the irritant. Supported by R01HL105635.

**Dry powder inhalation exposures of single rat lungs to fluticasone furoate: Control of dose, substance consumption and pharmacokinetics.**


Inhalation exposures of rats to respirable particles is an important part of air pollution studies and the development of new inhalation therapies. One problem with such studies is the availability of methods allowing controlled inhalation exposures to respirable particles using only small amounts of study material. The purpose of the current study was to evaluate the dosing precision, substance consumption, and resulting pharmacokinetics when using the Dustgun exposure platform to expose both the isolated, perfused rat lung (IPL) and endotracheally intubated rats (EIR). Using female C57Bl/6j mouse as the experimental model, the DB response was measured during exposure to L-menthol (6, 12, 18 or 32 ppm) in combination with either 2 ppm acrolein or 1500 ppm cyclohexanone, two cigarette smoke irritants. Whereas acrolein is an agonist of the irritant receptor, TRPA1, fluorescent Ca2+ imaging experiments in cultured murine sensory neurons identified cyclohexanone as a specific agonist of TRPV1, the capsaicin receptor. L-menthol significantly reduced the irritation response to acrolein, with an EC50 of less than 6 ppm. This effect was diminished by AMTB, an antagonist of TRPM8. The inhibitory action of L-menthol on respiratory irritation was maintained in Trpa1-/- mice. L-menthol significantly reduced the irritation response to cyclohexanone, but the EC50 was approximately 20 ppm.

The resulting PK of FF demonstrated a similar pulmonary absorption rate of this sparingly soluble steroid in the IPL and the EIR. Together were 18 lungs exposed and the total substance consumption was 55 mg, including determination of particle size distribution and preparative filter tests. Results indicated the usefulness of the IPL for predicting the pulmonary absorption of particulate solutes in rats in vivo, provided that similar sized aerosols and ventilation patterns were used during the exposures. Inhalation exposures of EIR to respirable dry powder aerosols can be performed as an alternative to intratracheal instillation of liquid-suspended materials using a similar animal treatment protocol.
Ozone (O3) is an air pollutant that is associated with cardiovascular and respiratory diseases. The aged population is considered to be more sensitive to pollutants such as O3; however, relatively few studies have demonstrated increased susceptibility in aged or senescent animal models. We employed a subchronic, intermittent O3 exposure protocol to study the susceptibility in young and aged Brown Norway (BN) rats. In one study, young adult (4 m) and aged (20 m) rats were exposed to 0 or 0.8 ppm O3 for 6 hr/d, 1 d/wk for 17 wks. Respiratory parameters assessed by unrestrained plethysmography the day after exposure revealed that O3 led to airway flow limitation (i.e. increased PenH). Young rats exhibited a greater rise in PenH over the 17 wk exposure. Bronchoalveolar fluid macrophages were reduced in aged rats and neutrophils increased in young rats. Age affected 20 of 58 serum analytes, including C-reactive protein, interferon, and macrophage inflammatory protein-2 (MIP-2). O3 affected 6 analytes, including fibroblast growth factor (basic), MIP-2, leukemia inhibitor factor, and haptoglobin. In another study, core temperature (Tc) and heart rate (HR) were monitored by telemetry in 8 and 20 m BN rats exposed for 6 hr/d, 2 consecutive d/wk to 0 or 1.0 ppm O3 for 12 wk. Aged rats were less responsive to aconitine-induced arrhythmia formation in a manner similar to 0.8 ppm O3, suggesting a latent O3-in-dependent autonomic modulation of cardiac function in rats. Rats implanted with a chronic indwelling cannula were exposed to O3 for a possible underrepresentation of chemical classes) a TTC value of 10 μg/kg for a possible underrepresentation of chemical classes) a TTC value of 10 μg/kg bw/d for maternal as well as developmental endpoints. These values are sufficient for a possible underrepresentation of chemical classes) a TTC value of 10 μg/kg bw/d for maternal as well as developmental endpoints. 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DEVELOPMENT OF A NEW NONCANCER TTC DATABASE FOR COSMETICS INGREDIENTS: A COLLABORATIVE PROJECT BETWEEN THE EC, COLIPA, AND ILSI EUROPE.

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The Seventh Amendment of the Cosmetics Directive foresees a deadline in 2013 for the replacement of animal testing of cosmetic products for repeated dose/reproductive toxicity and toxicokinetics. Currently, there are no accepted alternative methods for the regulatory assessment of repeated dose toxicity. As part of the research initiative to address the knowledge and technology gap, the European Commission (EC) and COLIPA jointly funded the COSMOS consortium.

The Threshold of Toxicological Concern (TTC) approach is being considered as one of the alternatives for the safety assessment of cosmetics ingredients. To improve the applicability of the TTC approach to cosmetics ingredients, COSMOS partners established two expert working groups in collaboration with ILSI Europe. The first expert group supports the development of a new noncancer TTC database for cosmetics ingredients, whereas the second group addresses the oral-to-dermal extrapolation. We report on a new noncancer TTC database (~650 cosmetics ingredients) compiled from various data sources. Also reported is the method to normalize the critical NOAELs from different study types. Cosmetics ingredients were defined as chemicals found in the EU COSING and US PCPC lists. About 90% of the non-cosmetics in the Munro dataset belonged to Cramer class III (high concern), whereas <50% of the cosmetics ingredients were in class III. Although preliminary, our results indicate that currently accepted TTC values may be adequately protective for cosmetics ingredients. The COSMOS noncancer database will comprise the single largest compilation of NOEL values in the public domain, and will serve as a sound basis for developing alternative methods for repeated dose toxicity. This abstract does not necessarily reflect the official position of US FDA, JRC, or EC.

COLIPA PROGRAM ON OPTIMIZATION AND VALIDATION OF HPLC-BIO-ANALYTICAL ASSAY TO EXPAND APPLICABILITY DOMAIN OF IN VITRO EYE IRRITATION RHT TEST METHODS FOR DYES.

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In vitro eye irritation assays using Human Reconstructed Tissue (RHT) test systems (SkinEthic™ HCE & EpiOcular™ EIT) are currently undergoing EVAM validation for detection of GHS irritants/non-irritants. In these test methods, irritation potential of a test chemical is determined by measuring cell viability in treated tissues by colorimetric MTT reduction assay. Cell viability is determined by enzymatic reduction of a yellow MTT tetrazolium salt to blue formazan. It is quantified photometrically with results expressed as % viability in the test chemical treated tissues relative to the negative control. Decrease in MTT reduction capacity is used to identify potential irritancy of test chemicals. A known limitation of the MTT test is possible interference of dye with absorbance measurement of formazan. The European Cosmetics Industry Association (Colipa) is conducting a program to address this limitation. The aims of the program are to: 1) optimize the preliminary analytical approach using High Performance Liquid Chromatography (HPLC) to quantitatively separate formazan from intrinsically coloured test chemical; 2) demonstrate application of the HPLC analytical method to evaluate dyes in RHT test methods; 3) demonstrate within and between laboratory reproducibility. Results presented describe validation using the FDA guideline for bioanalytical method and the multi-centric study (6 chemicals tested in 3 labs). This work has supported development of a Standard Operating Procedure that can be applied to extend the applicability domain of RHT test methods.

METAIBOILICS IN VIVO—APPLICATION FOR FORMING CHEMICAL CATEGORIES.

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BASF SE and metanomics GmbH have developed a metabolomics data base (MetaMap®Tox) containing more than 500 data rich chemicals, agrochemicals and drugs. This metabolome database is based on 28 day studies in rats with blood sampling and metabolic profiling after 7, 14 and 28 days of test substance treatment. More than 100 patterns (i.e., set of changed metabolic levels) have been established for different toxicological modes of action. With these patterns early detection of toxicological effects becomes possible and will help to accelerate development of new compounds with a better toxicological profile with a reduced number of animal studies. Thus this technology contributes to animal welfare by reduction through refinement. Following the requirements of the new European chemicals legislation REACH, a high number of animal studies has to be performed. On the other hand, REACH demands the conduct of animal experiments only when no alternative exists to fill a data gap. Amongst the most promising methods to reduce the number of animal experiments are “read-across” and “grouping”, i.e., forming of categories of compounds based on information justifying that these chemicals will have a similar toxicological profile. Structural similarity or QSAR models are often used to define such chemical groups, however, with known limitations. Omics technologies (e.g., metabolomics) can optimize the chemical grouping process by providing biological information for equivalence. In case of MetaMap®Tox, this is based on the recognition of modes of action through specific metabolome patterns and by comparing the entire metabolome of a chemical with those of the reference compounds available in the MetaMap®Tox database. Therewith, metabolomics provides a powerful tool for going from QSR to QBAR (quantitative biological activity relationship) through biology based grouping of chemicals.

PHARMACOKINETIC, TISSUE DISTRIBUTION, AND EXCRETION BALANCE OF PARABENS AFTER TOPICAL SKIN APPLICATION IN RATS.

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Parabens (PB) are preservatives widely used in topically applied drugs and personal care products. Our study compared the ADME profiles of radiolabelled methyl-, butyl- and propyl-PB following single oral, dermal or subcutaneous administration to Sprague-Dawley rats. PBs were administered in ethanol at a dose of 100 mg/kg. Blood total radioactivity Cmax and AUC were at least 10-fold higher when parabens were administered by the oral route when compared with those after other administration routes; Tmax was from 1 (oral gavage) to 8 hours (dermal application) after dosing. Methylparaben produced approximately 2x higher levels of radioactivity in all pharmacokinetic compartments when compared with other tested parabens. Whatever the administration route, no chromatographic peak of any paraben was detected in plasma samples; all radioactivity co-eluted as a single peak corresponding to that of para-hydroxybenzoic acid (PHBA). Following oral or subcutaneous administration, urinary excretion was predominant (>70 %, mainly during the first 24 hours), less than 4% were eliminated in the feces, and 2% were retained in the tissues and/or carcasses. Following dermal application, near to 50% of the dose was not absorbed, 14 to 27% and <2% were respectively excreted in the urine and feces, and <2% retained in the dissected tissues, whilst >20% of radioactivity was recovered in the remaining carcasses, likely located in the fur, paws or muzzle. These results suggest that parabens are partially absorbed through rat skin, extensively metabolized to PHBA, and rapidly excreted, mainly through the urine, with low retention in tissues.

REFINING ACUTE INHALATION TOXICITY TESTING: THE FIXED CONCENTRATION PROCEDURE.

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Acute toxicity testing is required under many regulatory frameworks. Acute studies are intended to support protection of human health, but are among the most criticized of regulatory toxicity studies for scientific and animal welfare reasons. A refinement method for acute inhalation toxicity has been proposed: the Fixed Concentration Procedure (FCP). The FCP avoids deaths as an endpoint by relying on ‘evident toxicity’, i.e. clear signs of toxicity such that it can be predicted that death or severe toxicity would occur if animals were exposed to a higher concentra-
tion. Testing at higher concentrations and the predicted severe effects can thus be avoided. Progress of the FCP through the OECD Test Guideline programme was suspended while work to address concerns raised by some member countries on three key points was initiated: The performance of the method in comparison with existing methods; the potential for sex differences in sensitivity to inhalation toxicity (as the FCP proposed testing females only); and, the perception that evident toxicity decision more transparent and consistent.

A mathematical objective system for recording clinical signs in acute inhalation studies has been developed to reduce the levels of certain tobacco leaf constituents.

The Globally Harmonized System of Classification and Labeling of Chemicals (GHS) represents an international approach to standardize the classification and communication of hazards for materials. One of the hazards addressed by GHS is serious eye damage and eye irritation. Institutional cleaning products, due to their surface-active chemistries, commonly have serious eye damage or irritation as a suspected health hazard. To support proper GHS classification, one approach is to conduct ocular testing. Current GHS criteria for classifying this endpoint is based primarily on interpreting in vivo data. However, due to animal welfare concerns, there is a shift in testing emphasis from in vivo to in vitro assays. Significant progress has been made in developing alternative ocular test methods. One such assay is the EpiOcular assay. EpiOcular is a corneal model consisting of human-derived epithelial keratinocytes cultured to form a stratified, squamous epithelium similar to the cornea. Two institutional cleaning products were evaluated using both in vivo and in vitro assays for eye irritation potential and subsequent GHS classification. For in vivo testing, the products were tested following OECD Test Guideline 405 and individual irritation scores were used to assign a GHS classification. EpiOcular served as the in vitro assay with tissue viability, cytokine release and histology evaluated. Tissue viability measured against time of exposure was used to determine the exposure time required to reduce viability to 50% of controls (FT50). The FT50, along with consideration of cytokine release and histology results, was used to assign a GHS classification. The in vivo data supported a GHS 2B classification (i.e., mild irritant) for both products. In contrast, the in vitro data for both products supported GHS 2A classifications (i.e., irritant). For this preliminary study, the in vitro assay provided a more conservative GHS classification for eye effects relative to in vivo testing; however, the limited number of test articles prevents drawing definitive conclusions.

As a person ages, the human body changes, and the efficiency of functions that ensure homeostasis is reduced. Altered function conditions (e.g., pharmacokinetics, pharmacodynamics) are physiologically and may have serious consequences on the capacity of detoxification and control of homeostasis, which reduces workers’ abilities to handle chemical stressors. Therefore, it is reasonable to expect that the impact on the ability of older workers to manage stress might be different in comparison to younger workers. The objective of this work was to analyze changes in physiological parameters that may influence the responses of older workers to chemical exposures from the perspectives of prevention, integration, and keeping them in their jobs regardless of their ages. For this project, we conducted a comprehensive literature review, using research tools such as MEDLINE, TOXNET, and other specialized medical books and reference volumes. We proposed grouping workers by stratum to compare different age groups (people aged between 18 and 70 years). This approach fills the gaps where there are sensitivities that reduce the body’s ability to handle chemical exposures. The results suggest that many physiological changes change with age. For example, the gastrointestinal tract transit decreases with age, thereby increasing the residence time of drugs and possibly the amount absorbed. This higher absorption can increase the interaction with a chemical product. A decrease in liver perfusion with age can increase the half-life of chemical reactivity; however, it does not seem that cytochrome P450 changes with age. A decrease in hepatic drug metabolism observed with age might be related to a reduction of the organ mass. Using this approach, the level of physiological and biochemical parameters are extracted, compiled, and compared. This research targets workers exposed to chemicals. Physicians and toxicologists in occupational health may use the results to identify constraints for aging workers with a perspective view for prevention.

Proteins and amino acids have been reported to be precursors for a number of potentially toxic constituents of tobacco smoke, including 2-aminonaphthalene, 4-aminobiphenyl and mutagenic heterocyclic amines. Therefore, a novel process has been developed to reduce the levels of proteins in cut tobacco. This process resulted in significant reductions in protein and total nitrogen (59% and 31% respectively) in tobacco leaf, which were associated with significant reductions (p<0.01) in tar normalised mainstream smoke yields of both nitrosamine containing and phenolic toxicants. In order to evaluate the biological effect of the treatment, test cigarettes containing up to a maximum of 80% of treated tobacco were compared against non-treated tobacco control product in a test battery of in vitro studies, and a 90-day subchronic nose-only rodent inhalation study. Cigarette smoke particular matter (PM) was tested in a Neutral Red uptake cytotoxicity assay, the Ames test, mouse lymphoma assay, and the micronucleus assay. Whilst toxicity was observed in all of these studies for all samples, there were no consistent qualitative differences between them. However, PM produced from cigarettes containing 80% treated tobacco demonstrated significantly lower mutagenic potencies (p<0.05) in Ames strain TA98 in the presence of S9.
In the inhalation study, the overall responses of the rats exposed to mainstream tobacco smoke from all cigarettes were found to be similar, although smoke from the test cigarettes showed reduced histopathology at one anatomical site (nasal 1) when compared to the control. In isolation, this limited change was considered minor rather than being indicative of a major biological effect.

The results of this test battery demonstrated that whilst the use of a tobacco containing reduced levels of proteins and polyphenols did reduce the genotoxicity associated with the presence of heterocyclic amines, it did not ameliorate any of the other biological endpoints (either in vitro or in vivo) investigated in these studies, when compared to an untreated tobacco control product.

253 A DECISION TREE APPROACH FOR THE SAFETY ASSESSMENT OF BOTANICAL COSMETIC INGREDIENTS.

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Natural and organic cosmetic and personal care products are becoming increasingly popular with consumers worldwide. Many of the new botanical ingredients being used in these topically applied products are novel to the industry and require a more rigorous safety evaluation. We present a decision tree approach to guide risk assessments for various botanical ingredients. A straight forward pathway can be followed for materials commonly ingested. The focus here is to first assure there is sufficient safety data to move to a cosmetic use and to then assess local tolerance, i.e., irritation potential, phototoxicity/phoroallergy, and Type IV sensitization risk. For materials found to closely resemble food or known herbal medicines the pathway to topical tolerance may be based on detailed characterization and should be supported by reliable data including in vitro, in vivo, in silico, or human data. However, other botanicals must first be characterized by: taxonomy, cultivar, content of macro and micronutrients, chemical markers, known naturally occurring toxicants, etc. Since extracts of botanical materials can differ based on extraction procedures, etc. they may require chemical fingerprinting or more detailed structural analysis to allow the use of ‘in silico’ tools such as FTYC, SAR, and metabolic profiling. For a chemical compound of known toxicity or for which a tailed structural analysis to allow the use of such tools; TTC, SAR, and in vitro testing may be required to complement in silico assessments and arrive at an adequate human safety assessment. A decision tree approach for botanical ingredient safety assessment is presented in this poster.

254 EVALUATION OF SUBACUTE BTAT TOXICITY IN RATS AND IN VITRO GENOTOXICITY.

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The sustainability of military training ranges is a necessary component of operational readiness. The development of new energetics for military use is ongoing with one of the objectives for a successful product being minimal or low negative environmental and human health effects. The elimination of compounds that are appreciably harmful to the environment and/or human health can provide significant economic savings associated with potential future clean-up and health costs. BTAT (Bis(2,2,2-trinitroethyl)-3,6-diaminotetrazine) is under consideration as a new energetic replacement for RDX. The AIPH initial health assessment investigations included an in vitro mutagenicity assay and an oral acute/subacute 14-day bioassay in rats. In a modified Ames microplate assay (Salmonella typhimurium test strains TA98, 100, 1535, 1537, E. coli test strains pKM101 and uvrA), BTAT was found to be not mutagenic in all strains (with S-9 activation) at concentrations below its water solubility limit of 100 μg BTAT/mL. Without S-9 activation, BTAT was mutagenic at ≥9 μg BTAT/mL to test strain TA100. In female rats, the acute oral LD₅₀ for BTAT was ≥2000 mg/kg body weight. Male rats were used in the subacute 14-day study. The subacute LC₅₀ was ≥1000 mg/kg/day. Statistically significant findings included effects on several blood chemistry and hematology parameters in dose groups ≥293 mg BTAT/kg/day, increased spleen weights at 1000 mg BTAT/kg-day, decreased kidney and liver weights and decreased food consumption at 1000 mg BTAT/kg-day, and decreased thymus weights and body weights at 500 and 1000 mg BTAT/kg-day. In summary, except for test strain TA100, BTAT was not mutagenic in the Ames assay. Acute BTAT toxicity was found to be low (LD₅₀ >2g/kg) and subacute toxicity was consistent with anemia and methemoglobinemia.

255 MUTAGENICITY AND SUBACUTE TOXICITY TO FEMALE RATS OF GUANIDINIUM 3, 4-DINITROPYRAZOLO (GDNP)*.


Sustainable use of training ranges requires the development of compounds that have a minimal impact to the environment when used in a weapon system. A new energetic, GDNP, has been developed that may have efficacy in specific systems. To ensure the health of potentially exposed personnel and the environment, initial toxicity investigations were conducted and the results compared with another widely used energetic (hexahydro-1,3,5-trinitro-1,3,5-triazine; RDX). In a microplate Ames assay, GDNP is not cytotoxic to Ames bacteria at concentrations less than 100 μg/mL. However, GDNP is mutagenic to 4 out of 5 bacterial strains, both with and without 39 metabolic incubation, at concentrations as low as 0.8 μg/mL. The mutagenicity of GDNP is likely due to the 3,4-dinitropyrazole component. Unlike RDX, GDNP did not have an affinity for the GABA receptor convulsant site, and was predicted to not induce seizure. After oral acute dosing in female rats, GDNP had toxicity similar to that reported for guanidine hydrochloride, i.e., at concentrations above 750 mg/kg. GDNP induced progressive sedation culminating in bradycardia, reduced lial histopathology (ALD) for both sexes was 5000 mg/kg. A 14-day toxicity study in rats was conducted with 4A2NT in the feed at concentrations of 0, 125, 250, 500, 1000, and 2000 ppm. The 2000 ppm concentration was selected as highest concentration for subsequent 90-day study. An oral 90-day subchronic toxicity study in rats was conducted with concentrations of 0, 500, 1000 and 2000 ppm of 4A2NT in the feed. The calculated consumed doses of 4A2NT in the feed were 0, 27, 52 and 115 mg/kg-day for males and 0, 32, 65, and 138 mg/kg-day for females. A no observed adverse effect levels (NOAEL) was not determined. The low observed adverse effect levels (LOAEL) was 27 mg/kg/day for males and 32 mg/kg/day for female rats based upon adverse histopathology of the liver (cytoplasmic vacuoles) and body weight decreases. Testicular atrophy (hypospermatia) was also observed in male rats at dose of 52 and 115 mg/kg. The toxicity data developed can be used to develop oral RD kinetics that can be useful for human health risk assessment.

256 TOXICITY ASSESSMENT OF 4-AMINO-2-NITROTOLUENE.


2, 4-Dinitrotoluene (2,4-DNT) is used in the manufacture of dyes, in munitions and in military explosive composition. It degrades into a number of nitro reductive products, including 4-amino-2-nitrotoluene (4A2NT) (CAS:119-32-4) through in vivo metabolism and in vitro bacterial systems. 4A2NT is also an environmental degradation product of 2, 4-DNT and is detected in soil and ground water at some ammunition plant manufacturing and disposal facilities. The toxicity data on 4A2NT is limited. Therefore, we developed acute, sub acute and subchronic toxicity data in rats to assess environmental and human health effects from exposures. The intraperitoneal lethal dose (LD₅₀) for both sexes was 5000 mg/kg. A 14-day toxicity study in rats was conducted with 4A2NT in the feed at concentrations of 0, 125, 250, 500, 1000, and 2000 ppm. The 2000 ppm concentration was selected as highest concentration for subsequent 90-day study. An oral 90-day subchronic toxicity study in rats was conducted with concentrations of 0, 500, 1000 and 2000 ppm of 4A2NT in the feed. The calculated consumed doses of 4A2NT in the feed were 0, 27, 52 and 115 mg/kg-day for males and 0, 32, 65, and 138 mg/kg-day for females. A no observed adverse effect levels (NOAEL) was not determined. The low observed adverse effect levels (LOAEL) was 27 mg/kg/day for males and 32 mg/kg/day for female rats based upon adverse histopathology of the liver (cytoplasmic vacuoles) and body weight decreases. Testicular atrophy (hypospermatia) was also observed in male rats at dose of 52 and 115 mg/kg. The toxicity data developed can be used to develop oral RD kinetics that can be useful for human health risk assessment.

257 ACUTE TOXICITY STUDY OF LIPOSOMAL ANTIOXIDANT FORMULATIONS CONTAINING N-ACETYLSCYSTEINE, α-TOCOPHEROL, AND γ-TOCOpherol IN RATS.

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The acute toxicity of a single dose of intravenously administered liposomal antioxidant formulation containing N-acetylcysteine (NAC) without or with tocopherol (α-T) or γ-tocopherol (γ-T) in rats was examined. Each group consisted of 5 male and 5 female Sprague-Dawley rats with a control group receiving empty DPPC liposomes (660 mg/kg DPPC, EL), and test groups receiving liposomes prepared from DPPC lipids with δ NAC (200 mg/kg NAC [L-NAC]), δ NAC and α-T (200 mg/kg NAC [L-NAC] and 83.3 mg/kg α-T [L-α-T]), δ NAC and γ-T (200 mg/kg NAC [L-NAC] and 71.4 mg/kg γ-T [L-γ-T]). These dose levels were determined from dose-range finding study and considered to be the Maximum Feasible Dose levels,
based on the volume of 10 mL/kg and physical properties/viscosity of the test articles that could be safely administered to rats by an I.V. injection. Two weeks after treatment (Day 15), all animals were sacrificed and submitted for gross necropsy, clinical pathology investigations (haematology, coagulation, clinical chemistry and urinalysis) with a comprehensive range of organs submitted for histopathological evaluations. The rats in the control group (EL) and three test groups exhibited no clinical signs of toxicity during the dosing period or during the 14-day post-treatment period. Weight gain and food consumption in all animals was appropriate for the age and sex of animals. Clinical pathology findings were unremarkable in all rats and in all groups. However, WBC counts in male rats treated with L-tyr-NAC formulation were below the lower end of the normal range although gross necropsy and histopathological observations were not of any toxicological significance. In conclusion, the results of this study showed no drug/treatment related toxicity in rats at the maximum feasible dose level by a single bolus intravenous administration. This work was funded by a grant (W81XWH-06-2-0044) from the Department of Defence, USA.

258 PLATELET COUNTING IN ANIMALS—BACK TO BASICS.


Rationale and scope: Platelet clumping is an important and common problem in laboratory animal species, particularly in Mini-pigs. In addition to reducing platelet counts, clumping may result in inaccurate gating of erythrocytes and leukocytes, pseudoleukocytosis, or trapping of leukocytes and erythrocytes. Our objective was to investigate the effects of pre-analytical variables on platelet counts, and the relevance of platelet clump flags generated by an automated hematology analyzer. Experimental procedures: In a study of Mini-pigs, blood was collected into tubes containing EDTA or CTAD and stored at -80°C. Three different experimental conditions were investigated. The Advia 120 analyzer was used to measure the platelet count (PLT) and the ‘Clump count’ (CC). May Grunwald/Giemsstained blood films were used to assess platelet morphology. EDTA samples were also investigated in dog, sheep and rabbit. Summary of results: In Mini-pigs the use of CTAD anticoagulated tubes resulted in higher PLT and lower CC when compared to EDTA. For EDTA and CTAD, storage at 4°C reduced the PLT by 10%, but had no effect on the CC; mixing (inversion or rotary mixer) had no effect on PLT or CC. The PLT was stable for 4 hours at room temperature. Blood smear examination confirmed the presence of platelet aggregates when the CC was elevated. Conclusions: In Mini-pigs, the use of CTAD as an anticoagulant results in fewer CC flags with lower CC and higher PLT than EDTA. In our hands, the CC flag was a useful alert regarding clumped platelets.

259 FLUOROALKYL METHACRYLATE COPOLYMER AQUEOUS DISPERSION: SUBCHRONIC TOXICITY EVALUATION IN RODENTS.

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A fluoralkyl methacrylate copolymer aqueous dispersion comprised of a short-chain methacrylate monomer, 2-chloroethyl methacrylate, a medium-chain methacrylate monomer F(CF2)6(CH2CH2OC(CH3))=CH2, was evaluated in one-gen reproto and repeated-dose toxicity studies. Four groups of young adult male and female Crl:CD(SD) rats were daily administered test substance by oral gavage that contained 0, 100, 500 or 1000 mg/kg/day of test substance for approximately 90 days. No primary test substance-related effects on mortality, clinical signs, neurobehavioral assessment, body weight, nutritional parameters or clinical pathology were observed at any dose. Treatment related adverse nasal lesions consistent with regurgitation rhinitis were observed in males and females at ≥100 mg/kg/day. Clearance of test substance and complete resolution of the associated inflammatory response did not occur following a 1-month or 3-month recovery period. Since posterior nasal regions were most affected, with foreign material present within the inflammatory exudates, reflux and retrograde aspiration of irritant material (possibly stomach contents with test formulation) into the nasal cavity were suspected. Therefore, nasal lesions are likely secondary to gavage reflux rather than primary target organ toxicity or gavage error. The underlying cause of the apparent reflux is uncertain. Nasal lesions similar to the repeat dose study were also observed in a one-generation reproductive study where P1 rats were administered test substance (25/sex/group) daily at 0, 20, 100, 500, or 1000 mg/kg/day for at least 109 days. In the one generation reproductive study, the NOAEL based on nasal lesions was 20 mg/kg/day in males and 100 mg/kg/day in females. No effects on reproductive endpoints at any dose were observed at any dose. The test substance is not a selective reproductive toxicant. Therefore, taking both studies together, the NOAEL for rats based on these type of nasal lesions was > 20 and < 100 mg/kg/day.

260 SUBCHRONIC INHALATION EXPOSURE OF RATS TO LIBBY AMPHIBOLE AND AMOSITE ASBESTOS.


Exposure to Libby amphibole (LA) is associated with significant increases in asbestos, lung cancer, and mesothelioma. To support biological potency assessment and dosimetry model development, a subchronic nose-only inhalation exposure study (6 h/d, 5/ wk, 13 wk) was conducted in male F344 rats. Rats were exposed to air (control), LA (LO, MED, HI; 1.01, 3.33, 10.08 mg/m3 gravimetric concentration: 43, 171, 280 particles/cc by APS, respectively), or amosite (AM; 3.35 mg/m3; 404 particles/cc). No clinical findings were observed during the exposures though average body weights were 4% lower than controls in the AM group for weeks 2-14. One day after final exposure, significant increases in BAL LDH (2-2.5x) and total protein (1.5-1.7x) were found in MED and HI LA groups, but no significant changes were found in the AM group at the same mass exposure as MED LA. BAL ALP and NAG concentrations were similar in all exposure groups including the control. Changes observed in BAL cells were significant decreases in percentages of macrophages (59% vs. 90%) and increases in percentages of lymphocytes (1.4x) and neutrophils (4x) in HI LA vs. control. Histopathological examination of the lung found a minimal increase in alveolus inflammation, interstitial fibrosis, bronchiolization, and foreign body presence in all rats (8/8) in LO LA, MED LA, and AM groups compared to controls. Alveolus inflammation was more severe (6/8) in HI LA rats. The HI LA group showed increasing bronchiolar epithelial hyperplasia (8/8 were minimal grade). Other groups of rats were necropsied 1 and 3 months post-exposure (also 8/group), with the final necropsy planned for 18 months post-exposure (50/group). Tissue fiber burdens are being determined to support dosimetry model development. Results show comparable inflammatory and fibrogenic responses 1 day after subchronic exposure of rats to LA and AM asbestos. (This abstract does not represent US EPA policy.)

261 UTILIZING A COMPREHENSIVE APPROACH FOR SAFETY ASSESSMENT OF COSMETIC INGREDIENTS: A CASE STUDY.

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Safety assessment of cosmetic ingredients is a critical part of cosmetic product safety review. Conventional safety assessment largely relies on toxicological data from animal testing. Due to recent European Union regulations and public support to stop testing cosmetic ingredients on animals, toxicologists face challenges of conducting safety assessment of new chemicals without employing animal testing. There are only a limited number of validated in vitro methods are available for risk assessment. In particular, lack of animal subchronic/chronic data prevents toxicologists from conducting the traditional Margin-of-Safety (MoS) based risk assessment. To address the challenges, we present here a comprehensive approach that combines investigative data with predictive toxicology to conduct risk assessment on a model chemical, diglycerin. Data on acute toxicity, skin and eye irritation, skin sensitization, and mutagenicity were obtained from investigatory studies. To fill the data gap of subchronic/chronic toxicity, an in silico approach was applied. The chemical was screened by DEREK and OECD QSAR Toolbox for structural alerts, and by US EPA T.E.S.T. and Acetelon's OSIRIS Property Explorer for potential reproto/developmental toxicity. In addition, three structurally similar analogues were identified by using the OECD QSAR Toolbox and US EPA AIM programs. The analogues’ subchronic/chronic and reproductive/developmental toxicity data were reviewed. The lowest no observed adverse effect level (NOAEL) among these studies was conservatively used as the point of departure in risk assessment. Exposure assessment was conducted to obtain systemic exposure dose by taking the actual skin penetration into account. Based on the calculation of MoS and the application of an additional uncertainty factor, we concluded that diglycerin is safe to use for the target product types and use levels. The framework proposed in this report represents a feasible approach to fulfill safety assessment in the absence of relevant systemic toxicological data.

262 EVALUATION OF STRAIGHT CHAIN UNSATURATED ALDEHYDES IN A LOCAL LYMPH NODE ASSAY.

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Local lymph node assays (LLNA) were conducted on hexen-2-al, 2-octenal, trans-2-nonenal, and 2-tridecenal to determine their potential to induce dermal sensitization. These materials are all straight chain unsaturated aldehydes with a variety of
uses which include application in fragrances. The materials tested have limited sen-
sitation data available and are all expected to have similar chemical reactivity; the
only difference between the materials is their carbon chain length (6, 8, 9, and 13
carbons, respectively). The variation in chain length can affect the potential for sen-
sitation as shorter carbon chains would be expected to have an increased volatility,
decreasing the amount of material available for absorption under non-occlusive
conditions. Each material was tested according to OECD guideline 429 in female
CBA/CA mice (5/group) at 5 concentrations ranging from 0.5 – 50% w/v in 1:3
ethanol/diethyl phthalate using on the standard LLNA dosing regimen. The auricu-
lary lymph nodes were excised and pooled for each experimental group and incorpo-
ration of 3HThdR was measured by β-scintillation counting and expressed as de-
niturations per minute (dpm) per lymph node for each experimental group. The
stimulation index (SI) values were calculated for each dose level, with values ≥3
considered a positive response. The estimated concentration (EC3) required to el-
icit a positive response was calculated and taken as a measure of relative potency.
The EC3 values for hexen-2-al, 2-octenal, trans-2-nonenal, and 2-tridecenal were
calculated to be 2.6%, 5.6%, 2.1%, and 3.8%, respectively. Based on these values,
chain length does not seem to have an effect on the LLNA results, as all the materi-
als tested were found to be weak sensitisers with very similar EC3 values.

263 ESTIMATION OF THE CONSUMER INHALATION RISK OF WATERPROOFING SPRAYS.
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Toxicology and Experimental Medicine, Hannover, Germany.

Waterproofing sprays are widely used consumer products containing for example
fluorinated polymers or silicon based compounds dissolved in alcohols or volatile
petroleum distillates. There have been repeated reports on cases of severe acute res-
piratory disorders following inhalation exposure when using these products for the
first time. It is hypothesized that impairment of the pulmonary surfactant by deposition of in-
haled volatile particles of the active compound is one of the main causes of the
acute lung injury. Since the inhalation toxicity cannot be predicted a priori based on
the physical and chemical properties of the formulation, proper test strategies
are required to ensure consumer safety.

We propose to combine screening tests addressing both, exposure and acute lung
toxicity. The exposure potential of the spray product is characterized by determin-
ing the release fraction of the active compound in the respirable particle size range
under conditions relevant for the product application. This is carried out by spray-
ing defined quantities of the product into a control volume and measuring the con-
centrations of health related size fractions. This procedure takes into account spray
ageing, especially size reduction of the droplets due to solvent evaporation.
The isolated perfused lung is used as a model for testing acute toxicity. Ventilated
rat lungs are exposed to aged aerosols with proper particle size of approximately 1
μm MMAD generated from the liquid spray formulation. Lung compliance and
lung resistance are continuously monitored during exposure. Dose dependent devi-
ations from the normal values (without exposure) are used as read-out parameters.
Using the combined procedure, different sprays could be ranked according to their
realistic exposure risk and, most importantly, sprays with known lung toxicity could
be uniquely distinguished from those that have been shown to be safe. In its current
stage of development the simple test method is recommended for screening of sub-
stances only.

264 TOXICITY ASSESSMENT OF THE INSECTICIDAL PROTEIN Vip3Aa20, PRESENT IN GENETICALLY
MODIFIED MIR162 MAIZE PLANTS.
Syngenta Crop Protection, LLC, Greensboro, NC.

The Bacillus thuringiensis-derived Vip3Aa20 protein has insecticidal activity against several lepidopteran species and is expressed by Event MIR162 genetically
modified maize for the biological control of agricultural pests. As part of an overall
safety assessment, bioinformatics homology searches for amino acid sequence simi-
larities between Vip3Aa20 and known toxins, in vitro digestibility studies, and in
vivo toxicity studies were conducted. There was no significant sequence similarity
between Vip3Aa20 and any proteins known or putatively known to be toxic to
mammals. Vip3Aa20 was readily digested after incubation in simulated gastric fluid
containing pepsin, as assessed by SDS-PAGE and Western blot analyses. Vip3Aa20
was administered as a single oral dose via gavage to groups of 5 male and 5 female
mice at 0 or 1250 mg /kg body weight (BW) to assess the toxic potential of
Vip3Aa20. Animals were observed for 14 days following dosing. There were no
deaths or treatment-related effects on body weight, food consumption, clinical ob-
servations, clinical pathology, select organ weights, gross necropsy findings or
histopathological findings. Therefore the NOAEL for this acute oral toxicity study
was > 1250 mg/kg BW. Additionally, this protein was tested for potential toxicity
when administered repeatedly for a period of 28 days. Vip3Aa20 was administered
via gavage to female rats at dosages of 0, 5, 50, and 500 mg/kg BW per day. An additional group was administered bovine serum albumin to con-
roll for any effects of dosing a high concentration of non-toxic protein at levels
matched to the highest dose level of Vip3Aa20. There were no deaths or treatment-
related effects on body weight, food consumption, clinical observations, hematol-
omy, clinical chemistry, select organ weights, or gross necropsy findings. These data
support the conclusion that Vip3Aa20 is nontoxic to mammals and that Event
MIR162 maize is safe for use as food or feed.

265 TOXICOLOGY ASSESSMENT OF ORTHO-
PHTHALALDEHYDE (OPA) FOLLOWING 90 DAYS OF
INHALATION EXPOSURE IN MICE AND RATS.
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Bau1, G. S. Travlos1, D. R. Gerrnolds1 and M. E. Wyde2. 1Division of National
Toxicology Program, NIEHS, NIH, Research Triangle Park, NC. ; 2Batelle Toxicology
Northwest, Richland, WA and 3Integrated Laboratory Systems Inc. , Research Triangle
Park, NC.

Ortho-phthalaldehyde (OPA) is a chemical sterilizer used on heat-sensitive medical
devices and surgical instruments. Despite the rising popularity of OPA as a poten-
tial alternative to glutaraldehyde, very little is known about its toxicity profile.
Studies at NIOSH indicate that OPA concentrations in air samples from hospital
sterilizing units range from 0.001 to 0.0135 ppm. Occupational exposure to the
marketed concentrate of OPA (5.75%) is also not uncommon among healthcare
workers. The objective of this study was to characterize the potential toxicity of
OPA following inhalation exposure. B6C3F1 mice and Harlan Sprague-Dawley
rats (of both sexes) were exposed to 0.44, 0.88, 1.75, 3.5 and 7.0 ppm of OPA for 6 hours/ day, 5 days/ week for 90 days. Endpoints evaluated included body and organ weights, hematological assessment, and
histopathology. Following 90 days of treatment, there were dose-related decreases in
body weight and decreased survival in the mice and rats exposed to ≥ 3.5 ppm
OPA. Relative lung weights were significantly increased in both mice and rats ex-
posed to ≥ 1.75 ppm OPA. The spectrum of lesions noted following OPA exposure
included inflammation and necrosis of the cornea, nose, larynx, trachea and
bronchus. A NOAEL for the nose in either sex/species was not identified. Additional lesions included inflammation and epidermal hyperplasia in the skin.
Hematological analyses in mice exposed to 1.75 ppm OPA indicated de-
creased red cell mass suggesting a suppression of erythropoiesis. These studies
demonstrate that subchronic exposure of mice and rats to OPA leads to inflamma-
ition of the respiratory system with the most severe effects being in the upper respi-
ratory tract. These are preliminary results that have not yet been peer-reviewed by
the NTP.

266 6:2 FLUOROTOLEUMER ALCOHOL: FOUR WEEK
INHALATION TOXICITY STUDY.
T. L. Serex, C. Morris, R. C. Buck, S. E. Loveless and M. P. Delorme. DuPont
Haskell Laboratories, Newark, DE.

6:2 Fluorotolem Alcohol (CAS# 647-42-7, 1-Octanol-3,4,5,6,7,8,8,8
tracecycliclururo,-6.2 FTOH) is a raw material used for manufacturing surfactant and
polymeric surface protection products. Inhalation of the 6.2 FTOH vapor phase is
a potential route of human exposure for toxicity determination. In this 4-wk study,
four groups male and female Crl:CD(SD) rats were exposed whole body 6 hr/day,
5 days week to 0 ≥ 0, 1.0 ± 0.0087, 10. ± 0.057 or 100 ± 0.02 ppm 6.2 FTOH for
a total of 22 exposures (mean ± SD). Exposure to 6.2 FTOH demonstrated no
adverse effects on body weight, body weight gains, food consumption or food effi-
ciency. No test substance-related or adverse clinical signs of toxicity were observed
on the course of this study. Male and female rats exposed to 100 ppm 6:2 FTOH
demonstrated increased mean serum bilirubin levels when compared to the control
group and several females at this exposure level exhibited increased alanine amino-
transferase (ALT). All animals exposed to 100 ppm 6.2 FTOH demonstrated in-
creased mean absolute liver weights as well as increased mean liver weight relative
to body and brain weight. Male rats exposed to 100 ppm 6:2 FTOH also demon-
strated decreased motor activity during the 4th week of exposure. Changes in
bilirubin, ALT, liver weights and motoractivity in animals exposed to 100 ppm re-
solved following a 4-week recovery period. Microscopic findings were limited to the
incisor teeth of male and female rats exposed to 100 ppm 6.2 FTOH observed as
basophilic striations within the inner dentin. This change is a common finding in
rats following exposure to fluorescence-containing compounds, was not associated with
changes in odontogenic epithelium and was considered non-adverse. Therefore,
derunder the conditions of this study, the NOAEL for 6.2 FTOH is 10 ppm. These
findings correlate well with the great weight effects observed in oral studies with 6:2
FTOH and indicate a lack of a route of entry effect. Therefore, route-to-route ex-
trapolation for systemic effects is appropriate for risk assessment of this material.

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Exciptents are pharmaceutically inactive compounds in drug products that serve different functions, such as, diluent, binder, coating agent. Various excipients are used based on the medication form and route of administration. Polyoxyethylene sorbitan fatty acid esters (polysorbates) are widely used in cosmetics, food products, oral, parenteral, and topical pharmaceutical formulations. Polysorbate 80 is a commonly used excipient in parenteral formulations as an emulsifier, solubilizer, and to stabilize aqueous formulations of medications. Formulations intended for intravenous administration are typically qualified for use using an in vitro hemolysis assessment. The hemolytic potential of polysorbate 80 concentrations in routinely used vehicles was assessed with rat and human blood. Briefly, mixtures containing blood substrate (blood diluted to give an absorbance value of 0.8 - 1.2 upon complete hemolysis) and each vehicle were diluted and incubated under static conditions for 30 minutes at 37°C. A hemolytic index for each vehicle formulation was determined by comparing the optical density determined spectrophotometrically in the test article samples to a scale between 0 and 100% hemolysis. Polysorbate 80 has been used at 0.1% in intravenous formulations for humans. The hemolysis assay performed with vehicles containing 0.1% polysorbate 80 caused severe hemolysis of rat blood. Subsequent titration experiments showed that the vehicle mixtures containing less than 0.006% polysorbate 80 were non-hemolytic to rat blood. Also, addition of rat plasma to the assay mix with 0.1% polysorbate 80 vehicle mixtures reduced the hemolytic potential suggesting partial binding to polysorbate 80 may limit the hemolytic potential. In contrast, vehicles containing polysorbate 80 failed to induce lysis of red blood cells obtained from human donors at concentrations up to 2.5% polysorbate 80. It will be useful to understand the hemolytic potential of polysorbate 80, a commonly used intravenous excipient, in other animal species to determine limits for use in preclinical studies.
in contrast to publically available assessments that suggest preservatives are prima-
arily used in adult-intended products. Results of animal studies conducted to deter-
mine the developmental effects of parabens suggest that this group of substances
may have developmental and reproductive effects when used in sensitive popu-
lations. Further studies suggested that parabens may also cause sensitization reac-
tions. Results of toxicology studies conducted using the preservatives MCI and/or MI
suggest that they may be acutely toxic via the oral and dermal routes of expos-
ure. Using the results of these studies and exposure scenarios developed by our
group, we analyzed the margins of safety for use of these preservatives in children’s
products. Our analysis suggests that these preservatives are often present in child-
intended products at levels that exceed their safe level for use. The results of our
studies suggest that several preservatives found in child-intended consumer prod-
ucts have the potential to be a safety concern following foreseeable (mis)use and
emphasize the need for realistic exposure considerations for these types of products.

272 GENETIC TOXICOLOGY DATA PLAY AN INTEGRAL ROLE IN THE SAFETY ASSESSMENT OF DIETARY SUPPLEMENTS CONTAINING NEW DIETARY INGREDIENT(S).
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Dietary Supplement Health and Education Act (DSHEA) of October 15, 1994
mandates the submission of a pre-market safety notification for dietary supplement
products containing new dietary ingredients (NDI). Other evidence of safety
includes published literature and unpublished toxicological studies including
genetic toxicity studies. The genetic toxicology data concerning the NDI can be
evaluated and interpreted in the context of documented history of safe use, if pro-
vided, and the proposed conditions of use. For example, if a botanical that is widely
used as a conventional food is positive in an in vitro genetic toxicity assay, using
the weight of evidence approach, its long history of safe use can balance the
signal. On the other hand, negative in vitro and in vivo genetic toxicity data on a test
substance derived from the root of a plant may be of questionable relevance for an
NDI derived from leaf because the two likely differ in their chemical composition.
Furthermore, a negative Ames test is of limited relevance for the safety assessment
of a botanical which contains methylleugenol and estragole that have been shown to
cause cancer in rodents but are also known to be negative in Ames and/or other
short-term studies. In general, these case studies indicate that genetic toxicology
data play an integral role in the safety assessment of a dietary supplement contain-
ning NDIs. In the draft New Dietary Ingredient Guidance for industry, the FDA
proposed genetic toxicity testing for some but not all NDIs.

273 IS DERMAL SENSITIZATION FOR 1, 2-
BENZISOTHIAZOLIN-3-ONE [BIT] IN CONSUMER
PRODUCTS A CAUSE FOR CONCERN?

1,2-Benzisothiazolin-3-one (BIT, CAS # 2634-33-5) is a preservative in widely
used consumer products, such as sunscreen, laundry and dish detergents, and hard
surface spray cleaners. Dermal exposure to BIT is known to cause skin sensitization
and allergic contact dermatitis in animal and susceptible humans following specific
doses. In general, once an individual is sensitized (e.g., receives an induction dose),
the excitation of a skin reaction may occur at lower exposures (e.g., μg/cm²). The
purpose of this study was to identify the concentration of BIT in various consumer
products where skin sensitization is unlikely to occur during normal use. It is well
established that the potential for skin sensitization is based on the mass of product
applied per unit area of skin (mg/gm²). In this study, the BIT exposure which was
thought to not pose a sensitization hazard (to those sensitized or not sensitized) was
estimated by dividing the NOEL for induction by a safety factor. Published data
from human patch test studies reported that the no expected sensitization induc-
tion level was 45 μg/ cm². We then assigned and applied safety factors for sunscreen
(300) and cleansing products (100) based on interindividual differences, the matri-
ces, and the product use. The amount of product applied to the skin (mg/cm²) and
the estimated “safe doses” were used to calculate the concentration of BIT for sun-
screen lotion, laundry detergent, dish detergent, and spray cleaner that would be
unlikely to induce skin sensitization in any consumer. Our quantitative risk assess-
ment results were compared to the BIT content in these consumer products to de-
termine if they are likely to be safe for a majority of users.

274 LOCAL TOLERANCE OF SUBCUTANEOUSLY
ADMINISTERED 2-HYDROXYPROPYL-β- CYCLODEXTRIN (HPβCD).
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Cyclodextrins (CDs) interact with drug molecules to increase solubility and stabil-
ity by forming inclusion complexes, thereby making CDs useful excipients for
pharmaceutical dosing. The nonclinical use of HPβCD has shown increased solu-
bilità and greater systemic safety relative to other CDs (primarily oral and IV data),
although data by the subcutaneous (SC) route are lacking in the literature. Local
tolerations to SC injection can be a challenge due to local high concentrations and/or
volumes of the drug and vehicle excipient. In acute local tolerances studies
using Sprague-Dawley rats, SC injection of HPβCD or other excipient vehicles (in-
cluding PEG400 and Poloxamer) resulted in injection site microscopic findings at-
tributed largely to the vehicle, volume of vehicle, and/or the trauma caused by the
injection procedure. In contrast to control vehicles (0.9% Saline, 5% Dextrose)
with findings limited largely to subacute inflammation, SC injection of the excip-
ient vehicle formulations resulted in varying incidence and severity of inflammation,
necrosis, and congestion at the injection sites. Adverse effects were most prominent
with SC injection of formulations in Poloxamer or PEG400. For HPβCD formula-
tions (25%, β2−HOH), SC injection was clinically well tolerated, while the severity
of inflammation and necrosis was slightly increased with higher concentrations of
cyclohextrin. The severity and extent of local findings were also greater at larger injec-
tion volumes (3.5 mL/kg) relative to lower volumes (1 mL/kg). Repeat-dose toxi-
cology studies in Sprague-Dawley rats and Beagle dogs confirmed the minor
injection site findings observed with 10% HPβCD at 1 mL/kg in local toleration
studies. Following drug-free recovery periods, inflammation and fibrosis persisted
at decreased severity whereas necrosis was absent at the injection sites. In conclu-
sion, a 10% HPβCD formulation was well-tolerated locally and systemically fol-
lowing repeated SC administration at a dose volume of up to approximately 1
mL/kg in rats and dogs.

275 POLYMERIC FLAME RETARDANTS: POSSIBLE LESS
HAZARDOUS ALTERNATIVES TO DECBROMODIPHENYL ETHER?
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Damages from the approximately 12 million fires per year in the United States,
Europe and China amount to nearly $317 billion. One contributing factor to the
increased incidence of fires is the widespread use of flammable plastics in electrical
and electronic devices found in residential and industrial products. Use of flame
 retardants (FRs) in plastics reduces the incidence of fire with the added benefit of re-
ducing potentially toxic smoke emissions from fires that do occur – e.g., PAHs,
PCBs, PCDs and PCDFs. Owing to health and environmental concerns, exten-
sively-used halogenated FRs such as decabromodiphenyl ether, or decBDE, are
being phased-out of commerce in the United States by 2013. As a consequence, FR
manufacturers have been considering alternatives to decBDE. An approach to for-
mulating newer and more environmentally-friendly brominated FRs is to produce
large molecular weight compounds – polymeric FRs – that are limited in their abil-
ity to cross biologic membranes and interact with cellular macromolecules. We re-
viewed the health and environmental effects of polymeric FRs, relying principally
on published data in the scientific literature. If information in the public realm was
lacking and/or available from industry sources – e.g., FR producers or suppliers – health
and environmental effects data were supplemented with such ‘in-house’ data where appropriate. Health effects studies reviewed included acute toxicity, sub-
chronic and chronic toxicity, mutagenicity/genotoxicity, and carcinogenicity. Environ-
mental effects studies reviewed included aquatic toxicity, environmental fate/transport, and environmental persistence/bioaccumulation. All health and en-
vironmental effects data for the polymeric FRs were compared to the corresponding
data for decBDE. Results of the analysis indicated that, by most measures, the
polymeric FRs displayed a more favorable health and environmental profile
than decBDE and thus may be a considered viable alternatives to decBDE in
consumer and industrial products that require FRs.
276 SAFETY ASSESSMENT OF CYTOSOL+™ BOTANICAL METABOLITES AS AN ANTIDIARRHEAL AGENT.
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Botanical polyphenols are one of the largest and most ubiquitous groups of secondary metabolites that are an integral part of the human diet. Clinical studies shown that polyphenol intake is associated with a reduced risk of cancers and cardiovascular and neurodegenerative diseases. Cytosol+™ comprised primarily of green tea and pomegranate polyphenolic extracts, demonstrated significant gastrointestinal health benefits and a broad-spectrum microbial inhibitory effect with very low daily intake. An E. coli 0149:K88 challenge study in pigs showed a significant reduction in the severity and duration of pathogen induced diarrhea, reduced fecal shedding, and increased proliferation of mucosal cells as evidenced by histometry. Increased villi crypt depth in treated animals also suggests direct protective effects on mucosal tissue. To evaluate the safety of Cytosol+™, a 28-day toxicity effect in weaned SPF piglets was investigated. Piglets (n=10) were randomly divided into three groups and orally inoculated either with water (control), or 50x or 500x of daily treatment dosage of Cytosol+™. Clinical disposition, blood chemistry, and histopathology were examined. Cytosol+™ did not show toxic effects at either dose levels after 28 days chronic use. No significant difference in histological analysis of the major organs was found. The safety and tolerability of oral administration of Cytosol+™ was further evaluated in a clinical trial in patients with infectious diarrhea. No adverse effects were observed in test subjects and results also indicated that Cytosol+™ is more effective than conventional anti-diarrheal pharmaceutical agents such as loperamide, anti-emetics and antibiotics. This study concluded that Cytosol+™ had no toxicological effect up to 500 times the daily treatment dosage and may serve as a safe and effective therapeutic alternative for gastrointestinal distress.

277 EFFECTS OF EXPOSURE TO ETHYL TERTIARY BUTYL ETHER AT LOW CONCENTRATIONS IN ALDH2 KNOCKOUT MICE.
Ethyl Tertiary Butyl Ether (ETBE) is used by mixing with gasoline for vehicles. ETBE is low toxicity and it’s No Observed Adverse Effect Level was thought to be 500 ppm in experimental animals. In our previous study, we found that in Aldh2 knockout mice, ETBE could induce damages in chromosomes and reproductive system even at 500 ppm. The present study was aimed at clarifying if ETBE has any effect on reproductive and other systems in mice at low concentrations. METHODS: Male C57BL/6 strain (WT), Aldh2+/+ (HT) and Aldh2-/- (KO) at 8 weeks were exposed to ETBE at 0, 50, 200 and 500 ppm, 6 hr/day and 5 days/week, for 9 weeks. Blood, liver, testes, epididymides and vasa deferentia were sampled 20 hr after the last exposure. Sperm from the cauda epididymides, and vas deferentia were released into a medium, and sperm motion was analyzed. RESULTS: There was no effect of ETBE exposure on the ratio of liver to body weight, and neither any change in the hematology in any exposure group. As for the reproductive system, the ratio of testes or epididymides to the body weight was not observed for any genotypes of mice. In WT mice, ETBE exposure did not affect the motility of sperm. In HT mice, there was a significant decrease in the motile percentage and sperm count. No adverse effects were observed in HT mice. The present study was aimed at clarifying if ETBE has any effect on the ratio of liver to body weight, and neither any change in the hematology in any exposure group. As for the reproductive system, the ratio of testes or epididymides to the body weight was not observed for any genotypes of mice. In WT mice, ETBE exposure did not affect the motility of sperm. In HT mice, there was a significant decrease in the motile percentage and sperm count.

278 HAZARD EVALUATION OF A SHORT-CHAIN FLUOROROTELOMER ALCOHOL PHOSPHATE MIXTURE.
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The toxicity profile of a short-chain fluorotelomer alcohol phosphate mixture was evaluated. This substance has low acute toxicity potential by the oral and dermal routes (LD50 >5000 mg/kg), is not an irritant to the eye or skin, and did not cause skin sensitization in the LLNA assay. It was not mutagenic in the Ames test or in the in vitro chromosomal aberration assay. The 96-hour LC50 in fathead minnows was > 120 mg/L, 96-hour LC50 in zebra fish was > 150 mg/L, 48-hour EC50 in Daphnid was 16.2 mg/L, and 72-hour EC50 in algae was > 120 mg/L. The 21-day no-observed-effect-concentration (NOEC) in Daphnid was 0.409 mg/L. The repeated dose toxicity potential was assessed by administering 0, 5, 25, and 125 mg/kg/day via oral gavage to SD rats for 28 days. Main study animals (10 rats/sex/group) were sacrificed at the end of the dosing period. One-Month Recovery (5/sex/group) and toxicokinetic (5/sex in the 5 mg/kg/day group) animals were sacrificed approximately one month after the dosing period. The toxic compo-

279 EFFECTS OF SUBCHRONIC ADMINISTRATION OF THE ETHANOLIC EXTRACT OF CARPOLOBIA ALBA STEM ON SOME HAEMATOLOGICAL AND SERUM LIPID PARAMETERS IN RATS.
The high cost of the potent antimalarials, artesminin combined therapies, coupled with the resistance of P. falciparum to other conventionally used antimalarials has left the poor masses of the tropical regions of the world heavily reliant on herbal preparations for the treatment of the devastating disease, without considering the possible toxic effects. This study was aimed at evaluating the effect of subchronic administration of the ethanolic extract of Carpolobia alba stem, an antimalarial herbal remedy in Southwestern Nigeria, on some haematological and serum lipid parameters that could serve as cardiovascular disease indices in albino rats. Twenty-five (25) albino rats were randomly divided into five groups (A, B, C, D and E) of five rats each. Ethanolic extract of Carpolobia alba stem was administered to rats in groups A, B, C and D at the doses of 31.25, 62.5, 125 and 250 mg/Kg body weight respectively for twenty-one days while group E, which served as the control, received the appropriate volume of the vehicle (5% DMSO solution). At the end of the experimental period, venous blood was collected from the animals and full blood count coupled with serum concentrations of HDL-cholesterol, LDL-cholesterol, triglycerides, and total-cholesterol were determined. Also the atherogenic index was calculated. The extract did not cause any significant alteration (P>0.05) in the red blood cell indices, white blood cell indices and serum concentrations of HDL-cholesterol, LDL-cholesterol, total-cholesterol and the atherogenic index at all doses administered compared to controls while it significantly increased (P<0.05) serum triacylglycerol concentration at the dose of 250 mg/kg body weight compared to controls. The results of this study suggest that Carpolobia alba stem extract may not predispose subjects to cardiovascular diseases when administered below 250mg/kg body weight.

280 TOXICOLOGICAL ASSESSMENT OF A PEDIATRIC ORAL REHYDRATION SOLUTION (PEDIAONE™).
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Diarrhea is a common illness in children, resulting in at least 200,000 U.S. hospitalizations and hundreds of deaths per year. UNICEF and WHO recommends pediatric oral rehydration solutions (ORS) for therapeutic treatment of dehydration as well as zinc supplementation. PediaONE™ is a safe and effective zinc-supplemented ORS which also provides a recommended percent daily value of vitamins and minerals typically lost during dehydration: Vitamin C, calcium, Vitamin B3, Vitamin B5, Vitamin B6, and Vitamin B12. To demonstrate the safety of PediaONE™, we conducted a series of toxicity tests at a GLP compliance laboratory. Acute oral and acute dermal toxicity tests were conducted in rats to determine the potential for PediaONE™ to produce toxicity from a single dose via the oral route and topical application. Under the conditions of these studies, the acute oral LD50 of PediaONE™ is <5,000 mg/kg, and acute dermal LD50 of PediaONE™ is <2,000 mg/kg. In a Primary Eye Irritation toxicity study in rabbits,
minimal conjunctivitis was observed 1 hr after instillation, however irritation decreased thereafter. The Maximum Mean Total Score of PediaONE™ 1.0 and was classified as minimally irritating to the eye. In a Primary Skin Irritation study in rabbits, very slight erythema was observed within 1 hr of patch removal, however severity of irritation decreased thereafter. The Primary Dermal Irritation Index for PediaONE™ is 0.2 and classified as slightly irritating to the skin. All animals were free of eye and dermal irritation by 48 hours. Throughout the studies all animals appeared healthy and there were no other signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior. These studies demonstrate the safety of PediaONE™, a novel pediatric oral rehydration solution.

281 SUBACUTE EFFECTS OF ORAL LEAD ACETATE ON BLOOD PARAMETERS AND GI MICROBIAL ANTIBIOTIC RESISTANCE IN JUVENILE CHICKENS.
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Lead (Pb) is a common heavy metal contaminant released into the environment and can result in growth inhibition, reproductive retardation, immune suppression, metabolic alteration, and impaired neurological function in chickens. Chickens are an important animal model due to their close association with humans through food production and consumption. Preliminary in vitro studies showed an unexpected increase in antimicrobial resistance in bacteria cultured with low levels of Pb. This study was conducted to determine if sub-acute oral exposure to Pb may similarly alter microbial antibiotic resistance in gut bacteria of white Leghorn chickens. Positive data would suggest increased difficulty managing pathogen outbreaks in flocks exposed to Pb. Fifty specific pathogen free Leghorn chickens (2 week old, male and female) were administered 0.0, 0.01, 0.1, 1.0, or 10.0 mM Pb acetate through drinking water for 14 days. Birds were bled and weighed, and cloacal swab samples were taken on day 0, 7, and 14. Birds were euthanized on day 14 and sections of the liver, spleen, kidney, and brain were collected. Blood physiologic parameters were not changed by Pb exposure with the exception of delta aminolevulinic acid dehydratase (d-ALAD) activity, which was suppressed by Pb. Unlike the in vitro results, no alteration in gut bacteria antibiotic resistance was observed in the Pb-exposed birds. This difference from the in vitro results could be due to the type (pathogen free) and age of the bird, the duration of Pb exposure, the form of Pb administered, or differences between in vitro culture and in vivo environments of the tested bacteria.

282 SOLVING THE DATA INTEGRATION PROBLEM FOR A SUPERFUND RESEARCH PROGRAM CENTER.
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Superfund Research Programs (SRPs) are multi-disciplinary and multi-institutional to leverage the best science and solve complex environmental health issues. Success depends on the center's ability to effectively integrate data across all research projects and cores. Our SRP Center has developed a system that integrates environmental monitoring data with the analytical core operational data and downstream bioinformatics/statistics to enable complete ‘source to outcome’ data modeling. The system incorporates commercial software for operational laboratory management (LIMS) and sample management in addition to custom software for bioinformatics, experimental data management and a web-store for sharing chemical standards to all researchers in the program. By creating a pipeline that integrates these systems together into a seamless workflow, we are able to use one set of identifiers throughout the workflow and keep data replication at a minimum. One successful example, the chemical standards repository web-store, uses the LIMS database to keep track of chemical inventory but maintains a separate data store for current requests and processing. For this workflow we’ve segregated the processing of sample IDs and chemical inventory so that we can easily incorporate other systems as necessary or easily replace any part of the workflow without rewriting the integration code. Through this and other data integrations we have been able to generate more repeatable and comparable results between projects while maintaining privacy and quality assurance compliance. We’ve also created a tool set that easily reports statistics and status to our collaborators and grantors through the web. These successes stretch our grant dollars by improving center efficiency and enhance collaboration between cores and projects at OSU and PNNL as well as case proposal collaboration with other organizations and governmental agencies. Supported by NIEHS grants P42ES016465 and P30ES00210.

283 CHEMICAL EFFECTS IN BIOLOGICAL SYSTEMS: A TOXICOLOGICAL DATABASE.
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The Chemical Effects in Biological Systems (CEBS) is a toxicological database with a flexible design that can accommodate a wide variety of data types. CEBS houses toxicological study data from the Division of the National Toxicology Program (NTP), National Institute of Environmental Health Sciences and other government laboratories, pharmaceutical companies, and academic laboratories. NTP carcinogenicity and toxicology, genetic toxicology, immunotoxicology, as well as some reproductive and developmental toxicology study data are all available in CEBS. Also housed are the chemogenomics database DrugMatrix® data, microarray study data, and phase I high throughput screening Tox21 data. CEBS is designed and modified to accommodate new data and to meet the needs of the scientists in viewing, downloading, and searching data. CEBS stores raw data and contextual information — such as protocol information, dosing regimen, and subject characteristics — on each of the studies. The user interface allows a user to browse through the list of studies, view studies, download data, and search for studies or subjects across the database that meet personally chosen criteria. CEBS allows a user to gather animal data from different studies together for analysis. The number and diversity of study types together with its searching capabilities makes CEBS different than most other scientific databases. One of the advantages of CEBS is that the raw data from studies is accessible to users. Raw data from toxicological studies can be viewed, extracted, and downloaded, permitting custom meta-analysis. CEBS also allows statistical meta-analyses on housed normalized data. As the NTP expands into different research areas such as epigenetics, genomics, and new technologies such as next-generation sequencing, designed flexibility will permit integration of these data within CEBS and continue to make CEBS a valuable tool for toxicologists.

284 USING CTD TO DISCOVER AND PREDICT MOLECULAR CONNECTIONS BETWEEN ENVIRONMENTAL CHEMICALS AND HUMAN HEALTH.
C. G. Murphy, A. P. Davis, C. A. Saraceni-Richards, M. C. Rosenstein, T. C. Wieggers and C. J. Mattingly, MDIBL, Salisbury Cove, ME.

The Comparative Toxicogenomics Database (CTD: http://ctdbase.org/) is a freely available resource (funded by the National Institutes of Environmental Health Sciences) that promotes understanding about the effects of environmental chemicals on human health. Biocurators manually curate three types of data from the scientific literature: chemical (C)-gene (G) interactions, chemical (C)-disease (D) relationships, and gene (G)-disease (D) relationships. These data are then internally integrated as well as externally combined with Gene Ontology (GO) and Pathway (P) annotations to infer possible connections between all five components (C, G, D, GO, and P). CTD has greatly expanded its curated content, in part, as a result of a collaboration with Pfizer, Inc., involving the curation within one year of over 50,000 additional toxicity publications selected for four disease targets (cardiovascular, renal, hepatic, and neurological defects). These data are now integrated and freely available to all CTD users. To help navigate and analyze this vast information, CTD provides statistical, analytical, and visualization tools that allow users to retrieve, explore, and maximize the utility of the data, including a new feature called “DiseaseComps” which complements our existing “ChemComps” and “GeneComps” by identifying similar diseases, chemicals, and genes based upon shared toxicogenomic profiles, respectively. We are also in the development phase of curating and integrating phenotype (non-disease) and exposure data. Collectively, CTD’s wealth of information, data integration strategy, and unique analysis tools make it an unparalleled and flexible resource for understanding toxicology and developing hypotheses about the molecular mechanisms underlying environmental diseases.

285 TOXML, A COMMON DATA EXCHANGE STANDARD FOR TOXICOLOGY.
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With rapid scientific advances in toxicology and changing regulatory requirements, the number of toxicity datasets available for those wishing to share and communicate knowledge, or to use for data mining and modelling is continually expanding.
The challenge with this ever-growing amount of data is that it exists in a multitude of different formats, depending on its source and original purpose. Different databases covering the same endpoint may omit or use widely differing conventions to structure and represent the same information. The issues of comparing or combining disparate data apply both to public and proprietary sources, and both historical and newly generated data. It is often laborious for individuals and groups to restructure their datasets in order to supply them to different investigators. The ToxML project addresses the need for a common data exchange standard that allows the representation and communication of this data in a well-structured electronic format to the user community. ToxML is an open standard based on the Extensible Markup Language (XML) format. We describe the utility of ToxML as a common data exchange standard for toxicology information, the mechanism for its dissemination and its community-based development in the future. An example of how ToxML is currently implemented and used in transactions involving large quantities of repeat-dose study data is described. The standard is open and maintained by a curation team overseen by the ToxML organisation. The standard is published on a website together with tools to view, edit and download it.

PS 286 EXTENDING THE DEREK-METEOR WORKFLOW TO PREDICT CHEMICAL-TOXICITY SPACE IMPACTED BY METABOLISM: APPLICATION TO TOXCAST AND TOX21 CHEMICAL INVENTORIES.

P. Volarath, M. Martin and A. Richard. US EPA, Research Triangle Park, NC.

A central aim of EPA's ToxCast project is to use in vitro high-throughput screening (HTS) profiles to build predictive models of in vivo toxicity. Where assays lack metabolizable capability, such efforts may need to incorporate the role of metabolic activation (or deactivation). A workflow combining two structure-based expert systems – Meteor (predicts bio-transformations) and Derek (predicts in vivo toxicity) – was previously developed and applied to identify ToxCast Phase I chemicals likely to require metabolic activation for rat carcinogenicity (Rat Carc). Positive and Negative Derek predictions for the parent structures [P+, P-] and for the Meteor-generated metabolites [M+, M-] were compared and validated using rat chronic study results from ToxRefDB. In cases where Derek has implicit knowledge of metabolic activation requirements associated with a structural alert (SA), the parent chemicals and associated metabolites are both predicted as rat carcinogens [P+/M+]. However, because the Derek knowledge-base is incomplete, some compounds trigger a positive Derek prediction only after the parent compound is metabolically transformed by Meteor, (i.e., [P-/M+]). We extended the workflow to combine metabolic activation-related knowledge implicitly contained in Derek SAs (through review of SA narratives) with that implied by the [P-, M+] outcomes of the Meteor-Derek workflow. In principle, the extended workflow enables the extraction of a set of meta-bolism (or deactivation) structural features in association with any Derek toxicity endpoint. This cheminformatics workflow is being applied to the full ToxCast Phase 1 & II (10,600 unique chemicals) and Tox21 (>8,600 chemicals) inventories to identify regions of chemical HTS space where in vitro to in vivo Rat Carc associations are more likely to be impacted by metabolism. These results will be used to inform future predictive modeling efforts. This abstract does not necessarily represent US EPA policy.

PS 287 REVISITING THE TTC APPROACH FOR CANCER ASSESSMENT: PART OF A ROAD MAP OF COMPUTATIONAL METHODS AT US FDA CFSAN OFAS.

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Alternative methods in risk assessment workflows are attracting attention due to international legislation on providing safety data for large numbers of chemicals. The Threshold of Toxicological Concern (TTC) is one method of interest and was developed based on the Threshold of Regulation (T0R) policy of US FDA Center for Food Safety and Application (CFSAN). TTC provides a mechanism for assessing the safety of low-exposure food-contact materials. The goal of this project is to aid regulatory risk assessment procedures by refining the TTC process. The first refinement is providing a cancer TTC dataset based on regulatory study review criteria. This process helps gain better regulatory acceptance and supports building a high-quality training set for other computational methods. The Carcinogenicity Potency Database (CDPB) was integrated into the Chemical Evaluation and Risk Estimation System (CERES) database at CFSAN. Study details and TD50 values were selected for the cancer TTC dataset only from studies in the CDPB that were in accordance with defined regulatory criteria (e.g., test substance administration routes, duration and sample size). To address gaps in available data, a first refinement is to incorporate known genotoxic mechanisms into the TTC decision tree. Positive and negative Ames assay data were curated from various databases. These data were then incorporated into and analyzed as part of the cancer TTC dataset. This analysis shows Ames positive carcinogens to be much more potent than Ames negative carcinogens. This analysis has two additional benefits. (1) The qualification of data through regulatory screening criteria increases the regulatory applicability and acceptance of TTC methods. (2) Our methodology provides a mechanism for incorporation of alternative and in vitro test methods into a regulatory risk assessment.

PS 288 CASE ULTRA: A NEW IN SILICO TOOL FOR QUANTITATIVE PREDICTION OF BIOLOGICAL ACTIVITY.

S. Chakravart1, R. D. Saiakhow1, M. A. Fuller1 and G. Klopman1, 2, 1MultiCASE Inc., Beachwood, OH and 2Department of Chemistry, Case Western Reserve University, Cleveland, OH.

Purpose of this study was to demonstrate a novel approach to build quantitative structure-activity relationships from diverse chemical structures with continuous biological activity data in a fully automated fashion. This in silico tool can be successfully used for assessment of potential toxicity or beneficial therapeutic effects of new drug candidates, impurities and metabolites. Case Ultra is a computer program that uses an algorithm to find structure-activity knowledge and can be used as both chemists and DRDBs completely automatically. Traditionally we have successfully used Case Ultra mainly in predicting binary activity outcomes (e.g. active/inactive or toxic/non toxic). Recently we have added a novel algorithm to Case Ultra to extend its capabilities to predict activity/toxicity on the continuous scale. This immediately makes the program equally useful in computational toxicology as well as in drug design discovery. The algorithm does not need any prior knowledge of different structural classes present in the training set and automatically divides the training set into representative classes and builds predictive QSARs for each class. We have applied Case Ultra to build predictive QSARs on very different benchmark datasets namely PTP1B inhibition, Daphnia LC50, DHFR inhibition, Estrogen Binders, COX2 Inhibitors etc. Besides being predictive Case Ultra also gives a very clear explanation of its predictions so that they are easily interpretable by chemists and toxicologists. The predictive performance and details of the QSAR models for different end points (e.g. PTP1B inhibition, Daphnia LC50, DHFR inhibition, Estrogen Binders, COX2 Inhibitors) are demonstrated. Various cross validation of the models shows a solid performance. For example average cross validated r2 of PTP1B (132 chemicals) and DHFR (397 chemicals) models are 0.66 and 0.52 respectively. Different chemical structural classes within each end point were also successfully identified.

PS 289 OPEN SOURCE PLUG’N PLAY PREDICTIVE MODELING METHODS USEFUL IN TOXICOLOGY.

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There is a wide range of different tools that can be used to create and use predictive models for toxicology related endpoints. For someone new to modeling and with an interest in applying models to her or his own data it might be quite time consuming to find which method and tools that are most adequate. Some of the existing tools are also quite costly and it is often not clear if there is any additional benefit with the increased costs. Furthermore, legislation like REACH demands a transparency which can be hard to fulfill for some of the commercial alternatives. We have developed a method to assess chemical liability of compounds based on data provided by the user of the system or by community models from for example OpenTox, which is called BioClipseDS. The method was implemented in BioClipse, using the Chemistry Development Kit, the Signature descriptor and libsvm. BioClipseDS has undergone more enhancements and can now be used as a plug’n play modeling and prediction platform, freely available to anyone with an open source that can be adapted to individual needs. The enhancements allow models to be built very quickly when datasets are provided in the SD-file format. Models can easily be built with thousands of compounds and based on different endpoints. When used for predictions, BioClipseDS shows an improved view and an overall assessment for all models. In an internal benchmark activity at AstraZeneca the method described here was the best in five out of ten cases. It was also the only method that could build a model on a data set with almost 100 000 compounds. Additionally, in this case this method also reduced the root mean square error on an external test set by almost 20% compared to the second best method. A selection of public datasets has been evaluated and the results are reviewed so that comparisons can be made of the performance of this method with other commonly-used modeling and prediction tools.
Male reproductive toxicants, manifest as testicular toxicants, and are among the top 6 target organ systems that result in project delays during the preclinical phases of drug development at AstraZeneca. Testicular toxicants are often not detected until pivotal 1 month studies are run during the preclinical phase and as a consequence, failures tend to be expensive, especially if the issue identified is related to the chemical scaffold of the development candidate. As such, there is a clear incentive to develop both in vitro and modeling methods to identify risks of testicular toxicity at a much earlier stage. For this purpose, we have developed a warning system aimed at testicular risk identification and mitigation. The warning system integrates data text mined from both public and internal sources via the PharmaConnect Knowledgebase. Using the knowledge base, compounds from public sources that relate to testicular toxicity were identified and integrated with internal reports (see poster on this method from Clark et al.). From these data, structural features that correlated with testicular toxicity were identified and form one part of the warning system as structural alerts. The second part is a predictive model of the in vitro RARa antagonist activity. RARa antagonism has been linked to testicular degeneration and can be used as an early marker for potential in vivo issues. The model is built on internal data and performance is in line with the experimental error in the range 65-80% correct classifications with sensitivity being in the lower range. In this study we present novel structural alerts related to testicular toxicity and show how these are used together with the QSAR model for risk assessment. We present performance statistics based on internal data and the application of a weight-of-evidence approach to give overall risk assessments.

**290 IDENTIFICATION OF TESTICULAR TOXICITY-RELATED RISKS IN EARLY DISCOVERY.**


Humans are exposed to chemicals based on the activities they perform. While the relationship between activities and near-field exposures have been greatly studied, an individual's near field exposures are difficult to ascertain because they vary based on that individual's personal product space. Key to understanding the relationship between activities and near-field chemical exposures is knowing which chemicals are in common household products. To achieve this goal, a workflow for quickly populating and curating a “personal chemical exposure” database linking products with their specific chemical components and ingredients was developed based on a large repository of publicly available Material Safety Data Sheets (MSDS) from a major retail chain (11,800 product MSDS). The resulting workflow utilizes Optical Character Recognition (OCR), chemical name and CAS identification software, and finally manually inspection through a straightforward multi-screen graphical user interface (GUI) to provide highly accurate identification of a products chemical components. Used in conjunction with ubiquitous computing (i.e. smartphone technology), 2D QR matrix codes, and bar-code scanner apps we demonstrate how a product chemical ingredient database can be used to inform an individual of the “Personal chemical exposure” by scanning real-life household/everyday products through the Augmented Reality for Exposure Awareness (AREA) tool. The workflow and tools are freely available allowing other to use the same techniques to create their own consumer product chemical database. The database, the first freely available and chemically searchable consumer product database, has been integrated into the Environment Protection Agency’s (US EPA) ACToR database for facile query by the public. This abstract has been cleared by the US EPA but solely expresses the view of the authors.

**291 CASE ULTRA: PREDICTIVE PERFORMANCE IN ASSESSING THE POTENTIAL CARCINOGENICITY, GENOTOXICITY, DEVELOPMENTAL TOXICITY AND ADVERSE IMPURITIES AND METABOLITES OF NEW DRUG CANDIDATES.**

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The purpose of this study was to apply novel approaches in the creation of a superior data driven expert system for in silico toxicology capable of dealing with large sets of diverse chemicals with variable structural complexity without the necessity for additional animal testing. Such a system could be used for the assessment of the potential carcinogenicity, genotoxicity, developmental toxicity and adverse effects of new drug candidates, impurities and metabolites. CASE Ultra advances the MCASE fragment based approach, which breaks training set molecules into fragments with a predefined number of atoms and a predefined branching pattern. CASE Ultra uses a proprietary algorithm that automatically identifies substructures relevant to toxicity without having any limitation on their size or branching pattern. This results in increased exploratory capabilities and predictive performance of the models. The non-proprietary training data used to build the models were available through a Research Collaboration Agreement entitled “Enhancement of In Silico Decision Support Tools for the Evaluation of Drug Safety” between MultiCASE Inc and The US Food and Drug Administration’s Center for Drug Evaluation and Research. The models within each expert system were grouped to represent assays recommended by regulatory guidelines. Validation experiments were performed for each model individually and for groups of related models using standard leave-group-out and y-scrambling methods. The predictive performance of CASE Ultra carcinogenicity, genotoxicity, developmental toxicity and adverse effects expert systems is demonstrated. The results of our validations demonstrate a solid predictive performance with more balanced sensitivity and specificity than MC4PC, including overall concordance for Salmonella mutagenicity prediction above 75%.

**292 PARTNERING PREDICTIVE TOXICOLOGY WITH MEDICINAL CHEMISTRY FOR STRATEGIC DRUG DISCOVERY.**

L. L. McIntosh and C. Fishburn. Exponent Inc., Menlo Park, CA.

Translational research involves targeted drug design to address specific clinical challenges, and avoids the screening of large compound libraries. Improved in silico capabilities enable optimization of chemical structures by allowing prediction of likely toxicities, sometimes replacing or supplementing early screening of compounds, and increasing efficiency.

**293 PERSONAL CHEMICAL EXPOSURE INFORMATICS: CREATION OF A CONSUMER PRODUCTS CHEMICAL INGREDIENT DATABASE.**

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**294 NBL: A KNOWLEDGEBASE OF NANOMATERIAL-BIOLOGICAL INTERACTIONS.**

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A risk characterization framework to classify nanomaterials based on their physical or chemical properties as well as their biological impacts is necessary to reduce the uncertainty around potential nanomaterial hazards. Structure-property relationships that can be used to predict nanomaterial impacts in lieu of empirical data can provide significant support for the nanotech industry in developing safer nanomaterials. Knowledge on the governing principles of nanomaterial-biological interactions can more effectively be utilized once computational tools are available for data integration and consensus analysis. The Nanomaterial-Biological Interactions (NBI) knowledgebase was developed to consolidate and integrate disparate data on
Today, drug discovery and development programs involve the generation of large amounts of in vitro and in vivo data, with the goal to ensure that potential drug candidates meet all the requirements needed to become new medicines. A significant part of the produced data comes from Non-Clinical Safety (NCS) organizations, whose efforts are aimed at ensuring that selected clinical candidates possess a suitable safety profile.

In modern drug discovery and analysis, knowledge generation, interpretation of results and hypothesis assessment are crucial. While in vitro data can be easily made accessible by a number of available IT solutions, the interaction with and the visualization of in vivo data generated by non-clinical safety (NCS) studies represent a real challenge. On one hand, each in vivo NCS study generates a number of specific data types, which are separately stored in independent Laboratory Information Management Systems (LIMS). Roche decided to commit to the Safety Data Integration (SDI) project with the clear goal to provide NCS experts with an application permitting rapid and easy access to all relevant in vivo NCS data, seamlessly integrated from active and decommissioned safety applications adopted in pertinent Roche sites worldwide.

The deployment of the SDI system to Roche scientists allowed them to have complete access to more than 3,500 in house in vivo studies and to perform highly complex queries such as: “Which compounds showed a 200% increase in Aspartate transaminase AND a 30% increase in liver weight AND necrosis in liver?” Roche, together with its partners, developed a fully integrated platform, which is currently unique in the non-clinical safety framework and represents the gold standard for in vivo NCS data repository, visualization and analysis.

The quality of environmental data collected and analyzed at a contaminated site is crucial in assessing risks to human health and the environment, as well as establishing cleanup levels. Data quality management should be implemented in all stages of a remedial investigation (RI), including field investigation, data evaluation and validation, database development and management, and data usability for risk assessment and other data users. Poor data quality used in risk assessments will result in expensive re-sampling, costly delays to remedial efforts, or worst yet, unnecessary remedial actions. Starting from the field investigation, a FORMS II Lite or Scribe software is used to minimize errors and shorten the sample documentation process. In addition, environmental data management software, such as Scribe and Environmental Quality Information System (EQuIS), provides automation for electronic data deliverable checks and submissions, including data verification of field parameters and validation of analytical data. Using these softwares and working closely with engineers, geologists, chemists, and other project team members throughout an entire RI will minimize errors and reduce costs. A case study demonstrates that an effective data management program has resulted in a significant cost saving. Most importantly, an effective data management in all stages of a RI ensures the high quality of data used in risk assessments; consequently, it provides technically sound and legally defensible risk management decisions.

The Department of Homeland Security (DHS) is mandated to develop and implement a capability for providing chemical threat awareness and assessment as applied to homeland security issues. The Chemical Security Analysis Center (CSAC), a knowledge management center under the DHS, was established to meet these needs. One area of focus for the CSAC is science and technology based assessments of specific hazards. The CSAC develops toxicologically informed comprehensive hazard assessments to determine expected public health impacts of the release of toxic chemicals. As part of the hazard assessment, the CSAC uses a series of computer modeling and dispersion tools to develop plausible scenarios that provide a realistic appraisal of acute public health consequences following the release of chemicals at various locations. Models predict population exposure and toxic effects for a given chemical, location, dissemination method, route of exposure, and amount of chemical released, in the presence or absence of effective countermeasures. Key modeling parameters are identified for each scenario that, when adjusted, produce larger or smaller numbers of exposures. Taking into account physical properties, mechanism of action, toxicity, environmental persistence, diagnostic and detection capabilities, and treatment options for each chemical, results of the consequence modeling are analyzed and used to guide medical mitigation decisions. This presentation will discuss a number of models the CSAC utilizes to develop scenarios such as release of chemicals in transportation hubs, office buildings, chemical production facilities, transportation vehicles, and in food or water. Models include the US Department of Defense (DoD) Hazard Prediction Assessment Capability model, the DoD Consequence Assessment Tool Set, the Sandia National Laboratories Facilities Weapons of Mass Destruction Decision Analysis Capability model, and the Los Alamos National Laboratory Quick Urban and Industrial Complex Dispersion Modeling System.
for the many thousands of environmental chemicals lacking comprehensive toxicological data and/or human health risk assessment (e.g., species, sex, endpoint, range of doses/concentrations, duration of study, etc.) associated with the >700 chemicals with derived US or California EPA human health toxicity values (e.g., cancer potencies, reference doses and concentrations). Second, the availability of additional in vivo and animal in vivo data for the same compounds was examined. The greatest overlap (~50-70%) was found to exist with databases of rat LD50, Ames genotoxicity, and in vitro cytotoxicity screening conducted by the National Toxicology Program. Next, we used the latter data as independent variables to develop both chemical structure-based and hybrid (chemical structure and in vivo and in vitro data) models for predicting toxicity values. We posit that values derived through such models can not only aid prioritization of yet untested compounds for additional study, but may support decision-making for environmental contaminants. As additional toxicological data are generated, their ability to refine provisional estimates can be evaluated in a value-of-information approach. Disclaimer: The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the US or California EPA.

300 NANOMATERIAL TOXICITY SCREENING IN DEVELOPING ZEBRAFISH EMBRYOS.


To assess nanomaterial vertebrate toxicity, a high-content screening assay was created using developing zebrafish, Danio rerio. This included a diverse group of nano-materials (n=42 total) ranging from metallic (Ag, Au) and metal oxide (CeO2, CuO, TiO2, ZnO) nanoparticles, to non-metallic nanomaterials (SiO2, nanoparticle and carbon nanotubes), as well as micro and ion counterparts. Overt toxicity (lethality, dysmorphology, and hatching) was assessed via visual inspection and/or high-content imaging analysis. At 5-7 hours post fertilization, individual embryos were treated in a 96-well plate with the testing material (8 doses, semi-log spacing, 3±3 at each dose). Solutions were renewed daily until 5 days post fertilization (dpf) when the larvae were placed into embryo rearing solution. A total of 6 dpf, dysmorphology was assessed via visual inspection. Following visual inspection, fish were euthanized and positioned, and images were recorded and analyzed on the ArrayScan IV automated microscopy system using the Zebrotix V3 bioscillation. Results showed little to no overt toxicity, with a few exceptions: some of the nano-Ag and nano-CuO compounds produced dose-related overt toxicity. This result was also seen in the Ag and Cu ions; however, in general, metal salts were more toxic than their corresponding nanoparticles at the same metal mass concentration. Also, nano-ZnO and ZnCl2 did not typically cause lethality or dysmorphology, but did delay hatching. By employing few larvae per dose, we were able to assess toxicity for a relatively large number of different materials, demonstrating the utility of high-content screening of whole organisms as a valuable platform for nanomaterial or other chemical toxicity screening. These results will be combined with data from other ToxicityScan screening assays for nanomaterials to more fully characterize bioactivity and toxicity. This abstract does not necessarily reflect US EPA policy.

301 PHOTOTOXICITY OF TITANIUM DIOXIDE NANO PARTICLES (NANO-TiO2) TO DAPHNIA MAGNA IS DEPENDENT ON SOLAR UV SPECTRUM.

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Phototoxicity of nano-TiO2 has become an important concern as the material can be photocatalyzed under environmental UV radiation and generate reactive oxygen species (ROS). In this study, we investigated photocatalytic ROS production and phototoxicity of nano-TiO2 to Daphnia magna under simulated solar radiation (SSR). Our hypotheses were that: i) phototoxicity of nano-TiO2 is ROS mediated and will be correlated to photocatalytic ROS production by the material; ii) photocatalytic ROS production and phototoxicity of nano-TiO2 is dependent on the UV spectrum within SSR (i.e., only certain wavelengths are able to photocatalyze nano-TiO2). D. magna was exposed to nano-TiO2 under different solar UV spectrum by applying a series of spectral filters (i.e., standard window glass (SWG), acrylic glass (AG), 345 nm, 360 nm, and 400 nm cutoff filters) within SSR. Photocatalytic ROS production was measured by AFS [3’ (p-aminophenyl) fluorescein] assay, a fluorescence-based ROS assay with great specificity to OH. Phototoxicity was evaluated by immobilization of D. magna. Intracellular ROS production was determined by H2DCFDA assay. Greatest phototoxicity occurred when no spectral filter was applied, with a 48-h LC50 of 180 µg TiO2/L. Immobilization of D. magna by nano-TiO2 at 500 µg/L under different spectral filters showed the following order (standardized to no filter): SWG(92%) > 345 nm(57%) > 360 nm(69%) > AG(6%) > 400 nm (0%). Photocatalytic ROS production by nano-TiO2 showed similar order (standardized to no filter): SWG(100%) > 345 nm(92%) > 360 nm(69%) > AG(6%) > 400 nm (0%). An excellent correlation (r = 0.94, p<0.001) was found between the immobilization of D. magna and ROS production. Intracellular ROS production was consistent with D. magna immobilization, strongly suggesting oxidative stress was involved in the phototoxicity. Our findings demonstrate that phototoxicity of nano-TiO2 can occur under natural solar radiation. We have also identified the activating wavelength for nano-TiO2 photoactivation – a critical component of risk assessment for TiO2.

320 ECOTOXICITY TEST PROTOCOLS FOR REPRESENTATIVE NANOMATERIALS—THE PROSPECT RESULTS FOR ZINC OXIDE.

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The OECD’s Working Party on Manufactured Nanomaterials has a Sponsorship Programme, where 13 nanomaterials are to be tested for a number of human health and environmental safety endpoints. PROSPECT (Ecotoxicity Test Protocols for Representative Nanomaterials in Support of the OECD Sponsorship Programme) is a public-private-partnership to generate data by addressing gaps in the current knowledge of physical-chemical and ecotoxicological properties of zinc oxide (ZnO). The PROSPECT data for four types of ZnO from the EU repository. ZnO was characterized as delivered and used in the test systems for the following properties: Agglomeration/aggregation, Water Solubility/Dispersibility, Crystalline phase, Dustiness, Crystallite size, Representative Electron Microscope picture(s), Particle size distribution, Specific surface area, Surface chemistry, Photocatalytic activity, Pour density, Porosity, Redox potential and Radical formation potential. To guarantee reliable results a harmonized handling procedure was established. ZnO was tested comparing different standardised preparation and dispersion protocols alongside bulk and soluble formulations. Exposure test was conducted with zebras (Danio rerio), rainbow trout (Oncorhynchus mykiss), the crustacean Daphnia magna and the sediment dwelling amphipod Corophium volutator. Bio-accumulation and uptake were measured using stable isotope fingerprinting and elemental analysis, combined with Transmission Electron Microscope and Coherent Anti-Stokes Raman Spectroscopy for label-free imaging of particles. Sublethal effects were explored across different levels of biological organisation, combining unbiased screening of the metabolome with more focused, functional studies of targeted pathways. Results highlight in particular subtle effects of ZnO on mechanisms of feeding rate and behaviour, with metal formulation a more relevant predictor of toxicity than size or aggregation.

302 EMBRYOLARVAL EXPOSURE TO TITANIUM DIOXIDE NANO PARTICIPANTS IN THE PBP RANGE PRODUCES PHOTO-DEPENDENT TOXICITY.

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Titanium dioxide nanoparticles (TiO2NPs) are able to absorb photons to produce electron-hole pairs that react with O2 and H2O to form reactive oxygen species (ROS). We found that exposure to 100-1000 µg/ml TiO2NPs when illuminated over a 5 d period resulted in mortality and sublethal morphological malformations as a result of photo-induced oxidative stress. In this study, we exposed embryonic zebrafish to environmentally relevant concentrations of two commercial brands of TiO2NPs for 23 d during metamorphosis. We show that a long-term, low-dose (0.001-10 µg/ml) exposure of embryos to photoactivated TiO2NPs increases toxicity in zebrafish larvae, whereas the same particles are relatively inert in the absence of light. Larvae were assessed for various endpoints of toxicity at 12 and 23 days post-fertilization (dpf). Exposures to illuminated particles produced dose- and photodependent mortality and delays in the metamorphic development process. Furthermore, exposures to both brands of particles led to increased levels of an oxidative stress marker in 12 and 23 dpf larvae when illuminated. Histopathological examination of the larvae demonstrated that illuminated TiO2NP exposures cause proliferative and necrotic branchiitis in the gills, as well as skin and hepatic necrosis.
Both brands of TiO₂ NPs were taken up into the developing larvae and were found most consistently in the liver, gut, and gills. Interestingly, we show that depuration of TiO₂ NPs is possible as Ti levels return to baseline, survival rates stabilize, and oxidative stress is reduced when larvae are placed into nanoparticle-free H₂O for 7 d following the 23 d exposure period. To our knowledge, this is the first study to show a photo-dependent toxic response to low doses of TiO₂ NPs during the metamorphic, exposed larval period. Our study reveals that the potency of TiO₂ NPs is much higher than previously shown and that the phototoxic effect is inherent to TiO₂ NPs regardless of the brand (Funded by NSEC).

TIO₂ nanoparticles (NPs) are used in a wide range of applications and concern has increased about the potential hazard of these particles to humans and the environment. At present, little information is available about the possible risks of exposure to TiO₂ NPs. We have investigated the effect of particle size on the toxicity of TiO₂ NPs in the zebrafish embryo. Three different sizes of citrate-coated TiO₂ NPs (primary particle size = 5, 12 and 19 nm) were used. Embryos (4-120 hpf) were exposed to graded concentrations of these citrate-coated TiO₂ NPs under either simulated sunlight illumination or in the dark. Citrate-coated TiO₂ NPs of all three sizes caused photo-dependent toxicity. Embryos exposed to 5 nm particles under illumination exhibited higher mortality than those exposed to 12 or 19 nm particles. To gain insight into the mechanism, we measured the hydrodynamic size of citrate-coated TiO₂ NPs aggregates and compared Ti uptake in the embryos. After 24 h the hydrodynamic diameters of TiO₂ NPs aggregates, measured by dynamic light scattering, increased from 28 to 63 nm (12 nm NPs) and from 22 to 111 nm (19 nm NPs). In contrast, the hydrodynamic diameter of aggregates of 5 nm NPs increased to a much smaller extent (from 15 to 23 nm). Ti body burden, measured by ICP-OES, in embryos exposed to 19 nm NPs appeared to be higher than the Ti levels in embryos exposed to smaller NPs when compared at the same particle number concentration, but was not different at the same citrate-coated TiO₂ NPs mass concentration. Aggregation can affect the degree of toxicity and uptake. Therefore further research is required on the correlation between aggregate size and toxicity. Our results indicate a photo-dependent toxicity of citrate-coated TiO₂ NPs to zebrafish embryos with the smaller particle being more toxic than the larger particle. This work was supported by NSEC (NSF grant No. DMR-0425880) and EPA Star Program (RD-8338601-0).

**Effect of Citrate-Coated TiO₂ Nanoparticle Size on Embryo Toxicity in Zebrafish.**

M. Kim, K. Louis, J.A. Pedersen, R.J. Hamers, R.E. Peterson and W. Heideman.

**Impact of Amphiphilic Functionalization on the Toxicity of Titanium Dioxide Nanoparticles in Zebrafish Embryos.**

S. Yang, K.M. Louis, O. Baz-Han, R.J. Hamers, R.E. Peterson, W. Heideman, J.A. Pedersen.

**Cadmium and CdTe-Quantum Dot Alters DNA Repair in Zebrafish (Danio rerio) Liver Cells.**


**Uptake of Quantum Dots in Fathead Minnows, Pimephales promelas, Ovarian Explant Cultures.**


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S. Yang, K.M. Louis, O. Baz-Han, R.J. Hamers, R.E. Peterson, W. Heideman, J.A. Pedersen.

**307 Uptake of Quantum Dots in Fathead Minnows, Pimephales promelas, Ovarian Explant Cultures.**


The need for understanding the interactions of nanomaterials (NMs) with biological systems is evident, as their use in a variety of applications including cosmetics, electronics, and biomedicine is increasing. Of specific importance is the biological fate of NMs in aquatic organisms, particularly in fish, for which much is not yet understood. Previous research has shown that particle characteristics such as size and surface chemistry (including plasma protein coatings) influence biological fate and ultimately the overall effects. One class of NMs of interest are quantum dots (QD) which are structurally composed of a semiconductor core and shell that can be modified by the addition of different functional groups. The goal of this study was to determine what particle surface characteristics and physiological conditions allow QD uptake of QDs into developing oocytes or their follicle cells in vitro. Oocytes were collected from mature female fathead minnows (FHMs) and transferred to a 12-well plate with approximately even numbers of oocytes in each well. Assay conditions and supplementation was methodically varied in regards to the presence of carp pituitary extract, bCG (25 U/well), QDs (80 nM, a plasma containing exposure) and QD-coated nanoparticles (60 nM, OC-E10, colloidal cysteine, may cause direct toxicity to the oocyte or perturb the functions of the follicle cells.
308 MULTIGENERATIONAL IMPACTS OF CARBON NANOMATERIAL EXPOSURE ON THE MODEL ORGANISM DAPHNIA MAGNA.

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We examined how carbon nanomaterial core structure and surface chemistry influence the toxicity of these materials to the progeny of an exposed F₀ generation of Daphnia magna. F₁ daphnids were exposed to various types of carbon nanomaterials with different core structures and functionalizations. Chronic toxicity was measured on the parent population by evaluating mortality and reproductive parameters over a 21-day period. In addition, the impact of carbon nanomaterials in the presence of additional environmental stressors was evaluated by running experiments under ideal population conditions or highly dense population conditions. The neonates produced by the F₁ generation were raised for an additional 21-day period over a 21-day period. In addition, the impact of carbon nanomaterials in the parent population by evaluating mortality and reproductive parameters was constant in the two cell lines. These results indicate that different dispersants affect MWCNT uptake into cells, and that cytotoxicity depends on the exposed dosage such as IC₅₀ value. It suggests a possible correlation that toxicity appears depending on time even if the exposure of MWCNTs is the low concentration because MWCNTs are biopersistent.

309 THE BIOLOGICAL RESPONSE OF MULTIVALLED CARBON NANOTUBES IN DIFFERENT DISPERSANTS.

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To date, there are many reports about the cytotoxicity of multi-walled carbon nanotubes (MWCNTs). However, the results are still controversial. As the one reason, various dispersants are used in each research. Therefore, we clarified influence of the dispersants of MWCNTs on the cellular uptake and the cytotoxicity. First, we examined the cytotoxicity, MWCNTs uptake and cytokine secretion to MWCNTs in three different dispersants (gelatin, carboxymethyl cellulose and 1,2-Dipalmitoyl-sn-glycer-3-phosphocholine) on human bronchial epithelial cells (BEAS-2B). Cytotoxicity was measured by alamar blue assay and cellular uptake of MWCNTs and cytokine secretion were analyzed using flow cytometry. Next, we researched that the relationship between the cellular uptake of MWCNTs and cytotoxicity using different two cell lines, BEAS-2B and human malignant pleural mesothelioma cells. We found that the cellular uptake of MWCNTs was different for each of the three dispersants and that the level of cytotoxicity and inflammatory response was dependent manner. It is also possible that cationic nanoparticles can interfere with the receptor assembly on lipid rafts, thereby disrupting cellular signaling.

310 THE IN VITRO EFFECTS OF SILICA NANOPARTICLES ON MOUSE MACROPHAGE MORPHOLOGY AND FUNCTION.


Silica (SiO₂) nanoparticles (NPs) are hard core, durable NPs that are being proposed as drug delivery devices for cancer therapy. SiO₂ NPs accumulate in macrophages of clearance organs after systemic administration and may not be fully cleared after administration. We determined if accumulation of SiO₂ NPs (7 nm) in mouse macrophages impact their morphology and function in vitro. RAW 264.7 and J774.A cells were exposed to SiO₂ NPs (0.001 g/L - 0.1 g/L) for variable time periods (24h - 72h) and their viability, proliferation, and function were evaluated. Our results show that exposure to high concentrations (0.01-0.1 g/L) of SiO₂ NP induced cell death as measured by plate based and flow cytometry viability assays. Lower concentrations (0.001 to 0.005 g/L) of SiO₂ NPs significantly reduced cell proliferation by ~ 4 fold as compared to control cells as shown by Coulter counter measurements. Interestingly, SiO₂ NPs induced a heterogeneous cell size distribution in RAW cells. Exposure of RAW cells to 0.005 g/L SiO₂ NPs also induced a 2 fold increase in the number of macrophages larger than 20 μm as measured by Coulter counter. The SiO₂ NP induced cell size increase was also confirmed by confocal microscopy. Cell surface activation markers (CD40, CD80 and CD86) did not show significant changes in expression when dosed with SiO₂ NP (0.0025 to 0.01 g/L). Phagocytosis assays in SiO₂ NP-treated macrophages were performed using fluorochrome conjugated E.coli. Bacterial uptake was assayed by flow cytometry and results show that SiO₂ NPs did not impair phagocytosis at 0.01 g/L. The results indicate that SiO₂ NP exposure can impact macrophage cell morphology and proliferation in vitro, but does not appear to impact activation markers and phagocytosis as indicators of macrophage function.

311 MODULATION OF IL-8 ACTIVITY UPON LIPID RAFT DISRUPTION AND NANOPARTICLE EXPOSURE.

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With the increase in production of engineered nanomaterials, researchers are discovering that there is a direct impact of these nanomaterials on the cell membranes. The cell membrane contains many different types of lipids and proteins responsible for numerous cell functions. The assembly of cholesterol and sphingolipids within the membrane forms microdomain structures known as lipid rafts. Lipid rafts contain many receptors that are involved in regulating cytokine production. Studies have shown that engineered nanomaterials can erode the membrane and create holes. We hypothesized that nanoparticles can interfere with cellular signaling by inhibiting the assembly of receptors on lipid rafts. Using two different cell lines containing an Interleukin-8 promoter Luciferase construct, we were able to evaluate the ability of charged nanoparticles to alter cytokine gene expression elicited by a secondary stimulus. We exposed cells to charged nanoparticles, followed with stimulation of the IL-8 promoter with the proinflammatory cytokine tumor necrosis factor-α (TNF-α). We then used endocytic inhibitors and cholesterol depleters to determine if the nanoparticles rely on endocytosis and membrane cholesterol to alter the TNF-α stimulated IL-8 promoter. Cell lysis products were collected to assess for intracellular proteins and IL-8 promoter expression. Additionally, cells were exposed to nanoparticles after adding TNF-α to determine if the nanoparticles can disrupt cellular signaling upon receptor activation. We found that cationic nanoparticles are able to reduce the IL-8 promoter activity despite exposure to the endocytic inhibitors. When membrane cholesterol is removed from the cells, all the effects of the nanoparticles disappeared. Furthermore, these nanoparticles can only disrupt signaling prior to receptor activation with TNF-α. Our studies demonstrated that cationic nanoparticles can disrupt cellular signaling in a cholesterol dependent manner. It is also possible that cationic nanoparticles can interfere with the receptor assembly on lipid rafts, thereby disrupting cellular signaling.

312 EVALUATION OF OXIDATIVE STRESS AND APOPTOSIS IN THE LIVER FOLLOWING A SINGLE INTRATRACHEAL INSTITUTION OF CEERIUM OXIDE NANOPARTICLES IN MALE SPRAGUE DAWLEY RATS.

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Cerium oxide (CeO₂) nanoparticles appear to exhibit antioxidant properties which have led some to suggest that these particles may be used to treat medical conditions that are associated with increases in oxidative stress. Conversely, other in vitro and in vivo work has suggested that exposure to CeO₂ nanoparticles can, at least under certain conditions, lead to increases in oxidative stress. Herein we attempt to explore the underlying mechanism of this finding. To this end, 7-week old male Sprague Dawley rats (n=72) were randomized to one of two groups: CeO₂ nanoparticle (20 nm diameter) instillation (7 mg/kg in 300 μl normal saline) or age-matched saline control (300 μl normal saline) or age-matched saline control (300 μl normal saline). After instillation, animals were sacrificed at 1, 3, 14, 28, 56 and 90 days (n=6/group). Compared to saline-control animals, the concentration of malondialdehyde (MDA) per gram of liver tissue in CeO₂ exposed animals was 25%, 31% and 20% higher at days 1, 3 and 90 post exposure (P<0.05) while the ratio of Bax to Bcl-2 was 55%, 62%, and 47% lower at days 14, 28 and 56 (P<0.05). Compared to saline-control, the levels of cleaved caspase-3 (17 kDa and 19 kDa fragments) were by 32% higher and 49%
higher (P< 0.05) at days 1 and 3 before being decreased by 35%, 25%, 20%, and 7% at days 14, 28, 56, and 90 respectively. These data suggest that the initial response of the liver to intratracheal instillation of CeO2 nanoparticles is characterized by increased oxidative stress and caspase-3 activation at days 1 and 3 post exposure. Whether these events are associated with hepatic apoptosis and subsequent tissue remodeling is currently under investigation.

**Comparative Study on Antibacterial Efficiency and Cytotoxicity of Various Types of Ion-Doped Titanium Dioxide Nanoparticles.**

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Visible light absorbing titanium dioxide (TiO2) has been widely accepted photosensitizer in harnessing solar energy for photocatalysis process. Doping TiO2 with non-metal or metal ions is one of the most efficient methods to shift the spectral response of TiO2 from UV to visible light region. Efficiency and safety of these materials are important selection criteria for further commercial uses. In this study, chromium (Cr-TiO2), nitrogen (N-TiO2) and carbon-doped TiO2 (C-TiO2) nanoparticles were investigated on their antibacterial efficacy in S. aureus and E. coli and their toxic effects in human skin epithelial (A431) cells. The nanoparticles were firstly subjected to physical and chemical characterizations such as light absorption by UV spectrophotometry, morphology by TEM, chemical composition by EDX, crystal structure by XRD, hydrodynamic diameter by DLS as well as photocatalytic activity by DPPH assay. The results showed that all types of ion-doped TiO2 nanoparticles can absorb both UV and visible light. N-TiO2 nanoparticles showed the highest photocatalytic activity in correlation to their antibacterial effects against S. aureus and E. coli under visible light followed by C-TiO2 and Cr-TiO2, respectively. Using CCK-8 and DCF assays, N-TiO2 and C-TiO2 demonstrated their ability to reduce cell viability and to generate ROS in A431 cells, respectively, in a concentration-dependent manner. The higher effects can be seen in Cr-TiO2. Beside their photocatalytic ability under visible light, it is interesting that non-metal-doped (N-TiO2 and C-TiO2) showed higher antibacterial efficiency but lower toxicity to A431 cells than metal-doped (Cr-TiO2) particles. The results from this study can be used as a selection guidance of ion-doped TiO2 nanoparticles for specific applications.

**Development of In Vitro Bioactivity Profiling of Alternative Sustainable Nanomaterials.**


Sustainable, environmentally benign nanomaterials (NMs) are being designed as alternatives based on functionality to conventional metal-based nanomaterials (NMs) in order to minimize potential risk to human health and the environment. Development of rapid methods to evaluate the potential hazard of alternatives before entering the marketplace is critical for informing material design and utilization. Cellular based high-throughput screening (HTS) assays, currently being utilized in the ToxCast chemical screening project, are valuable to evaluate the differences in the bioactivities of conventional NMs and their alternatives. Preliminary research has focused on development of nanoparticles (NPs) from natural biopolymer materials that will maintain integrity for intended applications and then rapidly degrade post-use. These NMs, infused with active components, would become inert after use and could serve as novel NM platforms in oral drug delivery or as environmental remediation. Biodegradable cellulose and lignin NPs have been synthesized by an environmentally-friendly water-based antisolvent precipitation process based on pH-jump. The hydroxypropyl methylcellulose phthalate NPs (~200-300 nm in diameter) dissolve above pH ~ 5.5 limiting their potential applications. However, the synthesized lignin NPs (~50-100 nm in diameter) have been stabilized up to pH ~ 9, allowing them to be viable in physiological conditions for their intended use and in the HTS assays (pH ~ 7.4). The next phase of research will focus on further characterizing lignin NPs and evaluating bioactivity of infused particles pre- and post-use using ToxCast assays. This research takes an innovative and
proactive approach to enhance the safety of materials, inform hazard assessment up-
stream, and move nanotechnology toward sustainability based on green chemistry
principles. This abstract may not necessarily reflect US EPA policy.

317 EFFECTS OF CERIUM OXIDE NANOPARTICLES ON
DIESEL EXHAUST PARTICLES—INDUCED
PULMONARY RESPONSES.
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Diesel exhaust particles (DEP) are the major constituent of ambient particulate that
are known to induce lung inflammation and injury. When using cerium compo-
sounds as diesel engine catalyst to lower DEP emission, cerium oxide nanoparticles
(CeO2) were detected in the exhaust. Our previous studies have shown that CeO2
not only induces pulmonary inflammation, but also lung fibrosis. In this study, we
investigated the effects of CeO2, DEP and their combination on pulmonary re-
sponses pertaining to lung inflammation and fibrosis. Male Sprague Dawley rats
were exposed to DEP with or without CeO2 (20% w/w) by a single intratracheal
instillation and sacrificed at 1, 10 and 28 days after exposure. Bronchial alveolar
lavage (BAL) was performed; cellular and acellular fractions of BAL fluid were ob-
tained; and particle-induced lung inflammation, cellular toxicity, and alveolar
air/blood barrier damage were determined through monitoring PMN infiltration,
LDH activity, and albumin content in the first BAL fluid. The DEP-induced re-
sponses were acute and transient, peaked at 1-day after exposure, but significantly
decreased at 10- and 28-day post exposure. However, CeO2+DEP induced inflam-
matory responses were found persistent throughout the 28-day exposure period.
DEP-exposed alveolar macrophages induced oxidant and nitric oxide generation
and proinflammatory cytokine, TNF-α and IL-12, production. The presence of
CeO2 markedly reduced DEP-induced cellular responses. At 28 days post exposure,
CeO2- and CeO2+DEP-exposed lungs showed significantly induced phospholipi-
dosis and hydroxyproline content in lung tissues. Morphological analysis showed
that both DEP and CeO2+DEP demonstrated granulomatous lesions. However, there
were more cells and collagen in CeO2+DEP- than DEP-exposed lungs. These
results suggest that exposure of rats to CeO2+DEP induced sustained inflammatory
lung injury and enhanced fibrotic development compared to exposure to DEP
alone. These findings suggest potential health effects of CeO2 when used as diesel
engine catalyst are of concern.

318 COMPARISON OF THE GLOBAL GENE EXPRESSION
OF RAT LUNG INHALED MANUFACTURED
NANOMATERIALS: ULTRAFINE NICKEL OXIDE, C60
FULLERENE, AND CARBON NANOTUBES.
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In this study, comparative analyses of gene expression profiling of the rat lung after
whole-body inhalation exposure to ultrafine nickel oxide (Uf-NiO), C60 fullerene,
multi or single walled carbon nanotubes (MWCNTs or SWCNTs) for 6 h, 4 days,
and 4 weeks were performed to gain insights into the influence of MNs on the pulmonary
system at a molecular level. DNA microarray analysis revealed that high expression
of genes associated with chemokines and response to oxidative stress were induced
by Uf-NiO at both 3 days and 1 month post-exposure. Mmp12 (macrophage met-
alloelastase) was significantly upregulated at both 3 days and 1 month post-expos-
ure. The results suggest that Uf-NiO lead to acute inflammation for the exposure
period, and the damaged tissues are repaired in the post-exposure period. Few genes
associated with chemokines were significantly higher than those of SWCNT or
C60 alone. These findings suggest potential health effects of CeO2 when used as diesel
engine catalyst are of concern.

319 LONG-TERM PHARMACOKINETICS AND
BIO DISTRIBUTION OF SILICA NANOPARTICLES
USING ACCELERATOR MASS SPECTROMETRY IN
VIVO.
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Nanoparticles and their use for commercial applications are continuing to increase,
especially for diagnostic and therapeutic purposes in the biomedical field. However,
to date, the toxicity and biological fate of nanoparticles have not been thoroughly
investigated; this information is critical for translation of nanoparticles for clinical
use. Silica nanoparticles are used for many biological applications including imag-
aging and as drug delivery vehicles. In this work, Accelerator Mass Spectrometry
(AMS), an ultra sensitive technique for quantifying long-lived radioisotopes, is used
to measure the long-term biodistribution and pharmacokinetic properties of silica
nanoparticles after administration in vivo. 14C-Labeled (t1/2=57.30 yrs) carbo-
ylated silica nanoparticles (33nm) were administered as a single bolus i.v. dose to
male mice. AMS was used to quantify the tissue distribution and pharmacokinetic parameters over an eight week period. Within eight hours after dosing, nanoparti-
cles were cleared rapidly from the bloodstream, but were retained in organs of the
reticuloendothelial system, including the liver, spleen, bone marrow and lymphatic
tissue. Small amounts of nanoparticles were also observed in other peripheral or-
gans and in excreta. These results demonstrate that silica nanoparticles can accu-
mulate in organs upon entering the blood stream and that AMS is a powerful tool
to assess the long-term pharmacokinetics and biodistribution of nanoparticles in
vivo. This work performed under the auspices of the U.S. Department of Energy by
Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344
and supported by LLNL CRADA No. PNNL/284.

320 TISSUE-SPECIFIC GENOTOXICITY OF TITANIUM
DIOXIDE NANOPARTICLES EVALUATED USING THE
IN VIVO COMET ASSAY.
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Nanosized titanium dioxide (nanoTiO2) is one of the most widely used nanomate-
rials. Although TiO2 is chemically inert, it can cause lung cancer in rats. The International Agency for Research on Cancer (IARC) recently classified TiO2 as
possibly carcinogenic to humans (class 2B), based on sufficient evidence in experi-
mental animals. Considering that nanosized TiO2 materials are much more reac-
tive than its bulk materials, a genotoxic evaluation of nano-TiO2 is necessary be-
cause the genotoxicity data are required for the cancer risk assessment. In the
current study, we evaluated the tissue-specific genotoxicity of 10 nm TiO2
nanoparticles and the possible mechanisms for their genotoxicity using in vivo
Comet assay. B6C3F1 (1H) male mice were treated by intraperitoneal injection
with 50 mg TiO2 nanoparticles /kg body weight daily for three days and sacrificed
4 and 24 hr after the last treatment. Liver, bone marrow, spleen and lung were col-
lected for the evaluation. The standard Comet assay was performed to detect DNA
breaks induced by the nanoparticles; and the enzyme-modified Comet assays by ad-
dition of human 8-oxo-guanine DNA glycosylase (hOGG1) and endonuclease III
(Endo III) were conducted to measure the oxidative DNA adducts due to the treat-
ment. The study revealed that the treatment significantly resulted in DNA strand
breaks in liver at both the 4 and 24 hr sampling times and in spleen at the 4 hr time
point, but not in other tissues. Statistically significant (p≤0.05) increases of oxida-
tive DNA adducts were observed in all tissue samples at all time points. The results
suggest that the 10 nm TiO2 nanoparticles can induce DNA damage including
DNA single strand breaks and 8-oxo-guanine, which could be the possible mecha-
nism for the induction is the oxidative stress caused by the treatment of the
nanoparticles.
cerium oxide nanoparticles (CNP) released in the exhaust. Previous studies from our laboratory have shown that DEP and/or CNP induced lung inflammation, damage and fibrosis. This study focuses on the effects of DEP- and/or CNP-exposed rats on the susceptibility to endotoxin. Male Sprague-Dawley rats were treated with intratracheal (IT) instillation of DEP (5 mg/kg body weight) and/or CNP (1 mg/kg body weight). After 3 days, the rats were exposed to lipopolysaccharide (LPS) by IT instillation (1 mg/kg body weight) and then sacrificed after 3 additional days. CNP, DEP, CNP+DEP exposures induced neutrophilia, enlarged macrophages, and released inflammatory mediators, interleukin-12 (IL-12) and osteopontin (OPN), into the bronchial alveolar lavage (BAL) fluid. The exposure to LPS further increased neutrophilia, but did not affect particle-induced cytoxicity and air/capillary damage. LPS exposure did not affect IL-12 and OPN production in rats treated with DEP or CNP+DEP but significantly increased IL-12 and OPN in BAL fluid of CNP-treated rats. Increased transforming growth factor beta (TGF-β), an important mediator of fibrosis detected in alveolar macrophages isolated from all particle-exposed rats. TGF-β induction was further enhanced in response to injury. A significant increase of collagen type I was seen in BAL from CNP- and CNP+DEP-exposed rats. Tissue inhibitor of metalloproteinase 1 (TIMP-1), a MMP-9 inhibitor, was markedly increased in particle-exposed groups. LPS exposure did not significantly alter particles-induced MMP-9, but markedly enhanced TIMP-1 levels. These results suggest endotoxin exposure significantly increased TGF-β production, but lowered the MMP-9/TIMP-1 ratio. These changes may lead to extracellular matrix damage and fibrotic development, leading to health concerns.

**324 RAT OVARIAN STEROIDOGENESIS EX VIVO IS STIMULATED DIFFERENTIALLY UPON EXPOSURE TO LOW CONCENTRATIONS OF NANOPARTICLES.** L. Larson1, 2, M. J. Carvan III1, 2, 3, R. Klapier1, 2, and R. J. Hunt1, 2, 3, 4, 5 1Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI, 2School of Freshwater Sciences, College of Natural Sciences and Mathematics, Milwaukee, WI, and 3NIEHS Children’s Environmental Health Sciences Core Center, University of Wisconsin-Milwaukee and Children’s Research Institute, Milwaukee, WI.

Gold nanoparticles (GNPs) have gained considerable attention for use in medicine, consumer goods, and industry due to their advantageous physicochemical properties. However, the effects of GNP exposure on female fertility remain unclear. The objectives of the present study were (1) to evaluate the effects of GNPs (primary diameter = 6.81 ± 1.25 nm; [Mean ± SD]) on testosterone (T) and estradiol-17β (E2) accumulation by rat ovaries in culture using radioimmunoassay; and (2) to identify the locus/loci whereby GNPs modulate ovarian steroidogenesis by our exploitation of multiple-reference gene quantitative real-time RT-PCR (qPCR) to evaluate expression of sterogenic (i.e., star, cyp11a1, hsd3b1, cyp17a1, and cyp19a1) and oxidative-stress (i.e., sod2 and bax) genes. We hypothesized that GNPs modulate P4 and E2 accumulation by affecting expression of one or more genes involved in steroidogenesis and/or oxidative stress. Ovaries were cultured under control or treatment conditions (31.7 pg/ml, 31.7 ppt, or 31.7 pg/gm GNPs) for either 12, 24, or 48 hours. Regression analyses indicated a positive relationship between either star (n=30 rats, p<0.05, r2= 0.278) or cyp11a1 (n=30 rats, p<0.001, r2= 0.366) expression and P4 accumulation upon exposure to 31.7 pg/gm GNPs, regardless of time. Additional analyses showed that E2 accumulation was positively repressed on hsd3b1 (n=24 rats, p<0.05, r2= 0.181) and cyp17a1 (n=23 rats, p<0.01, r2= 0.301) expression, independent of incubation period, upon exposure to 31.7 pg/ml and 31.7 pg/gm GNPs, respectively. These results suggest that GNPs stimulate differentially, depending upon concentration, rat ovarian steroidogenesis in our ex vivo model, via a complex mechanism that involves transcriptional regulation of star, cyp11a1, cyp17a1, and hsd3b1.

**325 EVALUATION OF THE APPLICATION OF STANDARD ASSAYS FOR ASSESSING GENOTOXICITY OF NANOMATERIALS.** T. Chen1, 2, 3, N. Mei1, 2, R. S. Woodruff1, 2, J. Yan1, Y. Chen1, W. Ding1, 2, J. A. Bhati1, R. Sadiq1, Y. Li1, 2, E. Rice1, Y. Zhang1, 2, A. S. Biris3, 2, P. C. Howard1, 2, T. Zhou1, 2, M. M. Moore1, 2, 3, 4, 5 1Division of Genetic and Molecular Toxicology, National Center for Toxicological Research, US FDA, Jefferson, AR, 2Division of Microbiology, Arkansas Regional Laboratory, US FDA, Jefferson, AR, 3Center for Food Safety and Applied Nutrition, US FDA, College Park, MD, 4Nanotechnology Core Facility, National Center for Toxicological Research, US FDA, Jefferson, AR, 5Nanotechnology Center, University of Arkansas at Little Rock, Little Rock, AR and 6Center for Veterinary Medicine, US FDA, Rockville, MD.

The unique properties of nanomaterials may cause adverse effects. It is very important to assure that the current genotoxicity tests are adequate to detect the potential genotoxicity of new nanomaterials. In this study, we evaluated whether the current genotoxicity assays were suitable for assessing the genotoxicity of nanomaterials. Genotoxicity of titanium dioxide nanoparticles (TiO2NPs) and silver nanoparticles...
(AgNPs) were evaluated using the Ames test, the in vivo and in vitro Comet assay, the mouse lymphoma gene mutation assay, the mouse Pgi-t mutation assay, and the in vivo and in vitro micronucleus assay. Mutagenic evaluation of 10 nm TiO2NPs and 5 nm AgNPs with the Ames test showed negative results. Five nm uncoated AgNPs induced mutations dose-dependently in mouse lymphoma cells via an oxidative stress mechanism. Micronuclei in TK6 cells were increased by AgNPs in a dose-, size- and surface coating-dependent manner. While 10 nm TiO2NPs were negative in the mouse lymphoma thymidine kinase mutation assay, the in vivo micronucleus assay, and the Pgi-t mutation assay, they induced DNA damage in TK6 cells, and in mouse liver, lung, spleen and bone marrow when measured using the Comet assay. Our results suggest that the Ames test may not be responsive to the treatment of nanoparticles while the Comet assay is very sensitive to the insults of the nanoparticles. More research, using a wider array of nanomaterials, is needed before drawing final conclusions as to the capability of the genetic toxicology assays for assessing the potential genotoxicity of nanomaterials.

### 326 HEMOPEXIN AS BIOMARKERS FOR ANALYZING THE BIOLOGICAL RESPONSES ASSOCIATED WITH EXPOSURE TO SILICA NANOPARTICLES.

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Recently, practical uses of nanomaterials are rapidly spreading to a wide variety of fields, such as cosmetics, foods, and medicine. However, potential harmful effects of nanomaterials and concerns about the long-term effects that they possess novel properties different from materials with submicron size. In this regard, we have attempted to establish risk assessment system by using biomarkers that estimate or predict the safety and toxicity of nanomaterials. We recently showed that haptoglobin could be a useful biomarker for analyzing the biological responses associated with exposure to silica nanoparticles (nSP). Here, we attempted to identify novel biomarkers by proteomics analysis. Initially, to identify protein biomarkers in mice, we analyzed changes in the level of plasma proteins following treatment with nSP by two-dimensional differential in gel electrophoresis analysis. The quantitative analysis showed that 82 spots displayed increased or decreased expression levels in the plasma of nSP treated mice compared with that of saline treated control mice. LC/TOF/MS analysis of the spots subsequently identified 7 different proteins. Then, we focused on hemopexin, one of the acute phase proteins. ELISA analysis showed that the levels of hemopexin in the plasma increase as the silica particle size decreases. These results suggested that hemopexin could be an additional biomarker for analyzing the biological responses associated with exposure to nSP. We believe this study will enable us to establish the development of safe forms of nanomaterials.

### 327 THE EFFECT OF IN UTERO EXPOSURE TO AMORPHOUS NANOSILICA PARTICLES ON NEONATAL IMMUNE FUNCTION AND NEUROLOGICAL FUNCTION.

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Nanomaterials (NMs) are increasingly being used for commercial purposes due to their unique properties, such as cosmetics, foods, and medicine. However, it is not understood sufficiently for their safety about potential risks to health. We have tried to establish safe assessment system of NMs by using predictable biomarkers for the development of safe NMs. Previously we identified some acute proteins as biomarkers of NMs by proteomic analysis. On the other hand, recent reports showed that microRNAs could be useful biomarkers for organ-specific injury and side effects of medicines. Here we attempted to examine the potential of microRNA as a safety biomarker of NMs. Because we already demonstrated that intravenous injection of silica nanoparticles with a diameter of 70 nm (nSP70) induced liver injury in mice, we focused on the liver-specific microRNA. Mice were intravenously injected with nSP70 and blood was collected at 4, 8, and 24 h after injection. Then, quantitative real-time RT-PCR analysis of microRNA was performed after the purification of small RNAs including microRNAs from plasmas. The expression level of liver-specific microRNA in nSP70 treated mice was higher than that of control mice. In addition, the sensitivity of the liver-specific microRNA was almost the same as the conventional hepatic injury biomarkers such as ALT and AST. These findings suggested that the liver-specific microRNA could be useful safety biomarkers of NMs in addition to ALT and AST. We are now trying to find other useful microRNAs as biomarkers of NMs. We believe that this study will provide useful information for the development of biomarkers of NMs.

### 328 MICRONRNA AS A BIOMARKER FOR THE SAFETY ANALYSIS OF NANOMATERIALS.

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Although zinc oxide nanoparticles (ZnONPs) have been applied in nanotechnology, their kinetics and tissue distribution in vivo are unknown. Here we compared the kinetics and tissue distribution of 10 nm 65ZnONPs, 71 nm 65ZnONPs, and 65ZnONPs injected into mice after intravenous injection. The areas under curve and half-lives in the second compartment of 65ZnONPs were greater than those of 65ZnONPs, the kinetic parameters were similar for both 65ZnONPs. However, the tissue distributions of the three forms were different. ZnONPs preferentially accumulated in the liver and spleen at 24 hrs. At day 28, 65Zn concentration was the highest and in bone and the proportion of recovered 65Zn radioactivity was the highest in the carcass; these had the same ranking, 10 nm 65ZnONPs >71 nm 65ZnONPs >65ZnONPs. Although more than 80% of the 10 nm 65ZnONPs had been excreted by day 28, greater amounts of the 10 nm 65ZnONPs than 71 nm 65ZnONPs or 65ZnONPs had accumulated in other organs (brain, lung, heart and kidneys). Zn ions seem to have a longer half-life in the plasma, but ZnONPs show greater tissue accumulation. Although the size of ZnONPs had no obvious effect on the kinetics, nevertheless the smaller ZnONPs tended to accumulate preferentially in some organs.

### 329 KINETICS AND TISSUE DISTRIBUTION OF NEUTRON-ACTIVATED ZINC OXIDE NANOPARTICLES AND ZINC IN MICE: EFFECTS OF SIZE AND PARTICULATE NATURE.

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Quantum dots (QDs), promising luminescent nanoparticles, have great potential medical applications, with the persistence of safety concerns about the human medical applications, with the persistence of safety concerns about the human...
their genotoxic effects remain under-explored. This study aims to elucidate the toxicokinetics and biological responses and provide the possibility of prediction for cancer therapy by attractive nanomaterial QDs. In this study, CdSe/ZnS core/shell QDs-induced cell death type in A549 cells was evaluated via MTI, apoptosis, and LDH assay and analyzed differential mRNA levels involved in apoptosis with/without UVA/UVB irradiation. The genotoxic effect of CdSe/ZnS QDs was measured, for the first time by comet and micronucleus assay based on human cancer cell line, for the present study. The extent of cell death was the most severe in CdSe/ZnS QDs treatment under UVB irradiation group, which indicated the strong induction of photocytotoxicity by CdSe/ZnS QDs with UVB and it led to both apoptotic and necrotic cell death. In the induction of olve tail moment and micronucleus formation, significant increases were also investigated in CdSe/ZnS QDs under UVB irrad- iation group. Here, we estimated the genotoxic effect and mechanistic details on the CdSe/ZnS QD-induced cell death pathway and suggest, furthermore, it might be used to apply for cancer therapy using UVB radiation.

331 ROLE OF DOSE RATE ON NANO PARTICLE-INDUCED INFLAMMATORY RESPONSES IN VITRO.

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The increased production of nanomaterials has caused a corresponding increase in concern about human exposures in consumer and occupational settings. Numerous in vitro studies in rodents have evaluated dose-response relationships following respira- tory tract (RT) delivery of nanoparticles (NPs) in order to identify potential hazards. These studies often use high doses and bolus delivery methods that do not reflect real world scenarios. We hypothesized that the delivered dose rate is a key de- terminant of the induction of the inflammatory response in the RT. These studies aim to re-evaluate the predictive power of in vivo bolus delivery for NP hazard identifi- cation. F-344 rats (175-270 μg) were exposed to the same deposited dose (200 μg) of TiO2 (80% anatase, 20% rutile; 21 nm) by high dose rate (bolus) intratra- cheal instillation or by low dose rate (aerosol) whole body inhalation over 4 hrs. Controls were exposed to saline or filtered air. The impact of dose rate was also ex- amined in the context of repeated instillation and inhalation exposures (one quarter the dose on each of 4 consecutive days). The greatest post-exposure (24 hrs) in- creases in high dose rate (HDRV) neutrophils were observed. The TiO2 instillate (31.1% ± 2.6% (SD)) and repeated (16.8% ± 3.6%) bolus TiO2 NP exposures. Aerosol delivery resulted in lower inflammation (6.2% ± 1.5%, single; 1.6% ± 0.6%, repeated exposure). For both bolus and aerosol delivery, there was an atten- uation of the neutrophil response when the dose was delivered over 4 days. Similar trends were found for changes in BALF lactate dehydrogenase and β-glucuronidase activities. We conclude that high dose rate NP delivery elicits significantly greater inflammation compared to realistic low dose rate delivery and that bolus delivery overestimates the NP-associated hazard. This research was funded by NIH R01CA134218, P30ES012473, T23E007026, and 5R2CE0518741.

332 SILVER NANOPARTICLE TOXICITY TO RETINAL PIGMENT EPITHelial CELLS IN VITRO IS INFLUENCED BY PARTICLE SIZE AND COATING, BUT NOT UVA RADIATION.


Silver nanoparticles (AgNP) are used in textiles, medical devices, cleaning products and ocular devices because of their antibiotic properties. Because the retina is the only part of the nervous system exposed to light and because other nanoparticles have demonstrated photocytotoxicity, human retinal pigment epithelial cells (ARPE-19) were used to test the potential cytotoxicity and photocytotoxicity of PVP- and citrate-coated silver nanoparticles (Ag-PVP and Ag-CIT, respectively). ARPE-19 cells were grown to confluence in DMEM/F12 + 10% FBS and dosed with 0, 3, 10, 30, 55, 100, & 200 μg/ml of 10, 50, and 75nm Ag-PVP or Ag-CIT(nanoComposition). For phototoxicity tests, the cells were exposed to 2 hrs of either UVA, visible light, or no light exposure 24 hrs after dosing. Cell viability(calcinel A/M apopmpidu iodide) was measured either 24 hrs after dosing (cytotoxicity) or 24 hrs after dark/light/radiation exposure (phototoxicity). Flow cytometry showed dose-related increased side-scatter consistent with cellular uptake of AgNP; silver particles were observed in the cytoplasm of ARPE-19 cells under darkfield microscopy. The cyto- toxicity of nano-silver was dose-dependent, and inversely proportional to particle size. Nano Ag-PVP was more potent than equivalent-sized Ag-CIT for 50 or 75 nm particles. No differences in cytotoxicity were observed between 10 nm Ag-PVP and 10 nm Ag-CIT. No differences were observed between cells treated 10 nm Ag-PVP or 50 nm Ag-CIT and co-exposed to dark (400) visible light or UVA. Thus, the tox- icity of Ag nanoparticles was influenced by particle size and coatings, but no evi- dence of photocytotoxicity was observed. This abstract does not reflect EPA policy.

333 IN VITRO MODEL TO MIMIC THE LUNG EPITHELIAL BARRIER FOR NANO-TOXICOLOGY STUDIES.

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Previous data have shown that upon alveolar deposition, dispersed carbon nanotubes (CNT) can penetrate into the interstitium or pleural area which may indi- cate a specific mechanism of CNT induced pulmonary toxicity. Our hypothesis is that physicochemical properties of CNT could play a key role in determining the penetration mechanism of CNT into deep lung tissue. However, this would be dif- ficult to determine in vivo. Lung epithelial cells typically form tight junctions which serve as a protective barrier against external particulates and can be moni- tored through resistance measurement in vitro. An experimental model was devel- oped using an immortalized human lung epithelial cell line (Calu-3 cells (HTB- 55), ATCC, Manassas, VA) and Transwell ® inserts (Costar 6.5 mm polysylymer membrane with 3.0 μm pores). Calu3 cells were cultured at 10, 20 or 50 x 104 per insert in Eagle’s Minimum Essential Medium (EMEM) containing 15% Fetal Bovine Serum (FBS). After two days, medium was changed to EMEM that contained 2, 5, 10, or 15% FBS to assess serum effects on tight junction formation. Resistance (ohms) of the cell monolayer was monitored every day from days 3 - 18 in culture using the Epithelial Voltomheter and STX2 electrode (World Precision Instruments). A subset of Transwell inserts were collected at various resistances and days in culture and tight junctions were immunofluorescence stained using the ZO-1 antibody. Light microscopy for light junctions was observed using an ophole the resistance reached 2000 ohms in all concentrations of FBS tested. Resistance reached and maintained > 4000 ohms in 5 days at 50k, in 12 days at 20k and 14 days at 10k cell density. Establishment of Calu3 cell monolayers with functional tight junctions is dependent on filter size, seeding density and serum concentration. The Transwell Calu-3 model represents a potential rapid assessment in vitro model to test the effects of nano-material exposure on cell tight junction integrity.

334 GENOTOXIC STRESS RESPONSE GENES ARE DYSREGULATED BY SILVER NANOPARTICLES IN TK6 CELLS.

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Silver nanoparticles (AgNPs) are presently the most used engineered nanomaterials due to their antimicrobial nature. AgNPs have been used in dressings to reduce in- fections in burns, and as antimicrobials in air fresheners, water purifiers, food stor- age containers, and in coatings for clothing. However, their genotoxicity has not been well examined. In this study, 4 genes involved in the genotoxic stress response, p21, GADD45, ATF3, and DDB2, were used to evaluate the genotoxicity of AgNPs. Cells were treated in triplicate for 24 hours with different concentrations of 5 nm AgNPs coated with polyvinylpyrrolidone (PVP) or tannic acid (TA). The cyto- toxicity of AgNPs increased as dose and the doses causing relative cell counts 50% of the control cell count were about 3 ug/ml for PVP-AgNPs and 4 ug/ml for TA-AgNPs. There were dose-dependent increases in the expression of all 4 genes except for those at concentrations causing very high cytotoxicity. The fold changes induced by the AgNPs were small and the highest induction over the con- trol was 2.5-fold that occurred in the p21 gene. The gene expression was signifi- cantly altered by the treatment of the two types of AgNPs for at least one concentra- tion in all the 4 genes (p < 0.05). The response of these genotoxic stress genes to the AgNP treatment was correlated well with our other genotoxicity studies showing that AgNPs induced DNA adducts, DNA breaks, micronuclei and mutations in TK6 or mouse lymphoma cells.

335 CEREBROVASCULAR TOXICITY OF PCBs BOUND TO NANOPARTICLES IN THE EXPERIMENTAL STROKE MODEL.

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Polychlorinated biphenyls (PCBs) are among the most persistent and widespread environmental pollutants. Recent evidence suggests that exposure to PCBs may in- crease the incidence of stroke and worsen stroke outcomes. PCBs released from en- vironmental sources are capable of binding onto nanoparticles present in the envi- ronment and be taken up by humans. However, very little is known about the toxic effects of PCBs assembled onto nanoparticles. In the current study, we hypothesis
that binding to nanoparticles can potentiate PCB-induced vascular toxicity and brain damage in an experimental stroke model through activation of toll-like recep-
tor 4 (TLR4), followed by stimulation of inflammatory responses, and alterations of
tight junction protein expression in brain capillaries. To study this hypothesis, TLR4-deficient (C3H/HeJ) and control (C3H/HeOuJ) mice were exposed to
PCB153-bound to nanoparticles (PCB153-NPs), PCB153, nanoparticles (NPs)
alone, or vehicle (PBS). PCB153 was administered in the amount of 5 mg/kg body
weight. PCB153-NPs-induced stimulation of proinflammatory responses and NF-
κB nuclear translocation were attenuated in TLR4-deficient mice as compared to
control mice. TLR4-deficiency attenuated the decrease in tight junction protein ex-
pression in response to PCB153-NPs treatment in mouse brain capillaries. TLR4
inhibition also protected against PCB153-NPs-induced disruption of tight junc-
tion proteins in cultured human brain endothelial cells. Importantly, PCB153-
NPs-mediated enhancement of the brain injury in an experimental stroke model
was also less pronounced in TLR4-deficient mice than in control mice. The results of
present study indicate the importance of the TLR4-mediated signaling in cere-
brovascular pathology of PCBs and nanoparticles. These findings may also be ap-
licable to other neuropathological disorders where organic pollutants and
nanoparticles, and/or BBB integrity play crucial roles.

336 TOXICITY EVALUATION OF MULTIWALL CARBON NANOTUBES IN RATS AFTER TAIL VEIN ADMINISTRATION.

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We have the limited knowledge about the chronic toxicity of nanomaterials,
whereas a growing number of studies have implicated the toxicological effects of
nanomaterials. Recently, some studies reported that maternally exposure to nanopar-
ticles may transfer to their fetuses and affect the development and function of the
central nervous systems. Further information is needed to clarify the potential for
chronic toxicity, particularly reproductive and developmental toxicity of nanomate-
rials. The aim of our study is to evaluate the biodistribution and reproductive and
developmental toxicity of nanomaterials via realistic exposure route (e.g., pul-
monary exposure). In prior to conduct above examinations, we need to develop the
sample preparation methods, and to determine dose and observation period. In
order to obtain the basic information for establishing above methods, we conducted
tail vein administration study of multi-wall carbon nanotubes (MWNTs) in fe-
male SD rats. MWNTs were added to blood serum obtained from female rats and
then ultrasonicated using an ultrasonic bath for 30 min. MWNTs were well dis-
persed into the serum. MWNT precipitation was not observed during injection.
The above MWNT suspensions were administered to two groups of five rats at
dose of 0.25 and 0.5 mg/kg bw. In both groups, body weight loss was observed at
1 day after administration. However, there are no other significant effects in these
groups. MWNT-related clinical signs of toxicity (e.g., abnormal behavior, irregu-
lar respiration, and pilorection) were not found in any groups of rats during the
observation period. Therefore, we have judged that 0.5 mg/kg bw is the allowable
dose for the ven administration of further examinations. Rats were dissected at 3
days after administration. MWNT deposition was not observed in the exami-
ned tissues (i.e., the heart, lungs, liver, kidneys, and spleen). In order to evaluate
biodistribution of MWNTs, we will perform light microscopic examinations of
MWNTs in rats.
suited in an increment of DNA damage in lung cells and especially in BAL cells which showed a 6-fold increase in comparison with the control group. However, CNTs induced no systemic genotoxic effects in peripheral leukocytes or bone marrow. An inflammatory effect, assessed by the number of neutrophils in BAL fluid and by immunohistopathological analyses, was seen in the same mice. Our findings suggest that long, needle-like CNTs induce both genotoxic and inflammatory effects in a murine model. In contrast, short CNTs induce only primary effects due to inflammation. (Funded by the Finnish Work Environment Fund)

### 340 GREEN TEA EXTRACT DIFFERENTIALLY AFFECTS ACETAMINOPHEN-INDUCED HEPATOTOXICITY BASED ON THE EXPOSURE PATTERN.


Green tea extract (GTE) is a widely used dietary supplement and has been promoted for a wide range of uses such as preventing cancer, aging, and weight loss. There are conflicting reports about the safety of GTE. Human studies suggest that GTE may have some short-term benefit in controlling obesity; but it has been also associated with clinical cases of hepatotoxicity. This study was conducted in B6C3F1 male mice to determine if GTE alters the toxicity of the widely-used over-the-counter analgesic acetaminophen (APAP). APAP was chosen since it is the leading cause of drug-induced acute liver failure in the US and certain conditions may increase its toxicity like chronic alcohol use or concurrent use of drugs or dietary supplements that induce cytochrome P450 activity. The first exposure scenario involved administering an oral dose of APAP (150 or 300 mg/kg) to mice followed by a single dose of GTE (500 or 1000 mg/kg) 6 hours later. The second exposure scenario involved administering once-daily oral doses of GTE (500 or 1000 mg/kg/day) for three days followed by an oral dose of APAP (300 mg/kg) on the 4th day. Hepatotoxicity was assessed 24h after the APAP dose in both scenarios. Based on serum ALT and AST levels, histopathology, and survival, GTE potentiated APAP-induced hepatotoxicity when administered 6h after the APAP dose. In contrast, GTE decreased APAP-induced hepatotoxicity when administered for 3 days prior to the APAP dose. These results and further mechanistic studies might help explain the wide-range of effects noted with GTE, and also highlight the potential for drug-dietary supplement interactions.

### 341 A SYSTEMS BIOLOGY APPROACH TO UNRAVEL MECHANISMS OF CYCLOSPORINE A-INDUCED HEPATOTOXICITY.

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There is a need for a better understanding of the mechanisms behind drug-induced hepatotoxicity which shall improve preclinical testing and diminish the number of hepatotoxic compounds going into animal experiments and clinical trials. Although transcriptomics, proteomics and metabolomics all give valuable information about toxicological responses, separately they don’t give you the complete story. By combining these different techniques in one toxicogenomic analysis, we aim to further unravel the system wide response to a toxic compound. Therefore, we combined transcriptomic, proteomic and metabonomic profiling of HepG2 cells exposed to Cyclosporine A. HepG2 cells were exposed to different concentrations of Cyclosporine A and cells were investigated at several time points. The mRNA and miRNA expression levels were investigated using array technology. The changes in protein expressions were studied using quantitative 2-D Differential Gel Electrophoresis and a metabolomic investigation was done using 1H-Nuclear Magnetic Resonance spectroscopy. The transcriptomics, proteomics and metabolomics analyses demonstrated many dose- and time-dependent changes in Cyclosporine A-exposed HepG2 cells. Cyclosporine A may induce ER stress and disturb the ER-Golgi transport. Moreover, patterns seen after cyclosporine A treatment can be related to cholestatic mechanisms. Our findings indicate that a systems wide approach combining metabolomics, proteomics and transcriptomics with bioinformatics can lead to a better understanding of the underlying mechanisms of drug-induced cholestasis and may help to improve the assessment of novel compounds for cholestatic properties at an early stage in drug discovery.

### 342 A STRUCTURE-TOXICITY-RELATIONSHIP INVESTIGATION OF 2-PHENYLAMINOPHENYLACETIC ACID ANALOGS.

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Diclofenac and lumiracoxib are NSAIDs possessing the 2-phenylaminophenylacetic acid backbone. Although structurally similar, they exhibit different pharmacological and toxicological profiles. Bioactivation of both drugs by Phase I and Phase II enzymes to reactive metabolites such as quinone imines and acyl glucuronides respectively have previously been studied and attributed to be the cause of hepatotoxicity associated with both drugs. However, the mechanistic differences in toxicity due to structural differences have not been previously studied. Therefore, the aim of this study was to investigate the effect of structural changes to the 2-phenylaminophenylacetic acid backbone on the cytotoxic profiles of the analogs. By substituting different alky groups and halogens at positions 5 and 2’6’ respectively, twenty-four analogs were synthesized. These analogs were tested for their cytotoxicity effects using TAMH, a metabolically-active mouse liver cell line and HuH-7, a human hepatoma cell line exhibiting low metabolic activity. The analogs were incubated at varying concentrations for 24 hours with the cells before carrying out the MTT assay to obtain IC50 values. Statistical analysis was carried out using one-way ANOVA. Results obtained showed that toxicity of the analogs were consistently greater in TAMH as compared to HuH-7, indicating that metabolism plays an important role in affecting cell viability. Increasing the size of the alky group at the position 5 resulted in an increase in cytotoxicity. It was also observed that a progressive increase in the size of the halogen at positions 2’6’ from fluorine to bromine resulted in an increase in cytotoxicity regardless of the substitution at the 5-position. These results demonstrate that structural changes at critical positions of the 2-phenylaminophenylacetic acid backbone exert significant effects on cytotoxicity. Further studies such as covalent adduction formation and metabolite profiling will provide mechanistic insights to allow design of safer analogs.

### 343 ACHATINA C-REACTION PROTEIN AMELIORATES LEAD-INDUCED TOXICITY IN RAT HEPATOCYTES.

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Lead is a ubiquitous toxic heavy metal having different sources of environmental exposure. Inhibition of the activity of several proteins through its binding to SH groups has been described as the major cause of lead toxicity (ATSDR, 1998). In the present study 20hM Pb(NO3)2, an environmentally relevant dose of exposure (Kansal and Singh, 1983), induced notable cytotoxicity in rat hepatocytes which was significantly ameliorated by treatments with individual subunits (60, 62, 90 and 110 kda) of C-reactive protein (CRP) isolated from a mollusc Achatina fulica. Isolated rat hepatocytes were incubated in vitro with Pb(NO3)2, Pb(NO3)2 plus each subunit (10tg/kg) for 4h maintaining a concurrent control. Cell viability was assessed by Trypan blue and MITT assay. Viability reduced significantly at 4h (78%) in Pb treated cells compared to control or cells pretreated with CRP subunits (92%). NBT and TBARS assays were performed to estimate O-2 and lipid peroxidation respectively. In TBARS assay microsomal fraction was heated with TBA- TCA-HCl and lipid peroxidation was measured at 535nm. GSH level was determined following the method of Sedlak and Lindsay (1968). In Pb treated cells there was a 26old increase in the rate of O-2 production and lipid peroxidation at 4h against control. Concomitantly GSH depleted rapidly (2old at 1h) in Pb treated cells. GSH depletion was found to be insignificant in rat hepatocytes pretreated with CRP subunits. Furthermore, reduced expression of HSP70 in CRP subunits treated cells clearly indicates amelioration of metal stress. Thus a new functional role of ACRP as a stress reducing agent in heavy metal toxicity in a vertebrate system emerges which warrants further research.

### 344 DIETARY FAT IS A PRIMARY SOURCE OF LIPIDS IN TCD2-ELICITED HEPATIC STEATOSIS.

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Lead is a ubiquitous toxic heavy metal having different sources of environmental exposure. Inhibition of the activity of several proteins through its binding to SH groups has been described as the major cause of lead toxicity (ATSDR, 1998). In the present study 20hM Pb(NO3)2, an environmentally relevant dose of exposure (Kansal and Singh, 1983), induced notable cytotoxicity in rat hepatocytes which was significantly ameliorated by treatments with individual subunits (60, 62, 90 and 110 kda) of C-reactive protein (CRP) isolated from a mollusc Achatina fulica. Isolated rat hepatocytes were incubated in vitro with Pb(NO3)2, Pb(NO3)2 plus each subunit (10tg/kg) for 4h maintaining a concurrent control. Cell viability was assessed by Trypan blue and MITT assay. Viability reduced significantly at 4h (78%) in Pb treated cells compared to control or cells pretreated with CRP subunits (92%). NBT and TBARS assays were performed to estimate O-2 and lipid peroxidation respectively. In TBARS assay microsomal fraction was heated with TBA- TCA-HCl and lipid peroxidation was measured at 535nm. GSH level was determined following the method of Sedlak and Lindsay (1968). In Pb treated cells there was a 26old increase in the rate of O-2 production and lipid peroxidation at 4h against control. Concomitantly GSH depleted rapidly (2old at 1h) in Pb treated cells. GSH depletion was found to be insignificant in rat hepatocytes pretreated with CRP subunits. Furthermore, reduced expression of HSP70 in CRP subunits treated cells clearly indicates amelioration of metal stress. Thus a new functional role of ACRP as a stress reducing agent in heavy metal toxicity in a vertebrate system emerges which warrants further research.

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50%, 60%, or 70% carbohydrate exhibited increased relative liver weights in all groups following gavage with 30 μg/kg TCDD for 168 h. Gas chromatography/mass spectrometry analysis of hepatic extracts identified a ~1.5-fold and ~2-fold FA increase in mice on the carbohydrate and fat diets, respectively. Interestingly, mice on the 5%, 10% and 15% fat diets exhibited a dose-dependent increase in the dietary essential FAs, linoleic acid (18:2n6) and linolenic acid (18:3n3). No dose-dependent increase in dietary FA increase was detected when comparing on-genotype TCDD-treated mice suggesting TCDD-induced steatosis is due to enhanced FA uptake rather than de novo lipogenesis. In Scd1 null C57BL/6 mice, that are incapable of desaturating stearate (18:0) to oleate (18:1n9), TCDD increased oleate 3-fold. This increase is also consistent with TCDD enhancing dietary FA uptake since oleate represents 81% of all monounsaturated FA in Harlan 79764 Teklad F6 rodent chow. Moreover, TCDD decreased serum C14 levels 1.7-fold, increased liver C14 levels 2-fold, and decreased parametral fat pad C14 content 3-fold, while having no effect on muscle C14 levels. Notably, C14 levels were >50-fold higher in TCDD-treated livers compared to other examined tissues. This is consistent with the induction of lipid transport genes Cd36 and Faslp in the intestine and G6D6, Vldlr, and Ldlr in the liver by TCDD. These results provide compelling evidence that increased uptake of dietary fat is a primary source of lipids in TCDD-elicited hepatic steatosis. Funded by SBRP P42ES04911.

345 TCCD-ELICITED EFFECTS ON LIVER, SERUM, AND ADIPOSE LIPID COMPOSITION IN SCDD+/+ AND SCDD−/− MICE.


Mouse studies have shown that the aryl hydrocarbon receptor (AhR)-mediated induction of stearoyl-CoA desaturase -1 (Scd1) alters hepatic lipid composition in 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-elicited steatosis. Comparative studies were conducted in fasted 4-week-old female Scdd+/+ and Scdd−/− mice treated with 30 μg/kg TCDD for 24, 72, and 168 h to investigate elicited changes in liver, serum and adipose lipid composition. Relative liver weights were increased in both Scdd+/+ and Scdd−/− mice at all time points while relative parametral fat pad weights decreased in Scdd+/+ (72 and 168 h) and Scdd−/− (72 and 168 h) mice. Mouse serum analysis identified a ~150% increase in total fatty acids (FAs) in both genotypes, and a 120%, 210%, and 135% increase in monounsaturated (MUFAs), saturated (SFAs), and polyunsaturated fatty acids (PUFAs), respectively, in Scdd+/+ mice. In hepatic lipid profile comparisons between genotypes, SFA and PUFA levels decreased 21% and 11%, respectively, while MUFA levels increased 234% in Scdd+/+ compared to Scdd−/− mice. Serum analysis identified a 7% and 11% decrease in absolute FAs in both Scdd+/+ and Scdd−/− mice, respectively. Serum lipid panels also revealed TCDD decreased total cholesterol and high-density lipoprotein particles by 25% at 168 h in both genotypes. GC/MS analysis of adipose EAMES at 168 h identified a TCDD-dependent 30% decrease in SFAs, MUFAs, and PUFAs in Scdd+/+ mice whereas SFAs and PUFAs increased 30% and MUFAs didn’t change in Scdd−/− mice. Adipose FA transporter, SCD2A1, was induced in both genotypes (3- to 7-fold) and AIPQ, involved in lipid catalysis, was increased 1.6-fold in wild-type mice. Collectively, these results suggest that, in addition to altered hepatic lipid composition, AhR mediates alterations in systemic lipid metabolism. Funded by SBRP P42ES04911.

346 A SURVEY OF MECHANISMS FOR DRUG-INDUCED LIVER INJURY.

V. Vijay and W. Tong.

Sponsor: W. Dekant.

Drug-induced liver injury (DILI) remains a major cause that results in termination of drug development programs or regulatory actions. Drugs that cause similar histopathological changes may induce DILI through similar mechanisms. Studying drugs that cause the same histological picture and performing comparative analysis of drugs with different histopathological manifestations could shed light on the mechanisms of toxicity and guide further research using emerging technologies. Currently, most histopathological terms used in the literature to describe DILI are free text, which has resulted in diverse histopathological terms being used to describe the same injury in clinical reports. This makes the analysis and interpretation of histopathological data difficult, especially for researchers without specialized training in pathology. We reviewed the histopathological terms used in the literature to describe various liver injury types associated with 115 drugs that were either withdrawn from the market due to hepatotoxicity or had indications of DILI in FDA-approved drug labeling. These terms were subsequently analyzed against the morphological abnormality terms in Systematized Nomenclature of Medicine (SNOMED), which resulted in a histopathological DILI ontology. The ontology characterizes different DILI types in humans with a controlled vocabulary (i.e., standard histopathological term) and the logical relationships (i.e., hierarchical tree) among them. We then mapped 115 drugs into the ontology terms. For each term, we determined the genomic signatures based on the drugs with the gene expression data available from Japanese Toxicogenomics database using ArrayTrack™. The implications of genomic signatures were interpreted through pathway analysis using KEGG and IPA. This ontology driven gene expression analysis linking to different levels of characterization of histopathology in DILI provides a new way to discern mechanisms of different DILI types. The preliminary results from this study will be discussed.
mation to 4'- and 5-hydroxy-Dcl, consistent with down-regulation of various Cyp550s (e.g. Cyp550C11) as evidenced by qRT-PCR. Hepatic gene expression analysis suggested activation of NFκB and MAPK pathways and up-regulation of co-stimulatory molecules (IL-1β, TNF-α, CINC-1) by LPS/Dcl and PIC/Dcl, but also down-regulation of protective factors (HSPr, SOD2). However, only LPS/Dcl lead to extensive release of pro-inflammatory cytokines (IL-1β, IL-6, IFN-γ, TNF-α) and factors thought to constitute danger signals (HMGB1, CINC-1). Our results show that cellular stress mediated by LPS but not by PIC or BSO reduce the threshold of diclofenac hepatotoxicity.

Organic halides have been used for syntheses of many drugs. In the present study, hepatotoxic effects of two brominated compounds, benzyl bromide and ethyl bromide, and their conjugation with glutathione (GSH) were compared in male Sprague Dawley rats. Animals were treated orally with benzyl bromide at 500 and 1,000 mg/kg in corn oil once or treated orally with ethyl bromide at 1,000 and 1,500 mg/kg for 24 hr. S-Benzyl GSH and S-ethyl GSH were identified in livers treated with benzyl bromide and ethyl bromide, respectively, by liquid chromatography-electrospray ionization tandem mass spectrometry. At the doses used, benzyl bromide showed increased activities of serum alanine aminotransferase and aspartate aminotransferase. Meanwhile, ethyl bromide was not hepatotoxic at the doses tested in the present study. Hepatic GSH levels were almost completely depleted by the treatment with benzyl bromide. However, the decrease in GSH by ethyl bromide was relatively marginal. The present results suggest that the decreased level of hepatic GSH, due to the formation of GSH conjugates, might be a critical process for the toxicity induced by low molecular weight brominated compounds. [Supported by a grant (2010-00266220) from National Research Foundation of Korea.]

**349 INTERSTRAIN DIFFERENCES IN SUSCEPTIBILITY TO LIVER INJURY INDUCED BY METHYL DEFICIENCY IS ASSOCIATED WITH PPARα EXPRESSION.**


Nonalcoholic fatty liver disease (NAFLD) is a major health problem and the leading cause of chronic liver disease in the United States and developed countries. In humans, genetic factors greatly influence individual susceptibility to NAFLD. Elucidating the molecular basis for these individual differences to disease susceptibility is critical in human patient care. In our own previous study, we demonstrated that Pparα expression is an important factor in determining individual susceptibility to NAFLD and one of the key mechanisms in protecting liver from injury induced by the methyl-deficiency.

**350 N-ACETYL-META-AMINOPHENOL, THE ALLURED NON-TOXIC ISOER OF ACETAMINOPHEN, IS TOXIC IN BOTH RAT AND HUMAN PRECISION-CUT LIVER SLICES.**

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N-acetyl-meta-aminophenol (AMAP) is generally considered as a non-toxic region-of-the well-known hepatotoxic acetaminophen (APAP). However, so far AMAP has only been shown to be non-toxic in mice and hamsters. To investigate whether AMAP could also be used as non-toxic analog of APAP in studies in rat and human, the toxicity of APAP and AMAP was tested ex vivo in precision-cut liver slices (PCLS) of mouse, rat and human. Based on ATP content and histomorphology, APAP was most toxic in mouse and least toxic in human PCLS. Surprisingly, although AMAP showed a much lower toxicity than APAP in mouse PCLS, AMAP was more toxic than APAP at all concentrations tested in both rat and human PCLS. In HepG2 cells, AMAP caused similar apoptosis and induced a higher Nrf2-mediated oxidative stress signal than APAP. The protein profile in the medium of AMAP-treated rat PCLS was similar to that of APAP, whereas in the medium of mouse PCLS it was similar to the control. Metabolite profiling indicated that mouse PCLS produced the highest amount of glutathione conjugate of APAP, while no glutathione conjugate of AMAP was detected in all three species. Mouse also produced ten times more hydroquinone metabolites of AMAP than rat or human. The marked species differences in APAP and AMAP toxicity and metabolism underline the importance of using human tissues for better prediction of toxicity in man.

**351 IDENTIFICATION OF GLUTATHIONE CONJUGATES OF BENZYL AND ETHYL BROMIDE IN MALE SPRAGUE DAWLEY RATS.**

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Acetaminophen (APAP) is a commonly consumed drug. Overdose results in acute liver failure in humans and in experimental animal models. The role of complement activation in APAP-induced hepatotoxicity has not been evaluated. Complement comprises more than 30 proteins that can participate in tissue injury and/or repair. Treatment of male, C57Bl/6j mice with APAP (200-400 mg/kg) resulted in liver injury as evidenced by increased activity of alanine aminotransferase (ALT) in plasma, hepatic necrosis and neutrophil infiltration in liver. C3 is a central component of the complement pathway. C3 concentration in plasma was significantly reduced by 6h after treatment with APAP. Immunostaining with anti-C3b antibody revealed accumulation of C3b around the centrilobular, necrotic areas. These results suggest activation of complement components by APAP treatment. Cobra venom factor (CVF) was used to deplete complement components. Pretreatment with CVF (15 U/mouse) abolished APAP-mediated C3b accumulation, and this was accompanied by a reduction at 24 h in plasma ALT activity, hepatic necrosis, hepatic neutrophil infiltration and expression of inflammatory genes (IL-6, IL-10 and PAI-1) relative to mice treated with APAP alone. Loss of hepato-cellular glutathione was similar in APAP-treated mice pretreated with saline or CVF, suggesting that CVF pretreatment did not affect APAP bioactivation. Mice deficient in C3 had reduced ALT activity in plasma 6 and 12 h after APAP administration compared to wild-type animals. These data implicate a key role for complement activation in hepatic inflammation and progression of injury during the pathogenesis of APAP-induced hepatotoxicity. [Supported by NIH grant R01DK087886]
was to evaluate S-S-adenosyl-L-methionine (SAMe) protection of APAP induced subcellular post-translation modification of proteins and polyamine homeostasis. The hypothesis was that S-adenosyl-L-methionine (SAMe) may prevent oxidative stress and protect polyamine pathways. Mice were divided into vehicle (VEH, 15 ml/kg water ip), SAMe (1.25 mmol/kg 5 ml/kg/ip), APAP (250 mg/kg/ip), SAMe and APAP (SAMe administered one hour before APAP) and livers were collected 2, 4, and 6 hours following APAP. Western analysis of hepatic subcellular fractions evaluated 4-Hydroxyenonal posttranslational modifications occurring after APAP treatment. APAP at 4h induced 4-hydroxyenonal (4HNE) adduction of carboxyl phosphate synthetase-1 (CPS-1). 3-Nitrotyrosine (3-NT) adduction of mitochondrial proteins was significantly increased 2 and 4h following APAP compared to VEH. The APAP associated rise in 3-NT adduction within mitochondrial fractions was reduced by SAMe at 4h post APAP. APAP depressed spermine (Spm) at 4 and 6h, while SAMe increased Spm levels, when given after APAP. In conclusion, we have identified novel protection mechanisms of SAMe against APAP toxicity. (Supported by NIH Grants INBRE 5P20RR016477-05S4 and 5P20RR016477 to the West Virginia Idea Network for Biomedical Research Excellence).

**354 PEROXYNITRITE IS CENTRAL TO INCREASED HEPATIC LEPTIN RESISTANCE IN XENOBIOTIC METABOLISM OF CYP2E1-INDUCED NASH.**

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We hypothesized that xenobiotic metabolism by CYP2E1 induced steatohepatitis of obesity increases circulating leptin and down regulates both OB-Ra and OB-Rb forms of leptin receptor through peroxynitrite mediated free radical mechanisms. Results indicated that steatohepatitis progression by cytochrome P450 was associated with increased bioactivation of CYP2E1 increased serum leptin levels significantly (48ng/ml in diet induced obese mice (DIO)) to 132 mg/ml in DIO mice with steatohepatitis. This was also associated with reduced stress in steatohepatitic livers, inhibitable by either, or NADPH oxidase inhibitor apocynin or iNOS inhibitor 1400W and in NADPH oxidase or iNOS KO mice. Macrophage depleted DIO mice had significantly decreased oxidative stress suggesting that resident macrophages were the seat of free radical induced inflammation. Since absence of both forms of leptin receptors OB-Ra and OB-rb has been shown in the liver, we explored the expression patterns of these receptors in the steatohepatitis model. OB-Ra and OB-Rb mRNA expression was significantly reduced in steatohepatitis of obesity (16% and 44% respectively) when compared mice with no steatohepatitis. Administration of FeTPPS alone increased the expression OB-Ra and OB-Rb(12 fold and 21fold respectively) while administration of apocynin or 1400W increased it 3 and 5 folds respectively when compared to steatohepatitic mice. Finally mice that were depleted for resident macrophages had increased expression of both OB-Ra and OB-Rb. Further FeTPPS induced increases in both OB-Ra and OB-Rb isoforms of the leptin receptor had a significant correlation with improved progression in steatohepatitis as evidenced by lesser tissue damage. Thus our data for the first time show that peroxynitrite mediated free radical mechanisms are key to induction of increased leptin resistance and steatohepatitic lesions in obesity following xenobiotic metabolism of CYP2E1.

**355 DEVELOPMENT OF A TOXICITY PROFILE FOR THE HEPATOCARCINOCGENIC CONAZOLES: CYPROCONAZOLE, EPOXICONAZOLE, AND PROPICONAZOLE.**

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Conazole are fungicides used in farming as pesticides and in medicine as Pharma- ceutical agents. The mechanism of conazole-liver carcinogenesis in mice has been the subject of intensive investigations. The motivation for this study was to apply toxicological and transcriptomic approaches to develop a toxicity profile for hepatocarcinogenic conazoles using cyproconazole, epoxiconazole and propiconazole. In the current study, male CD-1 mice were fed dietary levels of cyproconazole (0, 50, 100, or 200 ppm), epoxiconazole (0, 50, 200, or 500 ppm) or propiconazole (0, 500, 1250 or 2500 ppm) for 30 d. Each conazole induced hepatoemalgenesis and hepato- cytotoxic hypertrophy, decreased serum cholesterol and hepatic levels of all-trans retinoic acid, and increased hepatic cell proliferation. Microarray-based transcriptional analysis revealed 330 differentially expressed probesets common among these conazoles, including strong dose-dependent responses for CYPs, glutathione S-transferases, and markers of oxidative stress. Further analyses identified an 80 gene subset common to the three conazoles that were functionally related to cancer. Within these genes were pathways associated with xenobiotic metabolism, oxidative stress, cell signaling, and cell proliferation. A common TGF-α-centric gene signaling pathway was identified that may be the mechanism produced by these tumorigenic conazoles. (This abstract does not reflect EPA policy and mention of trade names is not an endorsement for any product).

**356 EFFECT OF DIETARY FAT/CARBOHYDRATE RATIO ON PROGRESSION OF ALCOHOLIC LIVER INJURY AND BONE LOSS IN RATS FED VIA TOTAL ENTERAL NUTRITION (TEN).**

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Diet is an important factor in the development of alcoholic liver injury. However, few studies have examined the effects of diet on the dynamics of injury progression or on alcohol-induced bone loss. In the current study, 300 g male Sprague-Dawley rats (N = 10/group) were intragastrically infused isocaloric liquid diets via TEN where fat content was either 5% (high carbohydrate, HC) or 45% (high fat) with or without ethanol (EtOH) substitution for carbohydrate calories to a final level of 13 g/kg/d. After TEN feeding for 14, 28 or 67 d, rats were sacrificed and liver and tib- bone analyzed. HC rats gained more weight and had larger fat pads than HF rats with or without EtOH (P<0.05), whereas blood/urine EtOH values did not differ. Steatosis developed more rapidly in EtOH/HC rats than EtOH/HF rats (P<0.05) accompanied by increases in mRNAs encoding genes involved in fatty acid (FA) synthesis (FASN, ACC1). However, over time control rats also developed steatosis associated with increased expression of FA transporter, CD36. Genes related to FA degradation were elevated by EtOH-independent of diet (P<0.05), but no ef- fects were observed on genes associated with VLDL synthesis. Despite higher levels of steatosis, HC/EtOH rats had no increase in lipid peroxidation until day 67 and no increases in necrosis related to controls. In contrast HF/EtOH rats had in- creased lipid peroxidation from day 28 and increased necrosis over controls and higher expression of CYP2E1 and CYP4A1 enzymes over HC/EtOH rats by day 67. In contrast, no dietary differences were observed on EtOH-induced loss of bone mineral density or strength. Supported in part by R01 AA08645 (TMB), R01 AA018282 (MR).

**357 THE ROLE OF SERINE/THREONINE KINASE (STK) IN LIVER MACROPHAGE ACTIVATION FOLLOWING ACETAMINOPHEN (APAP) INTOXICATION.**

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Classically activated macrophages and mediators they release play an important role in promoting APAP hepatotoxicity, while, alternatively activated macrophages are involved in wound repair. Hepatocyte-derived macrophage stimulating protein functions to promote alternative macrophage activation by binding to its receptor, STK. STK--/-- mice were used to analyze the role of this protein in regulating alter- native macrophage activation following APAP intoxication. STK--/-- mice were signifi- cantly more sensitive to APAP than wild type (WT) mice. Thus, while adminis- tration of APAP (300 mg/kg, ip) to WT mice resulted in 8% mortality at 24 h, in STK--/-- mice, mortality was 44%. Surviving STK--/-- mice exhibited severe hemor- rhagic cirrhotic hepatic necrosis with injury extending into the midzonal and portal regions of the liver, a response which was not evident in WT mice. This was accompanied by significantly greater increases in serum transaminase which were apparent within 6 h of APAP intoxication. Increased hepatotoxicity was not due to differences in APAP metabolism, as measured by serum levels of free APAP and APAP-glucuronide. However, a persistent depletion of hepatic glutathione was noted in livers of STK--/-- mice when compared to WT mice. This was correlated with increased expression of heme-oxygenase-1, a marker of oxidative stress. In WT mice, APAP intoxication resulted in a time related increase in expression of macrophage chemoattractant protein-1 (MCP-1) and tumor necrosis factor-α (TNFα), which are known to be important in recruiting alternatively activated macrophages into the liver and initiating tissue repair. Expression of these mediators was signific- antly reduced in STK--/-- mice. These data demonstrate that STK plays an impor- tant role in regulating alternative macrophage activation and tissue repair in the liver following APAP intoxication. Supported by NIH GM034310, ES004738, CA131624, AR055073 and ES050522.
Cocaine is a local anesthetic and well known to be one of major abuse drugs. It is known that cocaine mediates organ toxicities including injury to the liver. We thus investigated a protective effect of auranofin against hepatic injury induced by cocaine. Cocaine (75 mg/kg) markedly increased serum alanine amino transferase (ALT) (4,130 IU/L) and aspartate amino transferase (AST) (1,730 IU/L) activities at 16 h after treatment, and induced hepatic necrosis surrounding central veins in mice. Concurrently, overexpression of HO-1 was identified at the edges of cocaine-mediated necrotic area. Auranofin (10 mg/mL, i.p.) significantly induced hepatic HO-1 protein in mice from 12 h after treatment. Interestingly, pretreatment with auranofin resulted in the prevention of the increase of serum ALT and AST activities in a dose-dependent manner. On the other hand, although cocaine increased tumor necrosis factor α (TNFα) gene expression in mouse livers, cocaine-induced liver injury was observed in TNFα deficient mice as well as wild-type mice. Auranofin-induced HO-1 gene expression was observed in human primary hepatocytes but not in mouse primary hepatocytes. The present findings suggest that auranofin is effective in preventing cocaine-induced hepatic injury, and HO-1 may contribute to protect against chemically-induced cytotoxicity.

EXACERBATION OF ACETAMINOEPHEN-INduced HEPATOTOXICITY BY THE ANTHELMINTIC DRUG FENBENDAZOLE.

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Fenbendazole (methyl 5-(phenylthio)-2-benzimidazole, Fen) is a broad spectrum anthelmintic agent commonly added to laboratory rodent feed to prevent or treat pinworm infestation. Potential interactions between Fen and hepatotoxins are unknown. In the present studies, we examined the interaction between Fen and acetaminophen (APAP). Mice were fed a control diet or a diet containing Fen (8-12 mg/day) for 7 days prior to APAP intoxication (300 mg/kg). We found that mice fed the Fen-containing diet were significantly more sensitive to APAP than mice on the control diet. Thus mice on the Fen diet exhibited exaggerated centrilobular hepatocellular necrosis and increased serum transaminase levels when compared to animals fed a control diet. Moreover, administration of APAP to mice on the Fen diet resulted in 63% mortality by 24 h compared to no mortality in mice fed a control diet. The Fen-containing diet alone caused no hepatotoxicity. Analysis of liver microsomal cytochrome P450s (cyp) revealed that Fen had no effect on the activities of cyp2e1 and cyp3a, the major enzymes mediating the first and second phase metabolism of the toxic APAP metabolite, NAPQI. In contrast, cyp2e1 activity was suppressed. A prolonged suppression in hepatic glutathione (GSH) was also noted in APAP-treated mice fed the Fen diet when compared to the control diet. These data demonstrate that Fen exacerbates the hepatotoxicity of APAP, an effect which is related to GSH depletion. These findings suggest a potential drug-drug interaction that should be considered in rodent colonies treated with Fen. Supported by NIH grants ES-009649, ES-019487, and DK-081461.

AURANOIN PROTECTS AGAINST COCAINE-INduced HEPATIC INJURY THROUGH INDUCTION OF HEME OXYGENASE-1.

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Auranofin, a disease-modifying gold compound, has been empirically applied to the management of rheumatoid arthritis. It is reported in vitro that auranofin suppresses the release of inflammatory cytokines from immune cells; moreover, the drug has an ability to induce heme oxygenase-1 (HO-1), a rate-limiting enzyme for heme degradation and an oxidative stress responsive protein. However, action of auranofin and effect against chemically-induced hepatic injury are obscure. Auranofin is a local anesthetic and well known to be one of major abuse drugs. It is known that cocaine mediates organ toxicities including injury to the liver. We thus investigated a protective effect of auranofin against hepatic injury induced by cocaine. Auranofin (75 mg/kg) markedly increased serum alanine amino transferase (ALT) (1,130 IU/L) and aspartate amino transferase (AST) (1,730 IU/L) activities at 16 h after treatment, and induced hepatic necrosis surrounding central veins in mice. Concurrently, overexpression of HO-1 was identified at the edges of cocaine-mediated necrotic area. Auranofin (10 mg/mL, i.p.) significantly induced hepatic HO-1 protein in mice from 12 h after treatment. Interestingly, pretreatment with auranofin resulted in the prevention of the increase of serum ALT and AST activities in a dose-dependent manner. On the other hand, although cocaine increased tumor necrosis factor α (TNFα) gene expression in mouse livers, cocaine-induced liver injury was observed in TNFα deficient mice as well as wild-type mice. Auranofin-induced HO-1 gene expression was observed in human primary hepatocytes, but not in mouse primary hepatocytes. The present findings suggest that auranofin is effective in preventing cocaine-induced hepatic injury, and HO-1 may contribute to protect against chemically-induced cytotoxicity.
Acid synthesis. Conversely, terpinelione-induced SREBP-1c activity was inhibited by rapamycin pre-treatment. These data provide evidence that terpinelione can cause fatty liver disease mediated by activating fatty acid synthesis regulated by SREBP-1c.

**Increased Susceptibility to Drug-Induced Toxicity in Nonalcoholic Steatohepatitis.**

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Adverse drug reactions (ADRs) often arise from inter-individual variation in drug metabolism and disposition, and resulted in 82,724 deaths in 2010. Hepatic drug metabolizing enzymes and transporters play a crucial role in determining the fate of most drugs. Alterations in these processes can place individual patients at greater risk for ADRs. We have shown that nonalcoholic steatohepatitis (NASH), the most severe form of nonalcoholic fatty liver disease (NAFLD), leads to increased systemic retention of acetaminophen and ezetimibe due to elevated expression of sinusoidal transporters and disrupted localization of canalicular transporters. These transporters (Abcc1, 2, 3, 4, Abcg1, and Abcg2) are responsible for the disposition of numerous drugs such as methotrexate, which has significant toxicity that varies between patients. The purpose of the current study was to determine the effect of NASH on the disposition and toxicity of MTX. Sprague-Dawley rats were fed either a control or methionine-choline deficient diet for 8 weeks to induce NASH, followed by administration of a single i.p. dose of vehicle, 10, 40, or 100mg/kg MTX. Blood, urine, and fecal samples were collected up to 96 hours, followed by tissue terminal collection. At the onset of MTX dosing, Abcc1, 2, 3, 4, Abcg1, and Abcg2 were elevated in NASH livers, whereas Abcc2 and Abcg1 appeared to be removed from the canalicular membrane. Importantly, 40 and 100mg/kg doses of MTX resulted in hepatocellular damage followed by initiation of repair (as evidenced by positive PCNA staining) only in NASH animals. Control livers, regardless of MTX dose, did not exhibit significant hepatocellular damage and repair. In contrast, intestinal toxicity in NASH animals tended to be less severe, possibly due to altered MTX hepatobiliary disposition. These data suggest that the hepatobiliary disposition of MTX, governed by expression and localization of efflux transporters, may be altered in NASH, resulting in increased susceptibility to MTX hepatotoxicity.

**L-Serine Attenuates Steatosis by Suppression of Hyperhomocysteinemia.**

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Increasing evidence showed that hyperhomocysteinemia plays an important role in the development of steatosis. In this study we evaluated the effects of L-serine (L-ser) on the ethanol-induced steatosis and elucidated the possible mechanisms of action in terms of the inhibition of the maturation of SREBP-1c through suppression of hyperhomocysteinemia. In *vitro* studies showed that treatment of AML-12 murine hepatocytes with methionine and homocysteine increased the intracellular homocysteine levels and triglyceride contents, whereas cotreatment with L-ser decreased the homocysteine and triglyceride accumulation. L-ser treatment increased the expression of insulin-induced gene-1 (INSIG-1) and inhibited the maturation and translocation of SREBP-1c to the nucleus. Transfection of the cells with siRNA for betaine homocysteine S-methyltransferase (BHMT) or cystathionine beta-synthase (CBS) increased cellular homocysteine and triglyceride level possibly due to the increase in the activation of SREBP-1. L-ser treatment reversed the SREBP-1 activation and triglyceride accumulation in BHMT siRNA-transfected cells, but not in CBS siRNA-transfected cells. Male C57BL/6 mice received ethanol (5g/kg body weight) by gavage every 12 hours for a total 3 times. L-ser (200mg/kg body weight) was administrated by gavage for the last two ethanol treatment. L-ser treatment significantly attenuated hyperhomocysteinemia. Ethanol-induced activation of SREBP-1 and accumulation of triglyceride in the liver were reversed by L-ser. These results demonstrate that L-ser inhibits hyperhomocysteinemia by accelerating the conversion of homocysteine to cystathionine which results in the amelioration of SREBP-1 activation and fatty liver.

**LYSOSOMAL DIVERGENT METAL TRANSPORTER 1 (DMT-1)-MEDIATED IRON RELEASE CONTRIBUTES TO ISCHEMIA-REFPERUSION INJURY IN RAT HEPATOCYTES.**

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Translocation of chelatable iron from lysosomes to mitochondria plays a key role in ischemia-reperfusion (IR) injury of rat hepatocytes. However, the mechanism of iron release from lysosome during ischemia remains unknown. Divergent Metal Transporter 1 (DMT-1) is a cation transporter that mediates translocation of Fe" across lysosomal membranes. Accordingly, our AIM was to investigate the role of DMT-1 in IR-induced lysosomal iron release and iron-dependent oxidative injury in rat primary hepatocytes. Methods: Overnight cultured hepatocytes were incubated anaerobically at pH 6.2 for 4 h and re-oxygenated at pH 7.4 to simulate IR. Chelatable Fe" and mitochondrial membrane potential were monitored by confocal fluorescence microscopy of calcein and tetramethylrhodamine methyl ester (TMRM). ROS generation and cell killing were assessed by chloromethyl dichlorofluorescein (cmDCF) and propidium iodide fluorescence. Results: Lysosome-induced quenching of cytosolic calcein was blocked by pretreatment with ebselen (20 μM), an inhibitor of DMT-1, whereas the antioxidant N-Acetyl-Cysteine (NAC, 0.5 mM) and the lipid radical scavenger DPPD (1 μM) had no inhibitory effect. Ferristatin (50 μM), another inhibitor of DMT-1, also suppressed quenching of cytosolic calcein produced by ischemia. In addition, ebselen and ferristatin inhibited quenching of calcein loaded into mitochondria, indicating that inhibition of DMT-1 blocked mitochondrial iron uptake. Importantly, ebselen and ferristatin added before ischemia attenuated ROS formation, opening of mitochondrial permeability transition pores and cell killing after reperfusion. Conclusions: Taken together, the results indicate that DMT-1 mediates lysosomal iron release into the cytosol during ischemia. Lysosome-derived iron then enters mitochondria and contributes to iron-dependent oxidative injury and cell killing after reperfusion. Thus, DMT-1 is a potential therapeutic target against IR-induced hepatotoxicity.

**Interstrain Differences in Oxidative Stress-Induced by a Lipotrope Methyl-Deficient Diet in Mice.**

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Nonalcoholic fatty liver disease (NAFLD), the most common cause of chronic liver disease in the US, includes a range of liver injury from steatosis to steatohepatitis, fibrosis, and cirrhosis, and can result in development of hepatocellular carcinoma. Although major research efforts have been put toward understanding the pathophysiological and molecular processes underlying NAFLD, the mechanisms of individual susceptibility to NAFLD remain largely unknown. It is widely-accepted that oxidative stress is one of the earliest events in pathogenesis of both human and rodent NAFLD. In this study, we used a panel of genetically diverse inbred mice (A/J, C57BL/6, C3H/HeJ, 129S1/SvImJ, CAST/Eij, PWK/PhJ, and WSB/Eij) to investigate the strain-specific differences in oxidative stress after being fed a methyl-deficient diet (MDD), a human relevant mouse model rodent NAFLD. Feeding MDD for 12 weeks resulted in the induction of oxidative stress in livers of mice in all seven strains, as evidenced by the decreased levels of reduced glutathione (GSH), increased levels of oxidized glutathione (GSGS), and a decreased GSH/GSSG ratio. Mice fed the MDD also have an increased expression of Gpx3 gene, a sensitive marker of oxidative stress. These changes were accompanied by an altered expression of genes involved in DNA base excision repair, a predominant pathway for repair of oxidative DNA lesions. The magnitude of oxidative stress differed significantly among the individual mouse strains; with the extent of oxidative damage being greatest in WSB/Eij mice and least in A/J mice. These results suggest that the individual susceptibility to NAFLD may be linked to the extent of oxidative stress-associated alterations in the livers.

**Sulforaphane Prevents Liver Injury Caused by High-Fat Diet and Olanzapine.**

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Olanzapine (OLZ), an atypical antipsychotic, is a first-line treatment for psychosis and mood disorders. The major side effects of OLZ use are weight gain and metabolic syndrome, including the hepatic manifestation of this disease (i.e., NAFLD).
It is now understood that obesity is a major risk factor for drug-induced liver injury. Although OLZ has been known to cause obesity, the effect of diet-induced obesity on OLZ-induced liver damage has not been tested experimentally. This gap in our knowledge is especially important to fill, as the obesity incidence in the psychiatric population is even higher than in the US population as a whole. The purpose of the current study was to test the hypothesis that obesity enhances OLZ-induced hepatic injury, and to test the potential protective effect of anti-oxidant supplementation. Eight-week-old mice were fed either high-fat diet (HFD) or control diet for 4 weeks. Mice also received either OLZ (8 mg/kg/d) or vehicle via osmotic minipumps. Some mice were co-treated with SFN (90 mg/kg/d, i.g.), an isothiocyanate that confers indirect protection by activating intrinsic ROS defenses. HFD caused weight gain and hepatic steatosis, as expected in this model. OLZ exacerbated body weight gain in the HFD group and doubled plasma transaminase release. Hepatic triglyceride accumulation caused by HFD was exacerbated by OLZ co-administration. HFD moderately impaired glucose uptake and utilization; this effect of HFD was exacerbated in the OLZ group. SN partially blunted the biochemical changes caused by HFD, which was blocked in the combination of OLZ and HFD treatment. Taken together, these data show that OLZ dysregulates glucose and lipid metabolism and exacerbates hepatic changes caused by HFD. These data therefore suggest that underlying obesity may be a risk factor for OLZ-induced liver injury. Activating intrinsic antioxidant defenses with SFN can partially prevent these effects of OLZ and may represent a useful strategy to protect against liver injury in the clinics.

**368 POLYCHLORINATED BIPHENYL 153 WORSENS NONALCOHOLIC FATTY LIVER DISEASE IN C57BL/6 MICE.**

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Introduction: Exposure to polychlorinated biphenyls (PCBs) including the ubiquitous PCB153 has been shown to increase odds ratio for serum alanine aminotransferase elevation, indicative of nonalcoholic fatty liver disease (NAFLD). To determine if PCB153 plays a causal role in the development of NAFLD, we undertook a study to examine if PCB153 either caused NAFLD by itself or worsened NAFLD induced by a high fat diet (HFD). Methods: C57BL/6 mice were fed either a normal chow or HFD (43% milk fat) for 12 weeks with or without PCB153 co-exposure (50 mg/kg i.p. X4). Liver and fat samples were taken for immunohistochemistry, RT-PCR, metabolomics and lipid analysis. Results: PCB153 treatment increased body weight for animals fed with HFD. PCB153 treatment caused micro-vesicular steatosis and worsened HFD-induced macro-vesicular steatosis. Serum adipokines were increased with HFD and this was augmented by PCB153 co-treatment. The adiponectin/leptin ratio was reduced in both HFD and PCB153 groups. The adiponectin mRNA level decreased in the HFD+PCB153 group. PCB153 treatment increased adipocyte area, liver triglycerides and cholesterol levels in HFD fed animals. Metabolomic analysis identified alterations in approximately 70 metabolites between HFD and HFD+PCB153 groups. These differences included reduced levels of glutathione and the spontaneous glutathione conjugate, S-(hydroxymethyl) glutathione, by 84% and 74% respectively, in the HFD+PCB153 group. In contrast, erythronic acid levels increased 30-fold. Conclusions: PCB153 treatment worsened diet induced obesity by acting synergistically with HFD. In concert, the metabolomics results suggested that PCB153 caused weight gain, adipocyte area increase, liver triglycerides and cholesterol levels in HFD fed animals. Metabolomic analysis identified alterations in approximately 70 metabolites between HFD and HFD+PCB153 groups. These differences included reduced levels of glutathione and the spontaneous glutathione conjugate, S-(hydroxymethyl) glutathione, by 84% and 74% respectively, in the HFD+PCB153 group. In contrast, erythronic acid levels increased 30-fold. Conclusions: PCB153 treatment worsened diet induced obesity by acting synergistically with HFD. In concert, the metabolomics results suggested that PCB153 may exacerbate oxidative stress within the cell. Funding sources: This research was funded by NIH grants from NCCR (SP2ORR024489), the NIAAA (K23AA18399, P101AA17103) and the NIEHS (P30ES01443-T35ES014559).

**369 INVESTIGATION OF DRUG-DRUG INTERACTIONS CAUSED BY HUMAN PREGNANE X RECEPTOR- MEDIATED INDUCTION OF CYTOCHROME P450 3A4 AND 2C SUBFAMILIES IN PBX-MICE.**

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The induction of cytochrome P450 3A4 (CYP3A) enzymes is one of the risk factors for drug-drug interactions (DDIs). Two major CYP enzymes of CYP3A4 and CYP3A subfamilies are known to be upregulated by the human pregnane X receptor (PXR) regulation, and the DDIs via the enzyme induction of CYP3A4 and/or CYP3A2 subfamilies have been reported to have a major impact in the clinical treatments. The purpose of this study was to examine the DDI between a model human PXR ligand, bupropion (PBX-mice®) and PXR agonists, (S)-warfarin (CYP2C9), and (S)-mephenytoin (CYP2C19). The induction of several drug metabolizing enzymes and transporters in the liver was also examined by measuring the enzyme activity and mRNA expression levels. Significant reductions in the plasma exposure levels of triazolam, pioglitazone, and (S)-mephenytoin were observed in PXB-mice, and the microsomal activity of CYP3A4, CYP2C8 and CYP2C19 were confirmed to be increased in in vitro study. In contrast to the other three CYP isoenzymes, the plasma exposure levels of (S)-warfarin did not decrease, although the microsomal activity of CYP2C9 was elevated after the rifampicin treatment. The discrepancy in the (S)-warfarin results between the in vivo and in vitro studies may be due to the relatively small contribution of CYP2C9 to (S)-warfarin elimination. In summary, the PXB-mice are a useful animal model to examine DDIs caused by PXR-mediated induction of CYP3A2 and CYP3A4.

**370 ISOFORM-SPECIFIC ALT MEASUREMENT CAN DISTINGUISH HEPATIC FROM EXTRA-HEPATIC INJURY IN HUMANS.**


Serum alanine aminotransferase (ALT) is used as a clinical marker to detect hepatic damage and hepatoxicity. Two isoforms of ALT have been identified, ALT1 and ALT2. Increased plasma ALT activities are detected and measured in human serum/plasma using classical clinical chemical assays. Differences exist in the expression patterns of the ALT1 and ALT2 proteins in different organs, which suggest changes in the proportion of ALT1 and ALT2 in plasma could arise and reflect damage to different organs in man. This has, however, not been previously studied due to the lack of a selective methodology that can quantitatively both ALT1 and ALT2 protein and ALT activity in the total serum/plasma samples. Here we show, for the first time, that during three clinically different liver injuries (fatty liver disease, hepatitis C and direct liver surgery) the leakage of ALT1 activity into plasma greatly exceeds that of ALT2. In contrast, during skeletal muscle injury, induced in volunteers experiencing physical exertion, leakage of ALT2 exceeds that of ALT1 and the proportion of ALT isoforms changes accordingly. These results show that the measurement of ALT activity in plasma may offer an opportunity to define whether extra-hepatic injury is occurring in cases where these may falsely have been interpreted as liver injury only.

**371 HCV CORE PROTEIN EXPRESSION AND ACETAMINOPHEN-INDUCED LIVER INJURY.**

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The Hepatitis C virus (HCV) is a hepatotropic pathogen of significant importance to public health. Chronic HCV infection pre-disposes patients to a variety of liver diseases and it has also been shown that hepatitis C infection was a predictor of acute liver injury among hospitalizations for acetaminophen overdose in the United States. Acetaminophen (APAP) overdose is the leading cause of acute liver failure in the United States and we used transgenic mice expressing the HCV core protein to explore if this increased susceptibility of animals to APAP induced liver injury. Treatment of C57BL/6 mice with APAP (200 mg/kg body weight) resulted in moderate liver injury at 6 h as indicated by elevated ALT levels, focal centrilobular necrosis and nuclear DNA fragmentation. HCV core protein over-expressing mice showed a variable response, with approximately half the animals showing significant exacerbation of all parameters of liver injury while the other half were protected. These effects were not mediated by changes in APAP metabolism, since neither cytochrome P450 protein levels nor early depletion of glutathione was affected in the HCV transgenic mice. Animals showing protection against liver injury had a substantial increase in liver glutathione content at 6 hours when compared to wild type animals, accompanied by protection against mitochondrial oxidative stress and prevention of mitochondrial AIF release. This was accompanied by an elevation in glutathione S transferase mRNA levels and activity, which suggests that an efficient clearance of the reactive intermediate is the mechanism of protection against APAP hepatotoxicity in these mice. In the case of animals showing exacerbation of liver injury, a substantial decrease in liver glutathione at 6 hours, elevated mitochondrial oxidative stress, and enhanced release of AIF from the mitochondria were observed. This was accompanied by induction of the ER stress response, which probably resulted in amplification of the mitochondrial oxidative stress and downstream cellular injury.
Hepatotoxicity from acetaminophen (APAP) overdose is the leading cause of acute liver failure in the United States. APAP-protein adducts are detectable in plasma, and quantification of these adducts represents a valuable tool for studying APAP-induced hepatotoxicity. The elderly population is of particular interest with respect to such toxicity since older patients have a high incidence of chronic APAP usage; however, the effect of age on plasma protein adduct concentrations has not been evaluated. The present study explored the possibility of age-dependent differences in plasma protein adduct concentrations in a mouse model. Male C57Bl6/J mice, 4 or 22 months of age, were treated with 0, 75, 150 or 300 mg/kg APAP, i.p., in saline vehicle. Plasma and liver tissue were collected at 2, 6, 12 or 24 h post-injection. HPLC-MS/MS quantification of plasma APAP-protein adducts revealed higher concentrations in 4-month-old mice compared to 22-month-old mice for the 300 mg/kg dose at 2 and 24 h. At 24 h, this coincided with more severe hepatic necrosis in 4-month-old mice than in 22-month-old mice, as determined by histology. To explore the origin of these differences, liver tissue was collected from non-treated mice of the same ages, and in vitro enzyme activity for major APAP-related metabolic reactions was assessed. APAP glucuronidation and sulfation rates were lower in 4-month-old mice compared to 22-month-old mice, which may have contributed to the observed accumulation of plasma protein adducts in young mice. In conclusion, age-dependent differences in plasma APAP-protein adduct concentrations were evident and could be attributed to altered APAP metabolism. Age is an important consideration for future work that utilizes plasma protein adducts to study APAP-induced hepatotoxicity. Supported by a grant from the University of Utah on Aging. Mice were obtained from the National Institute on Aging and reared colony strains.

L-FABP MEDIATES IMPAIRED LIPID UPTAKE AND TRAFFICKING IN A RODENT MODEL OF EARLY ALCOHOLIC LIVER DISEASE.

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Chronic ethanol consumption is a prominent cause of liver disease throughout the Western world. Among the predictable and prominent histologic abnormalities resulting from ethanol ingestion is hepatosteatosis (fatty liver). Utilizing a modified Lieber-DeCarli liquid feeding regimen, male C57Bl6/J mice were fed for a period of 6 weeks. To assess the extent of liver damage, standard biochemical assays revealed increases in serum and liver triglycerides, increased ALT activity and increased immunohistochemical staining for the reactive aldehyde, 4-hydroxyxenonale (4-HNE). Gene array analysis revealed significant alterations in the expression of genes involved with lipid trafficking and metabolism, including liver fatty acid-binding protein (L-FABP), peroxisome proliferator activated receptor-alpha (PPAR-α), carnitine palmitoyltransferase 1a (CPT-1a), and sterol carrier protein 2 (SCP-2). A significant decrease in the cytosolic and nuclear expression of L-FABP was observed, as well as expression and activity of PPAR-α. Immunohistochemical evaluation of liver sections also revealed considerable changes in lobular expression and localization of L-FABP. Protein-protein interactions between L-FABP and PPAR-α/CPT-1a were also investigated utilizing co-immunoprecipitations. Collectively, these data demonstrate dramatic effects of ethanol consumption on L-FABP at the gene and protein level. L-FABP is responsible for activating PPAR-α, and subsequent transcription of FA metabolism genes like L-FABP and CPT-1a. An alteration in the expression of L-FABP suggests a cyclic demolition of the regulatory events of changes in lipid trafficking. The observed alteration in L-FABP expression provides mechanistic insight into the dysregulation of lipid homeostasis in the pathogenesis of ALD. (R37 NIH/NIAAA009300; NIH/NIAAA F31 AA18898-03)

EVALUATION OF HEPATIC FLAVIN-CONTAINING MONOOXYGENASE 3 (FMO3) GENE REGULATION, PROTEIN EXPRESSION, AND ENZYME ACTIVITY IN RESPONSE TO ACETAMINOPHEN TOXICITY.

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Flavin-containing monooxygenase 3 (Fmo3) catalyzes the NADPH-dependent oxygenation of nitrogen, sulfur, and phosphorus-containing xenobiotics in human liver. Members of the Fmo family of genes have distinct gender, development- and tissue-specific expression patterns in mice. Until recently Fmo3 was thought to be noninducible. We have previously shown that toxic acetaminophen (APAP) or alpha-naphthylisothiocyanate treatment, as well as bile duct ligation in mice markedly increase Fmo3 gene expression. The purpose of this study was to evaluate if the pronounced changes in Fmo3 mRNA levels by APAP treatment translate into increases in protein expression and functional activity of the enzyme. Hepatic Fmo3 mRNA, protein expression, and enzyme activity were quantitated at 48 h in male mice treated with APAP (400 mg/kg). This treatment resulted in mild hepatic necrosis as evidenced by plasma ALT values of 237.9 IU/L. Fmo3 mRNA levels increased significantly by 25-fold while protein levels increased just 2- to 3-fold. Liver microsomes from APAP and control treated mice were generated and assayed for oxygenation of methimazole (MIM), a selective functional substrate for Fmo3. The Kcat value for liver microsomes from APAP treated mice increased from 486.2 to 610.6 (nM min-1-mg-1), although this difference was not significant. The Kd value for microsomal samples from APAP mice decreased from 51.2 to 25.9 µM, which causes a statistically significant 2-fold increase in the catalytic efficiency (Kcat/Kd 23.6 10-3min-1uM-1) of Fmo3 for MIM. In summary, the profound increase in Fmo3 gene expression produced by APAP treatment was not accompanied by changes in protein and functional activity of a corresponding magnitude. The reason for this disparity in gene, protein expression and function is currently being investigated. Supported by NIH DK069557.

PROTECTION AGAINST ACETAMINOPHEN CYTOTOXICITY BY PPARα AND RXR AGONISTS: DETERMINING THE ROLE OF CHANGES IN VANIN-1 mRNA EXPRESSION.

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Activation of the peroxisome proliferator-activated receptor alpha (PPARα) prevents acetaminophen (APAP) hepatotoxicity in mice. A gene array analysis of livers from mice resistant to APAP toxicity by clofibrate (CFFB) treatment identified Vanin-1 (Vnn1) as a potential gene contributing to this protection. Vnn1 is as a cytoprotective gene responsible for the production of the antioxidant cysteine. We previously analyzed the susceptibility of Vnn1 knockout mice to APAP toxicity. As predicted, mice lacking Vnn1 were more susceptible to APAP hepatotoxicity. The current study aimed at developing a strategy to protect mice from APAP toxicity through the use of a protective agent with an in vivo role of protecting against APAP toxicity using the human hepatocyte cell line HC04. Treatment of HC04 cells with APAP resulted in dose-dependent cytotoxicity. Exposure of HC04 cells to 1.0µM CFFB resulted in a ten-fold increase in VNN1 mRNA expression. However, considerable cytotoxicity occurred at this CFFB concentration. By contrast, treatment with the peroxisome proliferator Wy-14,643 (100µM) resulted in a four-fold induction of VNN1 mRNA levels in the absence of noticeable cytotoxicity. Treatment with Wy-14,643 conferred partial protection against APAP cytotoxicity as shown by a 25% reduction in lactate dehydrogenase (LDH) leakage into media in comparison to cells treated with APAP alone. Due to cooperative interaction of PPARα and the retinoid x receptor (RXR) in gene transcription, we tested the ability of hexareten (Bex), a RXR activator, to protect against APAP cytotoxicity and upregulate VNN1 expression. Treatment of HC04 cells with Bex (1.0µM) alone or in combination with Wy-14,643 resulted in virtually the same magnitude of protection from APAP toxicity. Interestingly, Bex by itself did not change VNN1 mRNA expression. In conclusion, these results document the utility of the bex in vivo system for studying the mechanistic role of VNN1 as a cytoprotect gene.

LYSOSOMAL IRON RELEASE PROMOTES HEMORRHAGE/RESUSCITATION-INDUCED LIVER INJURY.

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Background: Despite recovery of hemodynamics by fluid resuscitation, liver, and other organs may progress after hemorrhage to develop multiple organ dysfunction syndrome. Recently, we showed that minocycline and doxycycline block iron uptake by mitochondria. Here, our aim was to determine whether iron plays a role in H/R-induced liver injury. Methods: Under anesthesia, C57BL6 mice were hemorrhaged to 30 mm Hg for 3 h and then resuscitated with shed blood plus half the volume of Ringer’s solution containing tetracycline, minocycline, doxycycline or vehicle. In some experiments, desferal and starch-desferal were administered intraperitoneally 1h before H/R. Serum alanine aminotransferase (ALT), liver...
10993-1 Biological Evaluation of Medical Devices - Part 1: Evaluation and Testing within a Risk Management Process. A completed semi-quantitative extractable studies on colorants as part of the safety assessment. The challenges and successes in demonstrating the safety of the colorants used in a short-term blood-contacting catheter are presented here. A color elution study was conducted on a stent delivery system catheter to demonstrate the potential bioavailability of the colorants. The sample devices were reflushed for up to 24 hours in acetone. These aggressive extraction conditions maximized the potential to extract colorant and were not intended to represent clinical use. Results were quantitated using UV-VIS spectroscopy against a five point calibration curve for each of five colorants. The estimated maximum amounts of extracted colorant ranged from 0.25 μg/ml (0.5 μg/device) to 5.0 μg/ml (10 μg/device). These values were used as reasonable “worst case” estimates in the safety assessment. Poor process spike recovery results and baseline noise caused by extracted polymer materials contributed to uncertainty in the reported results. The safety assessment was conducted following ISO 10993-17: Establishment of allowable limits for leachable substances. The history of clinical use, existing toxicology and biological safety data were included in the risk assessment. The threshold of toxicological concern (TTC) was applied for colorants with insufficient toxicological data. We concluded that the colorants used in this short-term BSC catheter were eluted from the device at toxicologically insignificant amounts when extrapolated to the clinical exposure.

377 CYTOTOXICITY STUDIES IN A549 CELLS CULTURED ON 2-METHACRYLOYLOXYETHYL PHOSPHORYLCHOLINE POLYMERS.

2-Methacryloyloxyethyl phosphorylcholine (MPC) polymer has a phospholipid polar group that mimics a biomembrane, and has been used in medical science, biotechnology, pharmaceutical industry, and environmental technology. In medical devices, MPC polymer shows good biocompatibility with the reduction of protein adsorption, and it is used as surface ornamentation agents with artificial organs, blood-contact medical tubes, catheters, and contact lenses. In this study, we compared the cytotoxicity of chemical materials in human lung-derived epithelial-like cell line A549 cells and Chinese hamster lung fibroblast (CHL) cells. A549 and CHL cells were cultured on the MPC polymer-coated plate (MPC plate) and a control plate, and examined in the cytotoxicity and genotoxicity test. On MPC plate, CHL cells formed spheroid and the growth rate was almost the same as control plate during the small spheroid. On the other hand, A549 cells gathered and grew up with a grape-like shape and the growth rate was less than half of the control. The genotoxicity test was carried out with the micronucleus test and no remarkable difference was observed. As a result of the cytotoxicity test for CdSO4 and ZnO (nano size) using a 96-well MPC plate, the cytotoxicity of CdSO4 in CHL cells was weaker on MPC plate compared with control, and the cytotoxicity of ZnO in A549 cells was stronger on MPC plate compared with control. This result was considered that the morphology of cells affected on the sensitivity for the chemical materials. Further molecular level analysis would help the better understanding of the living body/material interactions and the mechanism of biocompatibility.

378 BIOCOMPATIBILITY EVALUATION OF A SILICONE-COATED SURGICAL NEEDLE COMPRISED OF A NOVEL TUNGSTEN-RHENIUM ALLOY.
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A new surgical needle for cardiovascular surgery was developed to provide improved mechanical performance and reduced penetration force over current surgical needles. This needle was manufactured using a novel Tungsten-Rhenium alloy with a new silicone-based lubricious coating. The biocompatibility of this needle was evaluated by conducting cytotoxicity, intracutaneous reactivity, sensitization, acute systemic toxicity, pyrogenicity, and hemocompatibility studies in accordance with ISO 10993 guidelines. Headspace analysis using Gas Chromatography-Mass Spectroscopy was conducted to determine if residual organic solvents from the coating process remained on the needle. The results of this program of biocompatibility studies indicated that this silicone-coated Tungsten-Rhenium alloy surgical needle was non-cytotoxic, non-irritating, not a sensitizer, non-acutely toxic, non-pyrogenic, and hemocompatible, and residual solvents were not detectable. Overall, this biocompatibility assessment demonstrates that the new coated surgical needle is biocompatible and does not represent any safety concerns for patients.

379 CHALLENGES IN CONDUCTING SAFETY ASSESSMENTS OF COLORANTS USED IN MEDICAL DEVICES.
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The medical device industry has recently experienced an increase in questions and concerns from the FDA regarding color additives (i.e., colorants) in many categories of devices. In response to the FDA questions and to be compliant with ISO 10993 guidelines, companies have been asked to perform safety assessments on colorants used in medical devices. These assessments are complex and require expertise in toxicology, pharmacology, and materials science. The challenges in performing these assessments include identifying the appropriate test methods, determining the appropriate dose levels, and interpreting the results in the context of the intended use of the device. In addition, there are regulatory requirements that must be met, such as establishing allowable limits for leachable substances. Despite these challenges, companies are working to meet the regulatory requirements and ensure the safety of colorants used in medical devices.
a rate of 1 μL/hr can be conducted. In our laboratory, we validated the iPrecio soft-
ware and the accuracy of the delivery of the micro-infusion pumps, to allow its use on regulatory compliant studies. Three infusion pumps were programmed to de-
liver 1 or 30 μL/hr for 30 minutes, and 2, 4, 8, 12, 24 and 48 hours, with several planned interruptions of the infusion over the course of the given period. Our vali-
dation study demonstrated the successful implantation of iPrecio® pumps, the ability to use these pumps in a number of dosing schemes, and that the software ac-
curately programmed the infusion pumps to deliver 1 or 30 μL/hr for 4-48 hours with an accuracy of +/- 15% of the intended volume. In conclusion, the iPrecio® implantable pumps were shown to be a suitable alternative for use on regulatory compliant studies when very low rates of infusion are required.

**382 EVALUATION OF AN IN VITRO HUMAN SKIN IRRITATION TEST FOR USE WITH MEDICAL DEVICE EXTRACTS.**

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In accordance with ISO 10993, the rabbit skin irritation test is used to assess med-
ical device biocompatibility, however, this method is time-consuming and expen-
sive. Our study’s goal was to determine if the Epiderm™ reconstructed human skin model (MatTek Corp.) could be an acceptable alternative. To that end, eleven medical device polymers and adhesives were selected for testing. Four extracts were prepared per test article, two each with saline and sesame oil as vehicles, resulting in 44 total matrices. Half of the extracts were spiked with known amounts of two R-
38 dermal irritants (lactic and heptanoic acid). A range-finding study determined the lowest concentrations that would produce skin irritation (LOAEL). Tissues were exposed for 24 hours. Cell viability was assessed by MTT and IL-1β release was measured by ELISA. The positive control (5% SDS) reduced cell viability to 8.5% of the negative control (PBS) and increased the release of IL-1β to 8.5% of the negative control (PBS) and increased the release of IL-1β to 8.5% of the negative control (PBS) and increased the release of IL-1β.

**384 RISK ASSESSMENT OF BISPHENOL A FROM BISGMA-
BASED COMPOSITE DENTAL MATERIALS.**

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Bisphenol A glycidyl methacrylate (BisGMA) is an important resin monomer that has been used in composite dental products for over forty years. BisGMA provides essential properties of mechanical strength, rapid polymerization, and low shrink-
age, and is currently used in more than 70% of all dental composites. Recently, the safety of BisGMA has been questioned in regard to its potential to release Bisphenol A (BPA), but data-based risk assessments on this topic are scarce. A quantitative risk assessment of BPA release from dental composites was performed to address this gap. BisGMA is manufactured from BPA in a multi-step process, and low residual levels of BPA may remain in the BisGMA used in dental products. Published infor-
mation from several sources indicates that BisGMA contains < 2 ppm of residual BPA. Polymerized BisGMA is not subject to oral enzymatic degradation; therefore, the only likely source of BPA from BisGMA is leaching of residual monomer. The following exposure assumptions were used in the assessment scenario: 1) 2 ppm of residual BPA is present; 2) the product contains a maximum of 15% BisGMA (w/w); 3) applied product weight is sufficient to restore 3 large molars in an adult or 1 large molar in a 10 year old child and 4) any leached BPA is completely ab-
sorbed by the dental patient. Dental composites are likely to remain in the mouth for more than 10 years; therefore, BPA exposure via leaching into saliva was calcu-
lated both on a worst-case short-term basis and a more clinically relevant long-term basis and these estimates were compared to authoritative BPA toxicity reference va-
ues. The resulting margins of exposure (MOE) for an adult patient were greater than 10,000 on an acute basis and greater than 320,000 on a chronic basis. The re-
sulting margins of exposure (MOE) for a pediatric patient were greater than 20,000 on an acute basis and greater than 500,000 on a chronic basis. These MOE values confirm the safety of BisGMA-based dental composites with respect to potential BPA exposure and support their continued use in adult and pediatric dentistry.

**385 MEDICAL SURVEILLANCE OPTIONS FOR PERSONS WITH ELEVATED COBALT-CHROMIUM LEVELS FROM HIP IMPLANTS.**

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Background: Persons with metal-on-metal (MoM) total hip arthroplasties can de-
velop elevated blood and urine cobalt (Co) and chromium (Cr) levels. Epidemiologic studies evaluating long-term effects of elevated Co and Cr levels in persons with MoM implants are lacking and sale blood and urine levels are not de-
defined. Rare cases of cobaltosis, characterized by hypothyroidism, cardiomyopathy, peripheral neuropathy, blindness, and deafness, have occurred, generally after mas-
ive joint deterioration. Hypersensitivity reactions leading to localized symptoms in-
cluding progressive osteolysis have been reported. Case description: A 69-year-old fe-
male with bilateral MoM hip prostheses implanted 6 and 7 years ago was consid-
ering implant removal because of toxicological concerns associated with ele-
vated blood Co (22.1 ug/L) and serum Cr (11.3 ug/L) levels. Ophthalmological as-
essment, echocardiography, hemoglobin, and thyroid, renal and hepatic function did not reveal findings of Co or Cr toxicity. Hip replacement surgery was not ad-
vised for toxicological indications. Rather periodic surveillance evaluations to de-
tect early manifestations of Co and Cr toxicity were recommended. Case discus-
sion: This case provides evidence for lack of systemic toxicity in a person with bil-
ateral MoM hip replacements and increased blood Co and Cr levels. Conclusions: The lack of an established no effect Co or Cr levels may result in per-
sions unnecessarily undergoing revision surgery for toxicological concerns even though MoM implant removal has not demonstrated to be of benefit in asympto-
matic persons. Medical conditions associated with Co toxicity can be caused by other conditions, especially in elderly populations, and need to be considered in any clinical evaluation. Medical evaluations for early manifestations of systemic toxicity coupled with ongoing monitoring of blood Co and Cr levels can be per-
fomed for persons considering MoM hip implant removal for systemic toxicologi-
cal concerns. Subsequent epidemiological studies of populations with MoM hip implants will provide data on health risks and management options.
**386 IN SILICO MUTAGENICITY PREDICTION FOR LEACHABLE COMPONENTS OF DENTAL DEVICES.**


In silico predictive toxicology models are routinely used for screening or predicting the hazards of pharmaceutical and industrial chemicals. The applicability of these tools to leachable components of medical devices is an increasing topic of discussion as regulatory agencies begin to consider the addition of in silico prediction tools to the suite of chemical evaluation procedures. The usefulness of any predictive model depends on the underlying chemicals in the dataset and their relevance to the components of interest. Mutagenicity potential is one critical endpoint for the evaluation of medical devices with prolonged or permanent contact. Eighty-two chemicals were reported in the literature as potential leachables from dental devices with prolonged or permanent contact. These chemicals were specifically identified and evaluated for their predicted mutagenicity potential in the bacterial reverse mutation assay using several commercially or publicly available in silico predictive tools including TOPKAT, Admet Predictor and USEPA TEST. The bacterial reverse mutation assay was selected as the appropriate test for comparison of predictions based on its feasibility of applying the TTC approach to safety assessment of polymers. The results of this exercise confirm the potential utility of the TTC approach for compounds classified as sensitizers.

**387 APPLICATION OF THE THRESHOLD OF TOXICOLOGICAL CONCERN APPROACH TO EVALUATION OF LEACHABLE COMPONENTS FROM POLYMER DENTAL DEVICES.**

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The Threshold of Toxicological Concern (TTC) is a proposed approach for assessing the human health risk of leachable medical device components when toxicological data are not available. Currently, there are no published accounts of applying TTC in the safety assessment of medical devices to inform the use of this approach. The feasibility of applying the TTC approach to safety assessment of polymers was considered necessary for its relevance for regulatory assessment of medical devices. The resulting predictions were compared across the models and to the available test data on the identified chemicals. The specificity and sensitivity of each model were calculated and compared. Preliminary results indicate a general concordance of predicted results among the three models, with a lack of agreement for some compounds. The chemicals were specifically identified and evaluated for their predicted mutagenicity potential in the bacterial reverse mutation assay using several commercially or publicly available in silico predictive tools including TOPKAT, Admet Predictor and USEPA TEST. The bacterial reverse mutation assay was selected as the appropriate test for comparison of predictions based on its feasibility of applying the TTC approach to safety assessment of polymers. The potential for conflicting predictions when estimating the toxicological hazards of a medical device ingredient and the advantage of using multiple predictive models.

**388 ASSOCIATION BETWEEN PAH EMISSION AND LUNG CANCER RATES AROUND THE WORLD.**

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Polycyclic aromatic hydrocarbons (PAH) are products of incomplete fuel combustion. Some of them (e.g. benzo(a)pyrene) have carcinogenic properties. Because of this, it is important to be able to measure and estimate human exposure to PAHs. Although other factors, such as cigarette smoking, can lead to lung cancer, PAH emissions also contribute to lung cancer rates. A multiple linear regression model was developed to investigate the potential contribution of gross domestic product (GDP), smoking rates, cigarette price, population and PAH emissions on the number of lung cancer deaths (NLCD) around the world. These data were collected from the World Health Organization, the International Agency for Research on Cancer, and other sources. Diagnostic procedures suggested general log transformation of the data, followed by use of a Poisson distribution. The indicator variables were used to investigate the contribution of PAH emissions on NLCD for seven major parts of the world. After accounting for smoking rates, GDP, cigarette price and geography, we found that PAH emissions contributed significantly to the number of lung cancer deaths and that this contribution varies for different parts of the world. In North America and Europe, a 10% increase in PAH emission was associated with a 5.6% and 2.9% increase in median NLCD, respectively. On average worldwide, a 10% increase in smoking rates was associated with a 5.7% increase in median NLCD. While a 10% increase in cigarette price was associated with a 4.7% decrease in median NLCD.

**389 INFLUENCE OF TRAFFIC-RELATED AIR POLLUTION ON EXHALED NITRIC OXIDE IN HEALTHY ADULTS: A SEASONAL FOLLOW-UP.**

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Ambient air quality is a major concern in Belgium due to the high degree of urbanization and dense traffic networks. It is known that exposure to traffic-related air pollution is associated with adverse respiratory effects. We investigated whether traffic-related air pollutants are associated with exhaled nitric oxide (eNO) in healthy adults. The study population consisted of 48 healthy non-smoking adults (25 men, age 16-65 years). eNO was analyzed in winter and summer using a portable Niox Mino device. Exposure to traffic-related air pollution was assessed for all subjects in both seasons by interpolated NO2, PM2.5, PM10 and O3 concentrations at the home address for various exposure windows before eNO measurement and by using geographical information. Associations between exposure parameters and eNO were tested using a multiple mixed-effects regression model with age, sex, and inflammation in the week before eNO analysis as covariates. Various short-term NO2, PM2.5 and PM10 exposure windows (2-10 day lagged averages) were significantly associated with eNO (p<0.05). eNO in winter and summer were highly correlated (Pearson r=0.68, p<0.000001) and eNO was higher in men compared to women (17.4 vs. 24.5 ppb, t-test, p<0.001). Exposure to ambient NO2 and PM10 at the home address was associated with eNO indicative for lower airway inflammation in healthy adults. This study was financed by the Flemish Government (Department of Environment Nature & Energy, Environment & Health unit), and the EU-FP6 project INTARESE.

**390 CUMULATIVE RISK MODEL FOR PM and O3 IN AMBIENT AIR.**

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The study deals with the assessment of air pollution attributed short-term cardiovascular and respiratory morbidity and mortality in Athens in 2003-2005. The data used included the daily number of hospital admissions and deaths due to cardiovascular and respiratory causes, hourly mean concentrations of O3 and PM and meteorological data from several monitoring stations from the area of study. The relations among these variables were analysed using Generalized Linear Models (GLMs) and Artificial Neural Networks (ANNs). Using GLMs, causal links between PM-O3 levels and respiratory mortality were identified (an increase of 10 μg/m3 in both PM and O3 corresponds to an increase in morbidity equal to 4.49%). No statistically significant association was obtained for cardiovascular mortality. An increase of 10 μg/m3 for PM and O3 corresponds to an increase in cardiovascular morbidity equal to 1.84%, while respiratory morbidity seems to be more sensitive to these changes (4.96%). The ANN model attributed slightly
higher Relative Risk values. To validate the derived models, the RR values were val-

idated against independent data for different years. Daily morbidity data were bet-

ter predicted than mortality data suggesting that other causes than air pollution in-
fluence significantly mortality. ANN models perform better than GLMs in predicting daily morbidity: Pearson coefficients are 0.734 and 0.717 respectively for respiratory and cardiovascular morbidity using ANN compared to 0.519 and 0.429 obtained with GLM. Concerning daily mortality the two models produced similar results with a Pearson coefficient of 0.29 for ANN and 0.31 for GLM. ANN models tended to perform better than GLMs in predicting daily morbidity and mortality. Comparison of the two methods indicated that the flexibility and the adaptation of the ANN's models provides several advantages when assessing non-linear problems, such as the u-shaped temperature effect, in predicting daily mortality.

**393 NEUROBEHAVIORAL EXPOSURE-EFFECT GRADIENT IN SCHOOLCHILDREN CHRONICALLY EXPOSED TO PESTICIDES.**

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Our group previously showed in-season reversible neurobehavioral effects of low-
level chronic exposure to pesticides in residents in rural communities in Israel. This study assessed the neurobehavioral effects in 8-12 years-old schoolchildren in these communities. Eighty-four percent of these schoolchildren are the second genera-
tion exposed to pesticides, given that at least one of their parents has been residing in this rural area for at least three decades and chronically exposed to pesticides. The studied group included two sub-groups: 51 children who reside and attend school in an exposed valley; and 45 children residing on the hills around the valley and attend school in the valley. The control group included 40 age- and sex-matched children residing in a different rural area in which the use of pesticides is restricted for decades. Parents' questionnaire included exposure assessment and Inattention Hyperactivity. Neurobehavioral tests included Digit Cancellation, arithmetic, Diamond and Trail Making Tests A and B; and Purdue Pegboard.

The performance of all of these tests showed pesticides exposure-effect gradient. The parents' Questionnaires indicated a similar gradient between children's exposure to pesticides and their inattention and hyperactivity. Neurotoxic effects of chronic exposure of children to pesticides were manifested in their executive skills, first and foremost in their attention span, visual scanning and execution speed. There may be environmental-susceptibility interactions and epige-
netic effects expediting the occurrence of ADHD.

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**394 PERFLUOROOCTANOATE, PERFLUOROOCtANESULFONATE, AND CHRONIC KIDNEY DISEASE: CLEARANCE COMES BEFORE CAUSATION.**

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A previous cross-sectional (C-S) study based on NHANES data reported a de-

creased estimated glomerular filtration rate (eGFR) and 2-fold increased risk in chronic kidney disease (CKD) associated with serum perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) (mean ~5 and 25 ng/mL, respectively). These authors suggested biological plausibility based on a 28-day gavage study in which microscopic changes in the rat kidney were observed at 20 mg/kg PFOA or PFOS (a 100% lethal dose for PFOA by day 26) and not observed at 5 mg/kg dose, at which serum mean PFOA and PFOS concentrations were 39,000 and 72,000 ng/mL, respectively. To further examine this association, a C-S study was done of 506 male 3M Co. workers in a 2000 medical surveillance program prior to the 3M purchase of Intercontinental. Median PFOA and PFOS concentrations were 1.097 mg/L (range 7-92.025) and 716 mg/L (range 24-6241), respec-
tively. Discordant covariates were adjusted for by ANCOVA and logistic regression. Median eGFR was 75.3 mL/min/1.73 m² (range 24.6–130.2). The adjusted mean differences between the eGFR in the 1st and 10th deciles of PFOA (median values 57 ng/mL and 4,941 ng/mL) and PFOS (median values 107 ng/mL and 3,069 ng/mL) were an insignificant increase of 1.7 mL/min/1.73 m² (95% CI -1.0, 4.4) and 2.0 mL/min/1.73 m² (95% CI 0.8, 4.8), respectively. The odds ratios for CKD were 2.107 (95% CI 0.60, 1.91) and 0.66 (95% CI 0.35, 1.17), respectively. There was no evidence for a decreased trend in eGFR or increased risk for CKD in this C-

S study. This suggests that the likely correct interpretation of the NHANES data is
that lower eGFR leads to higher serum PFOA and PFOS based strictly on physiological factors and is non-causal. This may explain other perfluoroalkyl associations with renal-cleared compounds, including C-reactive protein and uric acid.

**395 A LONGITUDINAL OCCUPATIONAL ASSESSMENT OF THE POTENTIAL ASSOCIATION OF CHANGES BETWEEN SERUM CONCENTRATIONS OF PERFLUOROOCANOATE AND PERFLUOROOCTANESULFONATE AND NON-HIGH-DENSITY-LIPOPROTEIN ChOLESTEROL.**

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Introduction: Cross-sectional studies of general, environmentally-exposed populations have observed a positive association between serum concentrations of perfluoroocanoate (PFOA) and perfluorooctanesulfonate (PFOS) with serum non-high-density-lipoprotein (non-HDL) cholesterol. The strength of these associations has been paradoxically not observed in occupational studies. Toxicological and mechanistic studies have demonstrated that PFOA and PFOS would be expected to reduce serum cholesterol; therefore, a causal basis for the associations observed in cross-sectional studies has been questioned. Cross-sectional studies cannot be used to infer causality. The study reported herein represents the first longitudinal assessment of these potential associations among individuals whose initial concentrations were at general population levels. Objective: To examine in a longitudinal occupational assessment whether changes in the serum concentrations of PFOA and PFOS are positively associated with non-HDL cholesterol. Methods: Baseline and end-of-project PFOA, PFOS, lipid and hepatic clinical chemistries were measured in 204 workers involved with the demolition of former perfluoropolyalkyl manufacturing facilities. Of interest were 130 workers whose PFOA and PFOS baseline concentrations were at general population (ng/mL) levels. Results: Among the 130 workers, their mean matched pair changes included: PFOA 46 ng/mL (p < 0.0001); PFOS 4.3 ng/mL (p < 0.0001); and non-HDL cholesterol -0.3 mg/dL (p < 0.0001). Non-HDL cholesterol and hepatic clinical chemistries were not positively associated with PFOA or PFOS in linear regression analyses. Conclusion: In a longitudinal assessment, significant positive associations were not observed between changes in PFOA, PFOS, non-HDL cholesterol and hepatic clinical chemistries. Therefore, the associations observed in cross-sectional studies likely have a non-causal basis.

**396 NEGATIVE ASSOCIATION OF ENVIRONMENTAL EXPOSURE OF BLOOD LEAD AND BLOOD CADMIUM WITH DIABETES IN THE NHANES 1999–2006.**

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Background: There have been reports suggesting an association of blood lead and blood cadmium with diabetes type 2. The objective of this study was to evaluate whether environmental blood lead level (BLL) and blood cadmium level (BCL) were associated with diabetes in the adult U.S. population.

Methods: We analyzed data from NHANES 1999–2006 participants aged 20 years and older for levels of blood cadmium and blood in relation to the prevalence of diabetes. Diabetes (n = 2121) was defined as self-reported, currently taking insulin, or diabetic pills to lower blood sugar or glycated hemoglobin (A1C) >6.5%. Blood lead and blood cadmium were categorized by weighted distribution in tertiles. Logistic regression models were adjusted for age, education, alcohol intake, smoking status, BMI, hypertension and serum creatinine (as a marker of kidney function).

Results: A significant negative association was found between BLL, BCL and diabetes. Individuals with BLL in the second (BLL = 1.11 – 1.90 μg/dL) and third (BLL >1.91 μg/dL) tertiles had significant adjusted ORs (0.67, 95% CI: 0.56 – 0.80; and 0.46, 95% CI: 0.37 – 0.56, respectively) to have diabetes compared to those in the first tertile. Individuals with blood cadmium in the second (BCL = 0.21 – 0.44 μg/L) and third (BCL >0.45 μg/L) tertiles compared to those in the first tertile had significant adjusted ORs (0.78, 95% CI: 0.64 – 0.94; and 0.69, 95% CI: 0.56 – 0.85, respectively). Analyses of lead and cadmium mixture found that individuals in the second and third tertiles of both BLL and BCL had a significant negative adjusted OR (0.50, 95% CI: 0.37 – 0.67; and 0.30, 95% CI: 0.21 – 0.42).

Conclusions: The findings, which are inconsistent with several previous reports, suggest that a potential role of blood lead and blood cadmium in the pathogenesis of diabetes is still unclear. Because of the cross sectional nature of the NHANES data further prospective studies are need to address these findings.

**397 EXPOSURE-BASED ESTIMATION OF THE GLOBAL FOODBORNE BURDEN OF DISEASE FROM CADMIUM EXPOSURE.**


Foodborne diseases constitute a serious worldwide public health threat. The World Health Organization (WHO) has launched an initiative to estimate the global burden of foodborne diseases, the Foodborne Disease Burden Epidemiology Reference Group (FERG). Cadmium (Cd) is a toxic element that is widely distributed in foods, and is one of FERG’s priority chemicals. Weight of evidence analysis was performed based on a thorough literature search and review. Renal and skeletal effects were identified as having a cause-effect association with Cd exposure. Urinary cadmium (U-Cd) was used as the indicator of chronic Cd exposure and assumed to follow a log-normal distribution. Next, dose-response relationships were modelled by fitting dichotomous models to selected epidemiological data. Disease prevalence rates were estimated based on the published U-Cd ranges in different WHO regions and the derived dose-response relationship. The Cd-attributable disease prevalence was estimated by subtracting the base prevalence of each endpoint. The estimated Cd-attributable disease prevalence was 0.25-6.6/1000 for microalbuminuria and 0.20-2.1/1000 for osteoporosis in different WHO regions. In areas close to industrial sites, the Cd-attributable disease prevalence was 12.0-33.7/1000 for microalbuminuria and 15.9-28.5/1000 for osteoporosis. While certain seafood and animal offal can accumulate high levels of Cd, the assessment of data from populations with high concentrations of seafood or animal offal did not show a large increase in Cd-attributable diseases. Therefore, Cd exposure from grains and vegetables grown on contaminated soil from industrial sites is a more significant contribution to Cd related burden of disease.

**398 IS SSRl ANTIDEPRESSANT USE IN PREGNANCY PROTECTIVE FOR RISKY ALCOHOL CONSUMPTION?**

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Background: Women who drink heavily in pregnancy have been shown to exhibit higher levels of depressive symptoms than women who do not. Selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed antidepressants. A Finnish study reported that mothers of children with Fetal Alcohol Syndrome (FAS) were seven times more likely to have taken SSRIs during pregnancy than mothers of normal children. The purpose of this study was to examine the association between alcohol consumption and SSRI exposure. Methods: Data were collected from 992 women who enrolled in the California Teratogen Information Service and Clinical Research Program (CTIS) cohort study between 1978 and 2005. For this analysis, women were selected who reported any alcohol consumption. Alcohol exposure categories included average daily volume, number of binge episodes, and greatest number of drinks in one occasion during each half of the first trimester and each trimester. The exposure of interest was reported SSRI drug use (citalopram, escitalopram, fluoxetine, paroxetine, and sertraline) any time in pregnancy. Results: SSRI exposure in pregnancy was significantly negatively associated with prenatal alcohol exposure. Specifically, SSRI exposure in pregnancy was associated with significantly lower number of average daily drinks during the second half of the first trimester (β =−0.34, p<0.01) and significantly lower number of binge episodes during each half of the first trimester and the second trimester (p<0.05). In addition, SSRI exposure was associated with significantly lower maximum number of drinks in one episode during the second half of the first trimester (β =−0.61, p<0.01). Conclusion: We did not have the data to examine severity of the depressive symptoms. However, our analyses suggest that women with major depression who are appropriately treated with antidepressants may reduce alcohol consumption during pregnancy. Future analyses should include examining alcohol consumption and timing of SSRI exposure.

**399 DRINKING WATER ARSENIC LEVELS PREDICT PLASMA LEVELS OF OXIDIZED LDL CHOLESTEROL (OXLDL) IN NAVAJO POPULATIONS EXPOSED TO URANIUM-CONTAMINATED MINING SITES.**


Numerous abandoned mines within the Navajo Nation contribute uranium, arsenic, radium and other heavy metals to the soil and groundwater. Environmental exposure to heavy metal contaminants may promote or exacerbate cardiovascular...
disease. To assess the potential impact of these contaminants of cardiovascular health of exposed individuals, we have begun to examine (IL-6, CRP) and novel (oxLDL and its receptor LOX-1) plasma biomarkers in a large community of the Navajo Nation. Samples and data were obtained through a culturally appropriate community-based participatory approach, incorporating data collection and outreach by Navajo community staff. Biomarker and self-report data were then linked to geospatial data on contamination sites using traditional linear regression and Bayesian models. Proximity to abandoned uranium mines was not a significant predictor of oxLDL or LOX-1. However, when we used a binary model for uranium and arsenic drinking water levels, we observed that the estimated annual intake of arsenic was a significant predictor of the logarithm of ox-LDL, and therefore of ox-LDL itself. Age, the occupational exposure score, and the distance-environmental exposure score are also significant predictors. However, a binary variable derived from the arsenic intake, with the three other predictors named, is a better predictor. It has more significance in its model, and its model fits the oxLDL data better. Neither metal was a significant predictor for LOX-1. These results indicate that arsenic intake may increase markers of cardiovascular risk. While still preliminary, these findings highlight the potential risks of using water with high levels of arsenic contamination. Ongoing work will help us understand the level of risk incurred by arsenic, providing the Navajo community and health practitioners with a more informed basis for decision-making.

**400 INCREASED LEVEL OF URINARY 8-HYDROXYDEOXYGUANOSINE RELATED TO ALDH2 POLYMORPHISMS IN WORKERS OCCUPATIONALLY EXPOSED TO STYRENE.**


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In this study, we examined the effect of occupational exposure to styrene on the level of urinary 8-hydroxydeoxyguanosine (8-OH-dG), which is a good indicator of oxidative stress in vivo, in 329 styrene-exposed workers and 152 unexposed healthy individuals in a reinforced plastic factory. In addition, we also analyzed the modifying effect of genetic polymorphisms of ALDH2 on styrene-induced oxidative stress. Urinary 8-OH-dG was measured by an automated high-performance liquid chromatography-electrochemical detector system and is normalized to creatinine concentration. Results showed that the level of urinary 8-OH-dG was significantly increased in styrene-exposed workers than unexposed individuals. We observed that there was a positive correlation between the level of urinary 8-OH-dG and years of employment in workers exposed to styrene. Individuals carrying variant allele ALDH2*2 showed significantly higher level of urinary 8-OH-dG than wild-type ALDH2*1/*1 in styrene-exposed workers, but not in unexposed healthy individuals. These data indicated that occupational exposure to styrene seems to induce oxidative stress in vivo, and this oxidative stress may be modulated by the activity of ALDH2.

**401 PULMONARY FUNCTION TESTING IN UTILITY WORKERS.**


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Utility workers may be exposed to petroleum combustion by-products, insulation chemicals, and other pulmonary toxics in the workplace that may affect pulmonary function (PF) and may require use of respiratory protection. This investigation used occupational health monitoring examination data to characterize PF in a population currently employed as utility workers in the state of Florida. PF tests for male workers (n = 227) who required health examinations to ensure fitness for continued respirator use were compared to NHANES III Raw Spirometry subjects (n = 5,866) to determine if decreased PF was associated with employment as a utility worker. Mean FVC and FEV1 values were determined and multivariate regression was used to evaluate the impact of utility worker status on PF after adjusting for confounders. Workers produced a higher mean FVC of 4.84L (95%CI 4.72-4.96) compared to a mean NHANES III subject value of 4.70L (95% CI 4.68-4.73) (p = 0.0023). No significant difference was detected between the worker FEV1 mean of 3.81L (95%CI 3.72-3.90) and the mean NHANES III subject value of 3.71 L (95% CI 3.69-3.73) (p = 0.0560). No significant differences were found between mean pulmonary function test values of workers and NHANES III study subjects when stratified by age, height, and smoking status except among older utility workers, who demonstrated modestly better pulmonary function values compared to their NHANES III counterparts. Multivariate regression analysis demonstrated significant predictors of FEV1 included age, height, pack-years of smoking, and utility worker status (all p-values < 0.0001). Significant predictors of FVC also included age, height, pack-years of smoking, and utility worker status (all p-values < 0.02). The direction of effect for utility worker status was beneficial for lung function (parameter estimates 0.17 L FEV1 and 0.12 L FVC). The modest increase in PF observed in utility workers in multivariate analysis is likely due to a combination of effective exposure controls in the workplace and the healthy worker effect among aging workers.

**402 ENVIRONMENTAL FACTORS ASSOCIATED WITH THE DEVELOPMENT OF BREAST AND CERVICAL CANCER IN WOMEN FROM NAYARIT, MEXICO.**

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The incidence of breast and cervical cancer varies widely among countries. In Mexico, for several years (1955-2005) cervical cancer mortality used to surpass breast cancer mortality in women. In 2006, however, the incidence of breast cancer increased and went ahead cervical cancer. The same year, the state of Nayarit occupied one of the first places having high incidence of cervical and breast cancer. The aim of this study was to analyze whether some environmental factors are associated with the development of cancer, as well as to describe the current trends of morbidity for breast and cervical cancer in women who attended the Cancer Center of Nayarit, Mexico during 2006-2010. The preliminary results show that the prevalence of cervical cancer is high in the poorest and most marginalized regions where woman start sexuality at an early age. Patients with breast cancer had a family history of cancer (45%) early menarche (11-13 years), advanced age of menopause (50-54 years), smoking and drinking habits (9 and 6%, respectively). The etiology of cancer is multifactorial from which 95% is environmental and hormonal. Environmental estrogens include polyyclic aromatic hydrocarbons which appear to be carcinogenic by genotoxic mechanisms and are found in tobacco smoke. Studies are in progress to explore the association between environmental factors and the incidence of both types of cancer.

**403 HEART RATE VARIABILITY (HRV)-BASED ASSESSMENT OF AUTONOMIC FUNCTION DISTURBANCES USING A RODENT TELEMETRY MODEL.**

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While heart rate variability (HRV) is widely recognized as an adequate marker for cardiovascular or autonomic function, it is not often considered for the preclinical evaluation of cardiovascular risk. In the present study we explored the use of HRV for the preclinical evaluation of drugs for potential disturbances in autonomic function. Five groups of 4 male Sprague Dawley rats surgically implanted with DSI T1L1N2-C50-PXT radiotelemetry transmitters for collection of blood pressure and heart rate were administered either autonomic system blockers (5 mg/kg Phentolamine; 1 mg/kg Atenolol; 0.5 mg/kg Atropine; and a combination of Phentolamine and Atenolol) or physiological saline by intravenous injection, and cardiovascular data collected continuously predose and for 24 hours postdose. HRV analysis was performed with Kubios analysis software, developed by the University of Kuopio, Finland. Variations in heart rate induced by reference compounds were calculated using the methods outlined by the North American Society of Pacing and Electrophysiology Task Force (1996) and encompassed time domain (statistical and geometric), frequency domain, and nonlinear methods. Results obtained with each method were analyzed statistically (one-way analysis of variance with a repeated measures model) focusing on either short term (one hour) or long term (24 hours) changes in HRV. For each method used, statistically significant changes were identified and a set of variables with potential as autonomic function marker were selected and discussed. The results support the use of a selected set of HRV endpoints to assess autonomic function in a rodent preclinical model.
**STUDIES OF THE QT PROLONGATION RELATIONSHIP IN GUINEA PIG, RABBIT, AND BEAGLE DOG IN THE QTc ASSESSMENT.**


QT interval prolongation of the electrocardiogram has been associated with the occurrence of life-threatening fatal ventricular arrhythmias. The recent withdrawal from marketing of several drugs due to potential drug-related cardiac arrhythmias has greatly increased concern about drug-related changes on the QT interval. To avoid the QT prolongation as to increase the safety margin of new drug candidates during new drug discovery and development, use of much rigorous methodology is critical to evaluate the potential of QT prolongation liability. To exploring the relationship between preclinical cardiac conduct assessments relatively to the clinical outcome, measurements of QT Prolongation by Sotalol in Guinea pig, Rabbit, and Beagle dog were conducted using standard 12-lead ECG. The results display a linear relationship of ΔQTc versus body weight in three testing animals. Interestingly, extending the relationship with the literature human data revealed that ΔQTc is better fitted with the body surface area. Additionally, crude extracts of several Traditional Chinese Medicines, which respectively consist of potential cardiotoxic alkaloids, dipterter lactone, and cardiac glycosides, were studied using this system. Most ΔQTc is linear fit with the body weights of Guinea pig, Rabbit, and Beagle dog. Those results are discussed.

**QT CORRECTION METHODS: CAN WE FURTHER OPTIMIZE METHODS IN CYNOMOLGUS MONKEYS, BEAGLE DOGS AND GOTTINGEN MINIGUS?**

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Correction of QT interval for heart rate is common practice in safety pharmacology. While standard correction methods are widely used, these methods suffer from species specific but also individual variations of the QT/RR relationship. Electrocardiographic data obtained from implanted and jacketed telemetry was used for analysis. A comparison of standard correction formulas and an individual correction method was conducted in Beagle dogs, cynomolgus monkeys and Göttingen minipigs. In all species, an individual correction method (QTca) provided the most stable QT values as a function of heart rate. When comparing standard methods, QT was optimally corrected with the Fridericia’s method (QTcF) in minipig, the Bazett (QTcB) in monkeys and QTcF or the Van De Water’s method (QTcV) for Beagle dogs. When using jacketed telemetry data, the mean slope of QTc as a function of heart rate in cynomolgus monkeys was 0.179 ± 0.232 msec/bpm for QTcF, 0.467 ± 0.070 msec/bpm for QTcB and 0.020 ± 0.010 msec/bpm for QTcV. The interval between calculations of QTc correction parameters for each animal may represent a success factor when using this method. Our results strongly highlight the beneficial impact of an individual correction method to improve the correction slope in all species but also to decrease variation of individual data within the same species, therefore increasing sensitivity of the models to detect QTc prolongation or shortening.

**RELIABILITY OF QT MEASUREMENTS COLLECTED FROM ROUTINE 28-DAY REPEAT-DOSE TOXICITY STUDIES.**

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There is a growing demand to include safety pharmacology parameters as a part of nonclinical toxicity studies. To meet this demand, various options such as jacket ECG monitoring or a subset of surgically implanted telemetryed dogs has been explored. Measurement of surface ECGs from limb leads I, II and III during a standard 28-day repeat dose canine toxicity study has been an ongoing practice for several years. However, due to study-related manipulations during study conduct, reliability and additive value of surface ECGs measured during toxicity studies is still unclear. In this case study, we have compared the ECG data (QT interval) obtained from dog telemetry studies (CV studies) to that of ECGs obtained from toxicology studies (TOX studies).

For comparison, vehicle control data (QT and RR interval) from three canine TOX studies and vehicle control data (QT and RR interval) from three canine CV studies were compared following Day 1, 2 or 3 postdose intervals. To assess the effect of handling/study-related manipulations during toxicity studies, pre-study surface ECG data (Day 1) was also compared with post-study data (Day 28).

The QT duration for beagle dogs from CV studies was 250 – 300 milliseconds (ms) and for the dogs from TOX studies was 215 – 240 ms (Day 1 and Day 2). The QT duration for dogs from CV studies was significantly greater than the QT duration for the dogs from TOX studies at all the time points however; the data were still within our laboratory historical range for CV or TOX study type and all the data obtained from the TOX studies from Day 1 and Day 28 were very consistent (within 215-240 ms). These results suggest that the surface ECG data following daily handling or study related manipulations of the animals did not affect the QT interval data. This comparison also shows that the surface ECG measurements should provide a reliable snap shot of potential QT effects following repeat dose administration without utilizing special (jacketed or telemeterized) dogs. Further investigations on the sensitivity of surface ECG measurement is ongoing.

**OPTIONS FOR BLOOD PRESSURE DETERMINATION IN EITHER DRF, MTD, OR REPEATED-DOSE TOXICITY STUDIES IN CYNOMOLGUS MONKEYS.**

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Sponsor: E.Vogel

Introduction: Various methods are available for determination of blood pressure in cynomolgus monkeys. Animal restraint is required for standard oscillometry (OSC) as well as High Definition Oscillometry (HDO), whereas animals are allowed to freely move in their homecage during data collection for both minimal invasive(MI) and full implant (FI) telemetry. In this study we compared the above blood pressure determination approaches in the cynomolgus monkey model when applied to different study designs. Methods: Blood pressure measurements were performed under different conditions and various methodologies as follows: OSC: Cardell; Veterinary Monitor; HDO: S+B medVet Memo Diagnostic; MI: PA-C10-TOX implant, FI: Koenigsberg T27F-2B. Results: For OSC the systolic pressure range was 143±22 (mean±SD), diastolic 90±25, and MAP 116±24 mmHg, for HDO the ranges were 138±21 mmHg for systole, 73±12 mmHg for diastole, and 96±13 for MAP on conscious, untreated animals. For the FI, systolic pressure range was 118±3, diastolic 86±3, and MAP 100±4. Overall, it has to be noted, that for both restraint procedures, the standard deviation is higher when compared to invasive approaches. Although non-invasive OSC and HDO indicate overall reliable results, detection of hypertension becomes difficult because of excitement and manually elevated base pressure values. The introduction of an implanted system with regard to detection of hypertension was clearly shown in animals after treatment with Torcetrapib (FI), and with Effortil (MI). However, it certainly needs to be considered that invasive approaches might be accompanied by unexpected side effects (e.g. micro infarts), but our data indicate only local irritations like intima thickening. Conclusion: The data suggest that especially for drug induced hypertensive effects, the use of either full implants (FI) or minimal invasive (MI) telemetry implants should be considered since data collection is feasible in conscious, unrestrained animals and can be collected for significantly prolonged periods.

**JACKETED EXTERNAL TELEMETRY IN GUINEA PIGS: A SCREENING TOOL FOR EVALUATION OF CARDIO-RESPIRATORY FUNCTION IN EARLY DRUG DEVELOPMENT.**


The guinea-pig is a well established animal model for the evaluation of QT lengthening. The guinea pig is routinely used as anesthetised model or as a conscious model using implanted telemetry. Implantation of telemetry devices in guinea pigs requires surgical intervention that is time consuming and expensive. Therefore, the purpose of the present study was to evaluate a newly established jacketed external telemetry model in freely moving guinea pigs. Female guinea-pigs were equipped with jacketed external telemetry external telemetry using DSI transmitters and placed in plethysmography chambers allowing simultaneous recording of ECG and respiratory parameters. To evaluate the sensitivity of the model, effects of low dose of sotalol (10 mg/kg) or vehicle (0.5% methylcellulose) on ECG were investigated in freely moving guinea pigs (6 animals per group). In addition, effects of clonidine (5 mg/kg) on respiratory parameters were investigated. Drug treatments were performed by the oral route and all parameters were recorded one hour before and for a minimum of 4 hours after treatment. Although a low dose-level was used in the present study, significant increases in QT and QTT (Bazett’s correction) were observed after administration of sotalol. When compared to the vehicle, clonidine produced significant decreases in tidal volume and increases in respiratory rate. The present study demonstrates that the jacketed external telemetry model allows for a reliable screening tool for the potential risk assessment of new chemical entities, biologics or anti-cancer drugs on cardio-respiratory function in early drug development process.
409 METHOD VALIDATION OF CARDIOVASCULAR AND RESPIRATORY SAFETY ASSESSMENT IN CYMONOLUS MONKEYS AND BEAGLE DOGS.


This study validates the methods of cardiovascular and respiratory safety assessment in cynomolgus monkeys and beagle dogs using telemetry system and respiratory flow system at Pharmaron’s Laboratory. The cardiovascular effects of moxifloxacin and xylazine were investigated in cynomolgus monkeys and beagle dogs implanted with telemetry transmitters. The respiratory effects of methacholine, 8% CO2 and buprenorphine were evaluated in conditioned cynomolgus monkeys with helmet and beagle dogs with face mask connecting to respiratory flow system.

Moxifloxacin significantly prolonged QTc intervals and increased QT and R-R wave intervals in the telemetered cynomolgus monkeys (given 50 mg/kg) and beagle dogs (given 60 mg/kg). Xylazine increased both the diastolic and systolic pressures immediately after dosing in telemetered beagle dogs (given 2 mg/kg). Methacholine (8 μg/kg) significantly increased respiratory rate and minute volume (MV) in beagle dogs. CO2 at 8% significantly increased tide volume (TV), MV and respiratory rate in cynomolgus monkeys. Buprenorphine significantly decreased TV and MV in beagle dogs (given 16 μg/kg) and decreased MV and respiratory rate in cynomolgus monkeys. The changes observed cardiovascular and respiratory effects are similar and/or comparable to the results reported in the literature [1-8].

In conclusion, the cardiovascular effects of moxifloxacin and xylazine and the respiratory effects of methacholine, 8% CO2 and buprenorphine reported in the literature were reproducible in Pharmaron’s laboratory.

410 IMPACT OF A NOVEL DENOISING ALGORITHM ON ECG ANALYSIS.

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Noise and artifact in ECGs collected from ambulatory preclinical subjects has been an obstacle to accurate and efficient automated analysis of ECG intervals. Most troublesome is noise that is within the bandwidth of the ECG signal (e.g., in-band noise) because it cannot be removed by traditional filtering techniques without distorting the ECG. In-band noise is most often caused by EMG and movement artifact. A new technology, Multi-Domain Signal Processing (MDSP denoising), has been shown to reduce the amplitude of in-band noise by up to 90% without distorting ECG morphology. In this work, the impact of MDSP denoising on analysis of ambulatory telemetered preclinical subjects using the EMKA ECGauto is evaluated. Five 24-hour recordings obtained using DSI D70-PCTs and Dataquest were analyzed by an experienced operator using EMKA ECGauto before and after MDSP denoising. Analysis results show that denoising using MDSP reduces the number of cardiac cycles rejected by the EMKA system due to excessive noise by > 90%. These results demonstrate that denoising of ECG recordings using MDSP prior to analysis with EMKA ECGauto can provide a significant increase in the number of cardiac cycles that can be automatically analyzed using the EMKA system, and may reduce the amount of labor required to review and edit results, especially when continuous beat-to-beat evaluation is required.

411 STATISTICAL SIMULATION FOR SENSITIVITY OF THE JACKETED EXTERNAL TELEMETRY ASSAY FOR DETECTING CHANGES IN ELECTROCARDIOGRAM AND BLOOD PRESSURE.

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A statistical simulation was performed to assess the sensitivity of the canine jacketed external telemetry assay incorporated to a toxicity study for detecting drug-induced changes in electrocardiogram (ECG) and blood pressure (BP). Two studies were conducted: 0.5% methylcellulose and dl-sotalol at 1, 3, and 10 mg/kg (p.o.). The dl-sotalol study had a 3-period fixed sequence design with 4 dogs; each received vehicle (period 1) followed by two different doses (period 2 and 3). The vehicle study had 4 dogs; each received 0.5% methylcellulose over several periods. The sensitivity/power was simulated for 3-period fixed sequence (n=3/seq. 2 or 3), parallel groups (n=6/group or 3) and 4x4 Latin square (n=1/seq.) designs. The simulations assumed a true effect profile and variability from the two studies. The data were 5 min. averages, every 5 mins. for 24 hours. Data were summarized via AUC[0 to 6 hrs] and analyzed using repeated measures ANOVA. The simulation results for the 3-period fixed sequence design (n=2/seq.) showed that the test system was capable of detecting significant (p-value <0.05) changes of 10-14%, 37-46%, 21-28%, 13-16% and 9-13% in BP, HR, PR, QRS, and QTc with 80-90% power, respectively. The sensitivity was improved by increasing the number of dogs to n=3/seq. (5.3-6.6% in QTc with 80-90% power in n=3/seq.) and changing study design to a 4x4 Latin square (5%-6% change in QTc with 80-90% power with n=1/seq.) or a parallel design with n=6 or 8 per group (3.6-4.2% in QTc with 80-90% power in n=6/seq.; 3.2-3.5% in QTc with 80-90% power in n=8/seq.). In the dl-sotalol study, the expected pharmacological changes (dose-response and time-course changes) were accurately detected; effects as small as a 14% decrease in HR, 8% increase in PR and 9% increase in QTc were detected. In conclusion, the canine jacketed external telemetry assay incorporated to toxicity studies, was able to detect small changes in ECG or BP and was considered acceptable as a cardiovascular model in the preclinical testing.

412 ASSESSMENT OF BOVINE SERUM ALBUMIN ADDED TO MODIFIED KREBS HENSELIEF BUFFER EFFECTS ON ECG, LEFT VENTRICULAR MECHANICS, AND CORONARY BLOOD FLOW IN THE GUINEA PIG ISOLATED HEART.


The Langendorf isolated heart perfusion assay is valuable in determining the effects of pharmaceutical compounds on changes in cardiac electrical conductivity and cardiac contractility. Perfusion of the isolated heart with protein-free solution may result in myocardial tissue edema which would alter hemodynamic function. Bovine Serum Albumin (BSA) is commonly used in preparations of Krebs Henseleit Buffer (KHB) to prevent tissue edema. The added protein increases oncotic pressure by drawing fluid into the circulatory system, mimicking conditions found in the in vivo considering albumin in blood is responsible for 79% of the total capillary oncotic pressure. The aim of this study was to determine the effects of 1% BSA added to the KHB in retrograde perfusion on the isolated guinea pig heart. Measurements of ECG, left ventricular mechanics, and coronary flow were compared for KHB solution alone, against KHB with 1% BSA added. Additionally, in increasing concentrations of terfenadine, a known QT prolonging agent, parameters were assessed utilizing the 1% BSA in KHB. Hearts perfused with 1% BSA demonstrated an increase in coronary flow rates over time while hearts perfused with KHB alone decreased. Effect of BSA on ECG was minimal between the two groups. The increasing concentrations of terfenadine caused reduced contractility parameters as well as QT prolongation in the presence of BSA. In conclusion, the isolated hearts perfused with 1% BSA demonstrated greater stability in the measured contractility parameters over time as compared to hearts perfused with KHB alone. The effects of terfenadine where similar in this preparation.

413 THE TELL-TALE HEART: WHAT CAN THE ISOLATED HEART TELL US ABOUT DRUG-INDUCED CARDIAC INJURY?

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Pharmaceutical drug attrition and failures after FDA approval remains an issue for the pharmaceutical industry and public safety. 30% of post approval drug failures are due to cardiac toxicity with 12% from arrhythmias and 18% due to compromised contractility. There is a need to engage in earlier, rigorous, preclinical cardiac testing. A cardiac toxicity assay should be cost effective, balanced specific and sensitive, translatable and predictive of acute and latent cardiac dysfunction and damage. The Langendorf model appears to meet most of these requirements. Our goal was to demonstrate the power and viability of the Langendorf model for predicting drug induced defects in cardiac contractility by combining multiple analysis end points including ECG, contractility, energetics, and biomarkers of injury. Male Sprague-Dawley rats were anesthetized with 50 mg/kg Nembutal, intratracheal, intravascular, and hearts were removed, placed in a cardiopulmonary solution for < 10 min, cannulated via the aorta, and perfused with Modified Henseleit Krebs solution at 37°C. A balloon was inserted into the left ventricle to measure left ventricular pressure (LVP) and electrodes were placed on the heart to measure ECGs. After equilibration, a baseline was measured followed by escalating doses of each class of drug for 20 minutes each dose. ECG and LVP were recorded continuously and effluent was collected from the heart at the end of each dosing period for analysis of biomarkers indicative of injury. Energy status of the heart was assessed by measuring high energy 31phos-
Cardiac toxicity is one of the leading causes of drug failures over the past two decades; withdrawals occurring during both development and after approval. Up to 30% of patients (children and adults) receiving chemotherapies such as Doxorubicin experience latent cardiac toxicity up to 15 years after initial treatment. The need to employ predictive, specific, sensitive, and cost-effective safety assays earlier in drug development and at times to retro test withdrawn drugs to determine mechanisms of unexpected toxicities has resulted in the necessity to not only extrapolate pharmacokinetic (PK) data between preclinical species, but also in vivo to in vitro studies. Data generated by traditional PK models apply only to the conditions and species from which the data was generated. This limitation has been most apparent in risk assessment analysis of environmental and industrial toxicants where human data is not usually available and Physiologically Based Pharmacokinetic (PBPK) models are used for species to species extrapolation of data from animal to human model based on physiological differences. Our goal was to apply this technique in the pharmaceutical drug arena to determine clinically relevant doses for potential cardiac toxicity assessment in the Langendorff Rat isolated heart model. Using Berkley Madonna™ software we developed PBPK models, human to rat species extrapolations, to determine clinically relevant doses associated with cardiac acute and latent toxicity for Doxorubicin, Sunitinib, Erlotinib, and 5-fluorouracil. We also extrapolated rat in vivo dosing to rat Langendorff concentrations, to determine relevant doses for acute cardiac injury for Isoproterenol, Verapamil, Carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP), and Caffeine. The doses determined will be used to induce both acute cardiac toxicity and mimic conditions shown to cause latent toxicity in the Langendorff preparation.

The hERG channel assay is routinely used to evaluate the effects of new chemical entities on the delayed rectifier potassium current (IKr). Although the methodology of such assay is quite standardized, the experiments are not systematically conducted at physiological temperature. However, some test substances can display different properties or potencies at physiological temperature versus room temperature. The aim of this work was to evaluate the influence of the temperature on the hERG channel blockade induced by erythromycin or terfenadine.

Whole-cell patch clamp method was used to record the sodium current (INa) encoded by hNav1.5 in stably transfected HEK293 cells (n=6 to 7 cells/group). Experiments were performed at either near-physiological (35°C) or ambient (22-24°C) temperature. At physiological temperature, erythromycin (from 30 to 900 μM) concentration-dependently decreased the amplitude of IKr (-70.3 ± 4.9% at 900 μM, IC50 = 211.1 ± 51.54 μM). In contrast, at ambient temperature, erythromycin (900 μM) only slightly decreased the amplitude of IKr (-17.0 ± 4.0%).

Terfenadine (from 0.01 to 10 μM) similarly decreased the amplitude of IKr whatever the temperature used (IC50 = 0.27 ± 0.02 μM at ambient temperature versus 0.17 ± 0.05 μM at physiological temperature, NS). These findings suggest that the hERG Channel blockade induced by erythromycin was greater at physiological temperature than at room temperature. Therefore, the highest sensitivity of the model at 35°C should allow detecting proarrhythmic risk of a substance at lower concentrations. Although all substrates are not temperature-sensitive, the use of physiological temperature in the hERG Channel Test should be recommended for screening or safety pharmacology studies.

A novel heart slice model was used to compare the responses of human and rat to the following cardiotoxic drugs: isoproterenol, a β-adrenergic agonist known to induce cardiac ischemia and hypertrophy, and etomoxir, an inhibitor of mitochondrial long-chain fatty acid oxidation that can cause cardiac hypertrophy. Gene and
protein expression patterns were analyzed to compare activated pathways in the two species. Heart slices (1-200 μm thickness) from human donors or adult male Sprague-Dawley rats were cultured for 24 hr with isoproterenol (0-1000 μM) or etomoxir (0-500 μM). Both drugs effected human and rat slice energy (ATP) levels, which were measured as an indicator of tissue viability. Human heart slices treated with isoproterenol or etomoxir caused changes in gene expression associated with cardiac hypertrophy (Keg, Reg30), mitochondrial function (Idh2-3, Sdhα), and stress response (Hyp/A), as well as inflammatory (IL-6) and remodeling (TIMP 2, 4) protein biomarkers. Rat heart slices showed changes in genes related to stress response (Hyp/A) and cardiac hypertrophy (Reg3β). Additionally, etomoxir altered gene expression of the fatty acid oxidation pathway (Acot, Acox) in human and rat, consistent with its mechanism of action. In summary, heart slices responded to the known cardiotoxic agents isoproterenol and etomoxir. The mechanism of toxicity of these drugs can be examined using gene and protein expression in combination with pathway analysis. This model will be useful to provide insight into the effects of drugs with unknown mechanisms of toxicity.

419 MULTIPLEX BIOMARKER APPROACH FOR DETERMINING DRUG-INDUCED VASCULAR INJURY (DIVI) IN RAT.


Vascular injury is a common finding during the pre-clinical safety study of drugs. A lack of understanding of mechanisms of drug-induced vascular injury (DIVI) in animals, the absence of accepted specific and sensitive biomarkers have become significant barriers in the development of new therapeutics. A number of promising biomarker candidates have been nominated with the need for further validation and evaluation. However, the lack of qualified high-throughput assays has imposed a hurdle to the progress in this area.

Based on Lumines® MAP® technology, we developed multiplexed immunoassays for a panel of nine DIVI biomarker candidates in rat. The biomarker candidates included Alpha-2 Macroglobulin, Alpha-1 Acid Glycoprotein, Cavolin-1, CINC-1/GRO, Connective Tissue Growth Factor, Haptoglobin, Tissue Inhibitor of Metalloproteinase-1, Vascular Cell Adhesion Molecule 1, and Von Willebrand Factor.

To establish the sensitivity and specificity of the rat DIVI biomarker multiplex assay, serum and plasma samples from fenoldopam mesylate (FM) induced rat DIVI models were analyzed with the assay and the levels of the nine biomarker candidates were quantified simultaneously. To induce vascular injury, rats were treated with the vasodilator compound fenoldopam mesylate (FM). FM was administered by continuous intravenous infusion with a dose of 6 mg/kg/h over 24h. Subsequently to the infusion, three rats were sacrificed and serum and plasma was collected at day 1, 3, and 7 each. Sterile 0.9% saline was used in the vehicle control cohorts. Separately, a group of animals were IP-injected with FM. Serum and plasma samples were collected 6 hour, 24 hour and 5 days post-injection. Significant blood level elevations of several candidate biomarkers were observed in the fenoldopam-treated animals compared to the vehicle-treated cohorts. The degree of biomarker elevation in blood correlated to the dose and time of FM treatment. Overall, these results demonstrate the feasibility of using the multiplex biomarker approach to determine the onset and severity of DIVI in pre-clinical drug safety studies.

420 CHARACTERIZATION AND UTILIZATION OF INDUCED PLURIPOTENT STEM CELL-DERIVED CARDIOMYOCYTES IN IDENTIFYING NOVEL MECHANISMS OF CARDIOTOXICITY.

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Cardiotoxicity is one of the leading causes of early and late stage drug attrition which has significant impact on patients welfare and in particular for cancer patients. Current in vitro models insufficiently predict cardiotoxicity and there is a definite need for alternate physiologically relevant in vitro models. Induced pluripotent stem cells offer the promise for development of tissue specific in vitro models. In here, we describe the use of induced pluripotent stem cell-derived cardiomyocytes (iCell CM) for identifying novel molecular mechanisms of cardiotoxicity. Towards this end, we characterized iCell CM using gene expression analyses over a 42 day period post thaw in culture. Based on our initial analyses iCell CM express relevant cardiac markers such as ion channels (SCN5A, KCNQ2, KCNQ1 and KCNQ3), cardiac tissue markers(MYH6, MYLFP, MYBPC3, DES, TNNI3, TNN2 and TNN3), cardiac transcription factors (NKKX2.5, GATA4 and GATA6) and lacked the expression of stem cell markers(FoxD3, GROX2, NANOG, POU5F1, SOX2, and ZFP42). We then selected ten in vivo structural cardiotoxicants which were not flagged by our current in vitro assays and generated gene expression data in response to these compounds in both iCell CM and rat heart derived cell line H9C2. To further evaluate the data, we used our internal Casual Reasoning Engine (CRE) platform, which analyzes experimental gene expression data, in the context of prior biological knowledge to generate testable upstream molecular hypotheses. Based on our initial analyses across all compounds, iCell CM exhibited increases in structural cardiotoxicity injury signals, inflammation signals and TGFB beta signaling pathways whereas H9C2 cells exhibited increase in muscle injury signals, decreases in both TGFB gene and inflammation signals. In conclusion, iCell CM appear to represent a better in vitro cardiomyocyte model for understanding cardiotoxicity mechanisms and further validation will be performed to confirm these findings.

421 MURINE-SPECIFIC VASCULAR TOXICITY OF A VITRONECTIN RECEPTOR ANTAGONIST: MECHANISTIC STUDIES.

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In previous studies, a potent, orally-active, non-peptide vitronectin (tvfβ3, tvfβ5) receptor antagonist (SB-273005) induced unique vascular lesions in mice, but not other species pharmacologically responsive to SB-273005 or other antagonists. Vascular smooth muscle cell (VSMC) necrosis was observed in the aorta of mice approximately 6 hours after dosing, followed by VSMC loss with adaptive VSMC hypertrophy but no endothelial damage. The lesions were non-progressive, as aortic changes were similar in mice treated for 4 days or 3 months, and were irreversible with no evidence of regeneration observed after drug-withdrawal. Since drug-induced direct chemical vascular toxicity is uncommon, especially in mice, involvement of VSMC–endothelial cell (EC) interactions in development of the toxicity was hypothesized. To explore this possibility, in vivo model systems of strain-specific primary murine aortic VSMC and EC were established and utilized to investigate the potential mechanism of cellular toxicity using both monocultures and VSMC/EC cocultures. Incubation of monocultures and cocultures with SB-273005 within a dose range and timeframe comparable to the in vivo studies showed a concentration-dependent decrease in viability, with subsequent increases in cytotoxicity, for both VSMC and EC monocultures and VSMC/EC co-cultures; however, VSMC monocultures responded at lower doses (were most sensitive), suggesting a direct effect on VSMC. EC are less susceptible than VSMC to the toxic action of SB-273005, and do not markedly affect VSMC toxicity. Further studies using VSMC monocultures revealed increases in Caspase 3 & 7 activity as early as 1 hr following treatment with SB-273005. These data suggest VSMC apoptosis is the primary mechanism of toxicity responsible for the murine-specific vascular lesions.

422 COENZYME Q10 RESCUES ENDOTHELIAL CELLS FROM NRTI-INDUCED MITOCHONDRIAL DAMAGE AND MITOPHAGY.

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Long-term use of nucleoside reverse transcriptase inhibitors (NRTI) is known to induce mitochondrial damage in endothelial cells, culminating in endothelial dysfunction, an initiating event in the pathogenesis of atherosclerosis. Though the mechanism of NRTI-induced effects on endothelial cells is not well understood, prior work suggests that a mitochondrially-compartmentalized oxidative stress may be involved. We thus investigated the potential of adjunct utilization of the antioxidant coenzyme Q10 (CoQ10) to combat NRTI-induced endothelial toxicity and further characterized the mechanism of acute NRTI toxicity in endothelial cells. Co-treatment of human umbilical vein endothelial cells (HUVEC) with CoQ10 was found to rescue cells from increases in ROS levels and decreases in ATP production and oxygen consumption induced by NRTI treatment alone. In addition, we found that NRTI increased levels of endothelin-1, a marker for endothelial cell activation, but this effect was also reversed by CoQ10 co-treatment. Finally, treatment of HUVEC with the NRTI zidovudine (AZT) induced selective mitochondria...
ial autophagy, or mitophagy, AZT treatment for 6-8 h increased lysosomal activity, while 8 h treatment resulted in increased mitophagy with lysosomes, an indicator of mitophagy. The LC3-II/LC3-I ratio, a marker of autophagosomal activity, was also increased in treated cells. These findings suggest that NRTI-induced autophagic degradation of mitochondria may be involved in NRTI-induced endothelial dysfunction, and that this damage likely results from oxidative injury. NHLBI R01 HL082472.

423 TEMPOL AND TEMPOL-H PROTECT CARDIOMYOCYTES FROM NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITOR (NRTI)-INDUCED MITOCHONDRIAL TOXICITY.
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NRTIs are essential components of the successful antiretroviral combination therapies used for treatment of HIV-1. However, during long-term therapy NRTIs may damage heart mitochondria, thus limiting clinical use of these drugs. Consequently, reducing NRTI-induced mitochondrial toxicity may benefit HIV-1-infected patients. Using H9c2 rat cardiomyocyte cultures exposed long-term to Zidovudine (AZT) or AZT plus Didanosine (ddI) we demonstrated NRTI-induced mitochondrial damage. In an attempt to protect mitochondria from this damage we have used the stable free radical Tempol and its metabolite Tempol-H, cyclic nitroxides with antioxidant properties. H9c2 cells were exposed to 50 μM AZT plus 50 μM ddI for 15 passages (P15) in the presence and absence of 150 μM Tempol or 150 μM Tempol-H. The AZT/ddI combination caused moderate growth inhibition (< 30 %), and co-exposure with Tempol or Tempol-H did not restore cell survival. Mitochondrial oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured using the Seahorse XF24 analyzer. As seen previously, AZT/ddI reduced maximal FCCP-uncoupled OCR. However co-exposure with Tempol and Tempol-H stimulated OCR, restoring the AZT/ddI-reduced uncoupled OCR by 22-91 %. Similarly, the uncoupled ECAR levels were increased by 15-46 % with Tempol or Tempol-H. Preliminary Western blot findings showed that Tempol and Tempol-H enhanced the expression of uncoupling protein-2 (UCP-2). Therefore Tempol and Tempol-H may protect cardiomyocytes from mitochondrial compromise induced by the NRTI combination AZT/ddI, and UCP-2 may play a role through mild uncoupling. We are currently evaluating potential mechanisms by which these compounds may act as mitochondrial protective agents.

424 OVEREXPRESSION OF CAVEOLIN-1 PROMOTES ATHEROSCLEROSIS THROUGH INCREASED VASCULAR CELL ADHESION MOLECULE-1 EXPRESSION IN PRIMARY VASCULAR ENDOTHELIAL CELLS.
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Persistent organic pollutants, such as polychlorinated biphenyls (PCBs), are well-known to cause toxicity in various cell types, including endothelial cells. Particularly detrimental, coplanar PCBs including PCB 77 and PCB 126 can activate the aryl hydrocarbon receptor (AhR) and subsequently induce oxidative stress and NF-κB activation. Increasing evidence has shown that lipid raft membrane microdomains, caveolae, may play an important role in the toxicity of coplanar PCBs. Caveolae are abundant in vascular endothelial cells and the cardiovascular system in general, which points to a possible major role for caveolae in inflammation and atherosclerosis. Our lab has reported previously that coplanar PCBs promote the up-regulation of genes related to the activation of endothelial cells and the initial stages of atherosclerosis. In this work we show evidence that the integral caveolae protein, caveolin-1 (Cav-1), may play an important role in PCB-induced activation of endothelial cells. In an effort to examine the impact of Cav-1 on the initiation of atherosclerosis, a Cav-1 gene overexpression vector was created and transfected into primary vascular endothelial cells. Vascular cell adhesion molecule-1 (VCAM-1) and Cav-1 protein levels were assessed using Western blot. The inducible vector system successfully promoted overexpression of Cav-1 in a positive dose dependent manner. It was determined that doubling the expression level of Cav-1 resulted in nearly a 3-fold increase in VCAM-1 protein expression. These results were consistent for both porcine and human endothelial cell lines. Increasing Cav-1 levels may alter cellular trafficking and or cellular signaling and subsequently promote the early stages of atherosclerosis. This work adds to the growing evidence that Cav-1 or functional caveolae may play an important role in endothelial cell activation and cellular dysfunction.

425 TYROSINE KINASE INHIBITOR SUNITINIB, INDUCED CARDIAC HYPERTROPHIC GENES THROUGH MITOGEN-ACTIVATED PROTEIN KINASES-DEPENDENT PATHWAY IN H9C2 CELLS.
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Sunitinib (SUN) is a recently FDA-approved antitumor tyrosine kinase inhibitor for the treatment of renal cell carcinoma and gastrointestinal stromal tumors. Although SUN has improved survival rates in cancer patients, cardiotoxicity has been reported as a significant side effect. Several mechanisms have been proposed by which SUN causes cardiotoxicity. Yet, the potential effects of SUN on cardiac hypertrophic genes and the role of mitogen-activated protein kinases (MAPKs), including p38, ERK and JNK, signaling pathway have never been studied. Accordingly, we hypothesized that SUN induces cardiotoxicity by altering the expression cardiac hypertrophic genes through MAPK-dependent mechanism. To test our hypothesis, rat cardiomyocyte H9c2 cells were utilized as an in vitro model to a) determine the potential effect of SUN on the expression of cardiac hypertrophic genes, particularly α-MHC, β-MHC and BNP, at the mRNA and protein levels and b) explore the molecular roles of MAPKs' signaling pathways in SUN-induced cardiotoxicity using specific pharmacological inhibitors. At the mRNA levels, our results showed that SUN induced the mRNA expression of all the three hypertrophic genes in dose- and time-dependent manners. The mRNAs of hypertrophic genes were detectable as early as 6 h and remained elevated for at least 18 h after SUN treatment. Interestingly, pretreatment of H9c2 cells with p38 MAPK inhibitor, SB203580, significantly restored SUN-mediated effect, whereas neither inhibitors of ERK nor JNK inhibitors altered the mRNA expression. At the protein level, the induction of hypertrophic protein levels by SUN were gradually decreased by all MAPK inhibitors. In conclusion, our study clearly demonstrated that SUN induced hypertrophic genes at the mRNA and protein expression levels through MAPK-dependent signaling pathway.
Acknowledgments: King Abdulaziz City for Science and Technology (KACST) and the Research Centre, College of Pharmacy, King Saud University.

426 SUNITINIB, TYROSINE KINASE INHIBITOR, INDUCES CYTOCHROME P450 1A1 GENE EXPRESSION IN RAT CARDIOMYOCYTE H9C2 CELLS: A POSSIBLE MECHANISM OF CARDIOTOXICITY.
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Sunitinib (SUN) is a new multitargeted oral tyrosine kinase inhibitor that has both antiangiogenic and antitumor activity. Although sunitinib has improved survival rates in cancer patients, cardiotoxicity has been reported as a significant side effect. In this regard, several studies have demonstrated a direct correlation between the cytochrome P450 1A1 (CYP1A1) induction and cardiotoxicity. Taken together, the possibility that SUN induced cardiotoxicity through the induction of CYP1A1 has never been investigated yet. Therefore, we hypothesized that SUN induced cardiac hypertrophic genes through the induction of CYP1A1 gene expression. To test our hypothesis, we examined the capacity of SUN to induce the constitutive CYP1A1 gene expression and its correlation with two hypertrophic genes, atrial natriuretic peptide (ANP) and cardioprotein-1 (CT-1) mRNA levels in H9c2 cells. Our results showed that SUN induced CYP1A1 mRNA, protein, and activity levels in a concentration- and time-dependent manner in H9c2 cells. This induction was associated with proportional increase in the mRNA expression of hypertrophic ANP and CT-1 genes, suggesting a direct involvement of CYP1A1 in SUN-induced cardiotoxicity. Furthermore, the inability of SUN to bind to and activate AhR translocation, not only suggests an aryl hydrocarbon receptor (AhR)-independent mechanism is involved, but also to suggest that SUN is not an AhR ligand. On the other hand, Transfecting H9c2 cells with siRNA against CYP1A1, significantly restored SUN-induced ANP and CYP1A1 mRNA levels, implying the direct role of CYP1A1 in the induction of hypertrophic genes in response to SUN. In conclusion, this is the first demonstration that the SUN induces hypertrophic genes through the induction of CYP1A1 gene expression in H9c2 cells via an AhR-independent mechanism.
Acknowledgements: The Research Centre, College of Pharmacy, King Saud University.

Rationale: Arsenic (As) contamination of groundwater is common throughout the world, and chronic ingestion of well water contaminated with inorganic As can produce toxic effects including blackfoot disease, various cancers, hyperpigmentation, and hyperkeratosis. Chronic As ingestion has also been epidemiologically associated with development of atherosclerosis and hypertension, yet cellular mechanisms by which both inorganic As and methylated As metabolites exert these toxic effects are not well elucidated. Scope: Both inorganic arsenite (iAs') and the human metabolite monomethylarsinous acid ('MMAs') are believed to affect the activity of the voltage-gated or 'L-type' calcium ion channel (LTCC), which is the most thoroughly characterized Ca2+ channel governing extravascular Ca2+ entry and a key facilitator of the development and maintenance of vascular smooth muscle (VSM) tone. Intracellular Ca2+ can regulate the activity of other classes of ion channels, including the large conductance Ca2+-activated potassium ion channel ('BK Ca'), a known modulator of cellular depolarization that has been recently implicated in the development of hypertension and restenosis disease. Experimental Procedures: The present study examined the effects of iAs and MMAs on expression and activities of LTCC and BKCa channels in acutely isolated and primary / tissue cultured rat thoracic aorta, as well as the experimental A7r5 rat thoracic aorta smooth muscle cell (SMC) line using whole-cell patch clamp, vascular contractility, real-time RT-QPCR, and fluorescence microscopy approaches. Data: Initial results indicate significant alterations in SMC morphology, viability, and responsiveness to phenylephrine-induced vasoconstriction upon acute and subchronic exposure to both iAs and MMAs. LTCC activity is also altered following iAs exposure. Conclusion: Exposure of VSM and SMCs to relevant concentrations of both iAs and MMAs affects tissue contractility, cell viability, and the activities of key ion channels governing the maintenance of VSM tone.

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Elevated saturated fatty acids including palmitate often occur in the patients from perturbations in the regulation of critical homeobox genes. Supported by NIH Grant ES006273.

Sponsor: F. Matsumura.
corporation into nanomaterials may increase bioavailability in unanticipated ways. Developing children and fetuses may be particularly vulnerable to toxins found in e-waste, and early epidemiological studies near informal e-waste recycling sites indicate potential developmental neurotoxicity. Understanding the hazards of e-waste, the impacts of its disposal, and the dangers of informal or careless recycling will help reduce or prevent disease outcomes associated with exposure to e-waste components.

**432 NIEHS CENTERS FOR NANOTECHNOLOGY HEALTH IMPLICATIONS RESEARCH: BUILDING THE SCIENTIFIC FOUNDATION FOR EVALUATING PUBLIC HEALTH IMPLICATIONS OF ENGINEERED NANOMATERIALS.**

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The NIEHS established the NIEHS Centers for Nanotechnology Health Implications Research (NCNHIR) consortium to gain a comprehensive understanding of how physical and chemical characteristics of engineered nanomaterials (ENMs) influence their molecular interactions with biological matrices and elicit biological responses. This consortium includes eight centers funded in 2010 through a multi-project cooperative agreement (U19) and research grant (U01) mechanisms, along with additional investigators supported by Nano-EHS research program at NIEHS. Together, the consortium and individual investigators are exploring a library of engineered nanomaterials selected by NCNHIR with an overarching research focus that integrates physical and chemical characteristics of ENMs with biological effects. The research projects at these centers are investigating how the physical and/and chemical characteristics of ENMs dictate biological interactions at the molecular and cellular level. This knowledge can be translated to predict biological responses in vivo such as absorption, distribution, metabolism, and elimination (ADME) as well as physiological and pathobiological events in target and secondary organs, using appropriate routes of exposure and dose metrics. This information can be used to develop and apply predictive models in hazard characterization and assessment due to accidental or incidental exposure to ENMs. This session will highlight some of the recent findings and efforts of the consortium and provide scientific leads for a better understanding of the potential adverse health effects associated with ENM exposure.

**433 PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR MICE, RATS, AND HUMANS ORALLY-EXPOSED TO CHROMIUM.**

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A multi-compartment physiologically based pharmacokinetic (PBPK) model was developed to describe the behavior of Cr(III) and Cr(VI) in mice, rats, and humans. Tissue compartments were included for gastrointestinal lumen, oral cavity, stomach, small intestines (duodenum, jejunum, ileum), blood, liver, kidney, bone, and a combined compartment for remaining tissues. Data from *ex vivo* reduction studies were used to characterize reduction of Cr(VI) in fed rodent gastric fluid as a second-order process. *Ex vivo* data for human stomach fluid were used to characterize reduction of Cr(VI) in humans as a pH-dependent process under fed and fasted conditions. Tissue time course data for total Cr were collected from rats and mice exposed to Cr(VI) in drinking water for 90 days at six concentrations ranging from 0.1 to 180 mg Cr(VI)/L. These data were used to supplement the tissue time course data collected by NTP for Cr(III) and Cr(VI). Clear species differences are identified for Cr delivery to the target tissue (small intestine), with higher concentrations achieved in mice, consistent with tumor formation upon chronic exposures, than rats, in which tumors were not observed. Plasma/RBC Cr ratios suggest that Cr(VI) entered portal circulation at 60 and 180 mg/L in rodents. Species differences were identified for distribution of Cr to the liver and kidney, with liver/kidney ratios higher in mice than in rats. Overall, the PBPK model provides a reasonable fit to the available data. Simulations in humans were conducted to evaluate diurnal variation in gastric pH, as a function of meal consumption, and its impact of Cr(VI) reduction. Additional simulations were conducted to address potentially sensitive subpopulations, including fasted individuals, individuals on proton pump inhibitor medication, and children. The predictions of internal dose using the PBPK model support dose-response assessments for cancer and noncancer endpoints and allow for interspecies extrapolations to humans at environmentally relevant exposure concentrations.

**434 GASTRIC REDUCTION OF Cr(VI) IN MICE, RATS AND HUMANS.**

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The gastric reduction of Cr(VI) is a critical step in the proposed mode of action for the small intestinal tumors in mice. To investigate the kinetic mechanisms of gastric reduction of Cr(VI), stomach fluid was obtained from fed mice and rats and from fasted and fed human volunteers, including patients on proton pump inhibitors (PPIs). Studies were conducted to ascertain the kinetics (rates and capacity) of the various gastric fluids to reduce Cr(VI). The pH dependence of the various kinetic factors was also investigated. The reduction of Cr(VI) in fed mouse and rat gastric fluid followed second-order kinetics, with dependence upon both the Cr(VI) concentration and the concentration of reducing agents. Approximately 16 mg equivalents of reducing agent per liter of fed stomach fluid (which contains gastric secretion and food) was found to be adequate for both the mouse and rat. The second-order rate constant (k) was 0.005 and 0.003 min⁻¹ for rat and mouse, respectively. Species differences in gastric fluid production rates and water intakes indicated a higher Cr(VI) load per stomach fluid in mice compared to rats for a given Cr(VI) drinking water concentration. The amount of Cr(VI) emptying from the stomach into the duodenum was modeled in the stomach by accounting for competing rates of Cr(VI) loading via drinking water, emptying, reduction, and absorption. At doses that induce small intestinal tumors in mice, significant Cr(VI) emptying into the small intestine is predicted. Rate of reduction is faster in human gastric fluid at lower pH. The second-order rate constant for Cr(VI) reduction is higher in humans (0.6 – 0.1 min⁻¹ at pH 1-4, respectively) than rodents. Reduction capacity and rate are lower in humans taking PPIs compared to those not on PPIs. These data can be used in a PBPK model to support a risk assessment for humans exposed to Cr(VI).

**435 PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING OF POLYETHYLENE GLYCOL-COATED POLYACRYLAMIDE NANOPARTICLE IN RATS.**

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Though the use of engineered nanoparticles (NPs) has increased exponentially, studies of potential health hazards remain limited. In particular, little attention has been given to NP bioavailability and biodistribution. The aim of this study is to develop a physiologically-based pharmacokinetic (PBPK) model to explore the biodistribution of i.v. injected polyethylene glycol-coated polyacrylamide (PAA-peg) NPs in rats in order to advance the understanding of NP behavior in the body. The model consists of 8 tissue compartments (blood, liver, kidney, lungs, heart, brain, immune organs and carcass) interconnected via the systemic circulation. Each compartment is described as sub-compartment of macrophages (MPs) which may phagocytize NPs in a saturable process. All MPs are assumed to behave in the same way, only the number of MPs might differ between organs. The maximum capacity of phagocytosis and the number of MPs per organ were adjusted by visual optimization to experimentally observed data collected on 30nm PAA-peg NP (Wenger et al., 2011). The predicted time courses of NP and tissue distribution agree well with experimental data. According to the model, the NPs were quickly captured by the MPs, whereas the tissue levels stayed relatively constant. The MPs thus serve as the major reservoir of NPs in organs (storing 7% of the i.v. dose after 120 hr). The fitted MP parameters — number per tissue and maximum capacity — are consistent with what is known about NP behavior. To our knowledge, this is the first PBPK model that captures NP biodistribution with this degree of detail. Our model may easily be extended to other NPs or extrapolated to other species including human.

**436 ASSESSING THE RELATIVE POTENCY OF REACTIVE ALDEHYDES IN CIGARETTE SMOKE USING TRANSIENT CFD/PBPK MODELS OF THE RESPIRATORY SYSTEM.**


Computational fluid dynamic (CFD) models of the respiratory airways of the rat and human extending from the nose or mouth to the lung were coupled with physiologically based pharmacokinetic (PBPK) models to describe the uptake and tissue disposition of three reactive constituents of cigarette smoke: acrolein, acetaldehyde and...
Exposure Versus Internal Dose: Respiratory Tract Deposition Modeling of Inhaled Asbestos Fibers in Rats and Humans.

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A dosimetry model to calculate deposited fractions (DF) in the upper and lower respiratory tract (URT and LRT) is the first step to a more comprehensive model addressing retained fiber burdens. We developed equations for external forces on fibers suspended in inhaled tidal air as a function of fiber dimensions and orientation. These equations were used to obtain fiber equivalent diameters, and replaced particle diameter in expressions for inhalability and deposition efficiency. Resultant local and regional DF were used to compute different dose(s) of inhaled fibers in the human and rat LRT for a monodisperse aerosol of 10 fibers/cc assuming a density of 3.0 g/cm3 and an aspect ratio (β) of 3, 5 or 10. Fiber diameter was a greater determinant of DF than fiber length. Deposited mass, surface area (SA), and number of fibers per LRT surface area was significantly lower in rats than in humans. By number, the fiber DF increased with decreasing physical diameter in the diffusion range, and in the impaction range reached a peak at 0.5 – 1 μm in rats and 2 – 5 μm in humans. The deposited mass and SA of fibers increased with physical diameter across the entire size range to reach a peak at ~2 – 3 μm in humans and ~0.6 μm for rats. LRT DF beyond these sizes dropped sharply to 0 due to URT filtering. The differences in DF with bivariate fiber distribution illustrate the value of dosimetry models to interpreting and translating data across species, and can be used to characterize the inherent uncertainty in risk assessments based on exposure versus internal dose. (The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.)
high body burden. These results indicate that our PBPK model, which was initially developed from animal studies, can be used to predict TCDD elimination in humans. Improvement of the model will require a systematic study with serial samples of maternal milk and blood across gestational and lactational stages. (This research was funded in part by the US Environmental Protection Agency [R82471], NIEHS [R01 ES07171], Regione Lombardia and Fondazione Lombardia Ambiente, Milan, Italy. This abstract does not necessarily reflect the views of these agencies.)

441 PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODELING TO EXPLORE POTENTIAL METABOLIC PATHWAYS OF BROMOCHLOROMETHANE IN RATS.

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Bromochloromethane (BCM) is a volatile organic compound and a by-product of disinfection of water by chlorination. Physiologically based pharmacokinetic (PBPK) models are used in risk assessment applications and a PBPK model for BCM, updated with F-344 specific input parameters, is used here for hypothesis testing to provide useful information for risk assessment of this compound. Inhalation vapor uptake data for F344 rats obtained from the literature were used for PBPK development. The initial chamber concentration ranged between 2000-4000 ppm to ensure that metabolic saturation was included in the experimental design. The two different metabolic hypotheses examined were: 1) a two-pathway model using both CYP2E1 and glutathione transferase systems, and 2) a two-binding site model where metabolism can occur via one enzyme, CYP2E1. Our computer simulations demonstrated that both metabolic hypotheses described the available experimental data in a similar manner. The two-pathway results were comparable to previously reported values (Vmax = 3.8 mg/hour, Km<0.35 mg/liter, and KGST=4.7/hr). The two-binding site results use Michaelis-Menten parameters to describe the first binding site, Vmax1 = 3.7 mg/hour and Km1=0.3 mg/liter, and KGST1=4.7/hr. The two-binding site results use Michaelis-Menten parameters to describe the first binding site, Vmax1 = 3.7 mg/hour and Km1=0.35 mg/liter, and a clearance rate to describe the second site, CL2 = 0.05 liter/hour. In addition, the sensitivity of different parameters for each model was explored using our obtained optimized values. At concentrations below 2000 ppm for both metabolic hypotheses, there was a peak in sensitivity towards Vmax measurement using liver concentration. In summary, the combination of PBPK modeling and sensitivity analysis is useful when studying different kinetic hypotheses using parameters optimized with closed chamber experimental data. (This abstract does not reflect EPA policy).

442 WHEN TO SAMPLE AND HOW TO INTERPRET BLOOD LEVELS IN THE OCCUPATIONAL SETTING: THE EXAMPLE OF STYRENE.

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Styrene (C8H8) is a ubiquitous organic chemical produced at many plants and factories around the world. It is used, for example, to make polystyrene, a type of plastic. Styrene has been classified as a human carcinogen and epidemiologic studies have identified an association between occupational exposure to styrene and certain cancers, including breast cancer. However, further research is needed to understand the magnitude of this association and its underlying mechanisms. The purpose of this study was to create and test a physiologically based pharmacokinetic (PBPK) model of styrene that can be used to predict the toxicokinetics of styrene in humans, and to evaluate the impact of environmental and personal factors on styrene exposure and toxicokinetics. The PBPK model was developed using a systems biology approach that integrates biochemical pathways, transporters, and physiological parameters using public terminologies (e.g. GeneOntology, Foundational Model of Anatomy, Mouse Pathology Ontology, etc.). Using the model, we investigated the impact of various factors on styrene exposure and toxicokinetics, including workshift type, lunchtime break and one hour after the end of shift were, respectively, 0.10 mg/L (0.05-0.15) and 0.13 mg/L (0.07-0.19). The existing BEI was similar to the 5th percentile end of shift blood level (0.28 mg/L). Overall, this study supports the adequacy of the BEI put forward by the ACGIH, but proposes two new sampling times that might be more representative of the average and peak exposures with ranges of blood levels associated with a TWA exposure of 20 ppm.

443 ABSOLUTE AND RELATIVE ORGAN WEIGHT DISTRIBUTIONS FOR FISCHER 344 RATS.

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The Fischer 344 (F-344) rat is the standard rat strain used in toxicology studies conducted by the National Toxicology Program (NTP). While numerous reports have been published on growth, survival and tumor incidence, no overall compilation of organ weight data is available. Importantly, organ weight change is an endpoint used by regulatory agencies to develop toxicity values (TVs) for use in human health risk assessments. Furthermore, physiologically-based pharmacokinetic (PBPK) models, which utilize relative organ weights, are increasingly being used to develop TVs. Therefore, all available absolute and relative organ weight data for untreated control F-344 rats were collected from final NTP feed, drinking water and inhalation studies reported though September 2011 in order to develop organ weight distributions. Results show that organ weights were collected more frequently in NTP studies at 3-month (160 studies) than at other intervals and more frequently from feeding and inhalation than drinking water studies. Liver, right kidney, lung, heart, thymus, right testis, and brain weights were most frequently collected. Infrequently weighed organs included adrenals, pituitary gland, thyroid gland, spleen, pancreas, urinary bladder, and female and male reproductive organs other than the testis. From 3-month study results, the mean and standard deviation for absolute and relative organ weights were calculated for nearly 20 organs based on results from approximately 1,400 male and female F-344 rats. The results show greater variability in weights for small organs, and suggest a general variability trend in absolute organ weights of brain < right testis < right kidney < heart < liver < lung < thymus. Given the number of studies (160 studies), number of F-344 rats (nearly 1,400 males and females) and number of organs (nearly 20 organs) included in this evaluation, the resultant organ weight distributions are likely to be among the most comprehensive and robust available, and are useful as historical reference values, and for parameterization of probabilistic and Bayesian PBPK models.

444 VIRTUAL LIVER: AN IN SILICO FRAMEWORK FOR ANALYZING CHEMICAL-INDUCED HEPATOTOXICITY.

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The US EPA Virtual Liver (v-LiverTM) is an in silico framework for the dose-dependent perturbation of normal hepatic functions by chemicals using in vitro data. The framework consists of a computable knowledge-base (KB) to infer putative pathways in hepatotoxicity and a cellular systems model to predict the dose-dependent effects of chemicals. We have synthesized diverse evidence about thousands of effects for 650 chemicals in the KB from ToxRefDB, DSSTox, -omics data and the literature using public terminologies (e.g. GeneOntology, Foundational Model of Anatomy, Mouse Pathology Ontology, etc.). Using the KB we related ToxCastTM in vitro bioactivity assay data on 68 rodent hepatotoxics (e.g. PFOA, Imazalil, Bisphenol A, Triclosan, Acetaminophen, Phenobarbital, WY-14643) to a putative network of events resulting in hepatocyte necrosis, apoptosis and proliferation, which were mediated by constitutive androstane receptor, aryl hydrocarbon receptor, pregnane X receptor, and p53/proliferator-activated receptors. In order to estimate the in vivo effects of these chemicals we encoded this network in a dynamic cell-agent based model that integrates micro-dosimetry, intracellular signaling and cell-cell interactions across the spatial extent of the hepatic lobule to predict histopathologic outcomes. We used experimental data on DNA synthesis following exposure to normal levels of growth factors and cytokines (e.g. Insulin, EGF and TNF-α) to calibrate the dynamics of cell cycle progression through S-phase. Next we used in vitro data (e.g. nuclear receptor activation, oxidative stress and c-Jun activation) to estimate key parameters required for simulating the dose- and time-dependent effects of 20 chemicals on hepatocyte proliferation, apoptosis and necrosis. We are using the v-Liver framework to analyze alternative mitogenic and cytotoxic pathways involved in non-genotoxic cancer. Abstract does not represent EPA policy.

445 ROLE OF THE ASIALOGLYCOPROTEIN RECEPTOR IN EXTRAPOLATING THE SYSTEMIC CLEARANCE OF ENB-0040 ACROSS SPECIES.

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Traditional allometric scaling approaches focus on the relationship between body weight and clearance (CL) with minimal consideration for the mechanism behind protein CL itself. This can lead to differences between allometrically scaled predicted and actual values when scaling across species. For glycosylated proteins such
Biofuel blends of ethanol and gasoline are increasingly common in the USA. PBPK models were developed for ethanol-alone (Martin et al., 2011) and soy to inform studies of developmental effects of ethanol-gasoline blends. Following collection of tissue pharmacokinetic (PK) data from exposed dams (21K ppm ethanol/6.33h/gestation days (GD) 9-20) and fetuses (GD20), our earlier model was extended to pregnancy and lactation periods by including dynamic changes in maternal body composition and moving from a compartment model representing maternal, placental, and fetal compartments to a PBPK model to further refine predictions of humans CL of therapeutic proteins.

The PBPK model was adapted for pregnant women for potential use in the National Children's Study to assess and quantify prenatal exposure to stress through maternal hair samples and stress questionnaires.

To address concerns about potential health effects of exposure to nanomaterials during development, a PBPK model was developed that describes C60 disposition during gestation and lactation. A multiscale modeling approach was used, focusing on extrapolation of in vitro cell-based information to predict in vivo nanoparticle disposition. In this multiscale approach, a cellular kinetic model description of C60 kinetics in vitro is incorporated into the whole body PBPK model. Each tissue containing C60 was modeled as a series of sub-compartments representing specific subpopulations of cells, including entrainment in tissue macrophages, binding to proteins, and post-distributional aggregation, as identified by in vivo imaging data. The PBPK model was extended to pregnancy and lactation periods by including dynamic changes in physiology and transfers of C60 from the dam to offspring through placenta and milk. The model simulated C60 distribution agreed well with data from pregnant and lactating dams, placenta, and milk, as well as in pooled fetuses and tissues in pups 24 hr or 48 hr after the IV exposure on gestation day 15 or postnatal day 8, respectively. Our results indicate no substantial change in maternal C60 disposition during pregnancy or lactation in the rat, since the same cellular parameters for C60 kinetics could be used for different life stages. However, accumulation of C60 nanoparticles in the placenta, fetus and neonate following maternal IV dosing suggests the potential for developmental effects. This modeling strategy can be readily adaptable to other nanomaterials to contribute to developing a tool to assess potential risks of nanomaterial exposure in early life (This work was supported by NIH/NIEHS Award #U19ES019525, but solely expresses the view of the authors).

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants generated by byproducts of natural and anthropogenic combustion processes. Dibenzo[def, p]chrysene (Dibenzo[def, p]chrysene (DBC) is a high molecular weight PAH classified as a 2B carcinogen by the International Agency for Research on Cancer (IARC) on the basis of sufficient evidence of carcinogenicity in animals. Notably, DBC has been shown to cross the placenta in exposed mice, causing carcinogenicity in offspring. Here, we present pharmacokinetic data of DBC in pregnant and non-pregnant mice. DBC (gestational day 17) and non-pregnant female B6129SF1/J mice were exposed to 15 mg/kg DBC in corn oil by oral gavage. Subgroups of mice were sacrificed over 48 hours post-dosing, and blood, excreta, and tissues analyzed for DBC and metabolites. The pharmacokinetics of DBC and its diol and tetroxol metabolites were significantly different in pregnant versus naïve mice, with elevated maximum concentrations of parent chemical and metabolites achieved in blood and other tissues. Using a pharmacologically-based pharmacokinetic (PBPK) model, we found that observed differences in pharmacokinetics could not be explained simply by changes in tissue volumes and blood flows that occur during pregnancy. Analysis of whole fetuses corroborated previous research indicating DBC and metabolites can move across the placenta. Concentrations of DBC in fetuses were generally one to two orders of magnitude below maternal blood concentrations, while metabolite concentrations closely resembled those observed in maternal blood. Supported by Award Number P42 ES016465 from the National Institute of Environmental Health Sciences.
Conflict results have been reported for associations between maternal exposure to perfluoralkyl acids (PFAAs) and human birth outcomes. Of particular interest are recent reports from epidemiological studies linking increased maternal blood concentrations of PFAAs with reduced infant size at birth and also a longer time to pregnancy for women who have higher serum PFAA levels. The purpose of this effort was to determine whether the observed epidemiological associations may be due to reverse causality, i.e., the relationships between plasma levels of PFAAs and various outcomes may be explained on the basis of pharmacokinetics rather than the chemicals exerting causal adverse effects. In order to understand how changing developmental physiology may affect the tissue distribution of a chemical in the mother, fetus, and neonate, we have developed physiologically-based pharmacokinetic (PBPK) models of pregnancy, perinatal, and postnatal life stages. In this work, the ability of the models to predict PFAA concentrations observed in epidemiological studies was examined using Monte Carlo analysis. We also evaluated whether the epidemiological associations noted above could be explained entirely by predictions made with the PBPK models. The models provided a good fit to observed PFAA concentrations in humans during pregnancy and lactation. The PBPK models predicted that plasma PFAA levels would be related to lower birth weight, and, among parous women, longer time-to-pregnancy, purely on the basis of physiological phenomena. Conflicting results have been reported for associations between maternal exposure to perfluoralkyl acids (PFAAs) and human birth outcomes. Of particular interest are recent reports from epidemiological studies linking increased maternal blood concentrations of PFAAs with reduced infant size at birth and also a longer time to pregnancy for women who have higher serum PFAA levels. The purpose of this effort was to determine whether the observed epidemiological associations may be due to reverse causality, i.e., the relationships between plasma levels of PFAAs and various outcomes may be explained on the basis of pharmacokinetics rather than the chemicals exerting causal adverse effects. In order to understand how changing developmental physiology may affect the tissue distribution of a chemical in the mother, fetus, and neonate, we have developed physiologically-based pharmacokinetic (PBPK) models of pregnancy, perinatal, and postnatal life stages. In this work, the ability of the models to predict PFAA concentrations observed in epidemiological studies was examined using Monte Carlo analysis. We also evaluated whether the epidemiological associations noted above could be explained entirely by predictions made with the PBPK models. The models provided a good fit to observed PFAA concentrations in humans during pregnancy and lactation. The PBPK models predicted that plasma PFAA levels would be related to lower birth weight, and, among parous women, longer time-to-pregnancy, purely on the basis of physiological phenomena.  

Reduced Acetaminophen-Induced Injury in LPR Mice Is Dependent on Increased Glutathione Synthesis and Enhanced Detoxification of Oxidant Stress

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Acetaminophen (APAP)-overdose is a classical model of hepatocellular necrosis, however, the mechanism of protection previously reported in Fas receptor-defective (LPR) mice remains unclear. Apoptosis has no role in the mouse model of APAP-overdose as demonstrated by the lack of morphological evidence and no observed protection by caspase inhibitors. From these findings it was unclear why preventing the signaling of an extrinsic apoptotic pathway could reduce APAP-induced injury. To better understand this mechanism of protection, LPR and C57BL/6 mice were treated with 300 mg/kg APAP for 0.5h, 6h and 24h. LPR mice showed a reduction in liver injury as measured by plasma ALT levels (~50% decrease) and reduced hepatic necrosis (~25% decrease) at both 6h and 24h. To eliminate the possibility of altered APAP metabolism, Cytochromes P450, protein expression, formation of APAP adducts and hepatic glutathione (GSH) depletion were compared demonstrating equivalent reactive metabolite generation. Isolated primary mouse hepatocytes from both strains showed equivalent GSH depletion and LDLH release after APAP treatment in vitro. Interestingly, in vivo post-APAP GSH recovery was higher in LPR than C57BL/6 (6h: 200% & 24h: 160% increase). Driving the increased GSH levels, mRNA induction and protein expression of glutathione-cysteine ligase (GCLC) were higher in LPR mice. The higher levels of GSH in LPR mice resulted in enhanced detoxification of reactive oxygen species measured by hepatic GSSG levels (6h: 165% & 24h: 600% of C57BL6 APAP-treated). Inducible nitric oxide synthase (iNOS) mRNA and protein levels at 6h were much lower in LPR mice, which correlated with greatly reduced nitrotyrosine staining at 6h. Conclusion: These data indicate the importance of non-parenchymal cells in the protection seen in LPR mice by stimulating hepatic GSH production and reducing peroxynitrite formation. These factors in combination result in the APAP-protected phenotype observed in LPR mice.

Tolerance of Robenacoxib Injectable and Tablets in 4 to 5-Month-Old Kittens


Robenacoxib (RXB) is a non-steroidal anti-inflammatory drug (Omnisar®) with approved indications in cats and dogs. A safety study of the interchangeable use of RXB tablets at 2.4 or 12 mg/kg (tablets) plus 2 or 10 mg/kg subcutaneous (SC) injection (each representing 1 and 5X the upper dose band for each route; the therapeutically guided dose for tablet and injection is 1.0 and 2.0 mg/kg, respectively). Male and female kittens (4-5 months old) were dosed by alternating 7-day tablet/3-day injection cycles for 37 days. Controls were given empty gelatin capsules or sterile saline SC (5X volume). On Day 16, one 5X group female showed constipation, thin body score, and behavioral changes that resolved with treatment within 7 days; this cat remained thin but healthy to the end of study. Injection site observations of all kittens included dose-dependent increases in edema, heat or erythema, resolving within 8 days, changes in preauricular lymph nodes close to injection sites, and/or scab formation. Body weight was reduced in each treated group despite unaffected therapeutic success, renal toxicity and hepatotoxicity have been reported in the clinic. Therefore, concern exists regarding the long term use of RXB. The goals of this study were to evaluate the molecular mechanism of TXD-induced toxicity in female BALB/c mice using an Affymetrix microarray and to correlate gene expression changes with plasma drug levels and other toxicology endpoints including clinical pathology and histopathology. TXD was orally administered by gavage daily for 91 days at 500 and 1000 mg/kg TID. Analysis of gene transcripts in the livers showed that Cdkn1a (p21, Cip1), a cyclin-dependent kinase inhibitor 1A, increased 3.7-fold compared with the controls on Day 92. The increase of Cdkn1a transcription level may have contributed to the inhibition of cell cycle progression at G1, causing cytomegaly in the liver. The kidneys from Day 92 TXD treated animals (1000 mg/kgTID) were histopathologically normal which was consistent with the microarray results which showed no significant differences in gene expression between the kidneys from TXD treated animals and the vehicle control animals on Day 92. Work supported by NIAID Contract N01-AL-70043.
food consumption. Electrocardiograms showed a slower heart rate and increased RR and QT interval in the 5X group compared to controls. Mild effects on some serum chemistries were seen most often in the 5X group, including increases in creatinine and amylase. Hematology and coagulation findings were unremarkable. Histopathology findings of injection sites in both treated groups included granulomatous changes and fibrosis. Renal vacuolation and degeneration of proximal tubules were seen in one 1X female and three 5X males. No treatment-related effects were observed in the liver, stomach, duodenum, jejunum, ileum, or colon. In conclusion, repeated administration of RXB tablets and injectable were well tolerated in 4-5 month old cats, with some treatment effects observed more often in the 5X dose group.

Serotonin (5-HT) and cannabinoid systems both direct neural circuit formation during brain development, and their dysfunction may underlie impaired social behavior in autism and other psychiatric disorders. Cannabinoid CB1 receptors are found on 5-HT neurons, and agonists such as WIN 55,212 inhibit 5-HT release. CB1 ligands and cannabinoid metabolites acutely alter murine social behavior, perhaps via direct 5-HT system interactions. We have found that in dorsal hippocampal, socially deficient BTBR mice have 40% higher 5-HT1A and CB1 receptor functional capacity than related 129S1/SvJm mice or more sociaizable C57 mice, as measured by agonist-stimulated [35S]GTPαS binding. However, CB1 receptor saturation binding (Bmax=958±117 fmol/mg protein) and affinity for [3H]A2A ligands and acetaminophen administration significantly increased levels of the endocannabinoid anandamide in the cingulate cortex of BTBR mice, but not in 129S mice, and so- aminophen administration significantly increased level of the endocannabinoid anandamide in the cingulate cortex of BTBR mice, but not in 129S mice, and so-ciability testing also increased this measure. These data support the hypothesis of anandamide in the cingulate cortex of BTBR mice, but not in 129S mice, and so-

The Wnt/β-catenin signaling pathway is one of the fundamental mechanisms that directly regulates cell proliferation, cell polarity and cell fate determination during embryonic development and tissue homeostasis. Abrupt activation of the Wnt/β-catenin signaling pathway is linked to a high frequency of numerous tumors. We screened the chemical library to develop the new drug for lung cancer therapy as a target protein of Wnt-2 using a cell-based reporter gene assay which was used to measure the transcriptional activity of β-catenin-TCP/LEF in A549/Wnt2 cells stably expressed wnt2. Among the screened compounds, we investigated one compound, GDK-100017, which was reduced to below 50% of the luciferase activity at IC50 concentration of the cell proliferation. GDK-100017 reduced the β-catenin-TCP/LEF dependent transcriptional activity in a dose-dependent manner with an IC50 value of about 10μM and decreased cell proliferation about 25% and 50% of IC50 values at 24, 48 and 72h, respectively, however, did not affect to human embryonic pulmonary cell lines, L132 cells. It was down-regulated the expressions of Wnt/β-catenin signaling pathway target genes, such as E2F and cyclin D1 which play critical roles in proliferation. FACs analysis showed that GDK-100017 significantly induced the G1 phase arrest in A549 cell lines and this G1 arrest seemed to correlate with inhibition of E2F and cyclin D1 expression. Altogether, these results showed that GDK-100017 possesses potential anti-cancer activity against lung cancer cells by inhibiting their proliferation and inducing cell cycle G1 arrest partly via the inhibition of Wnt/β-catenin pathway.

Aim of the present study was to investigate the effects of polyethylene glycol 400 (PEG 400) as a vehicle on the rat gastrointestinal tract. Crl: CD(SD) rats (5 males and 5 females in each group) orally received 0, 5, 50 or 100% of PEG 400 at a volume of 5 mL/kg/day for 15 days. After the 15 days, the whole parts of the gastrointestinal tract were examined histopathologically. The animals in the receive of 50 and 100% groups sometimes excreted loose stools during the 15 days. Although the fecal change had made us suspect some morpho-

The Wnt/β-catenin signaling pathway is one of the fundamental mechanisms that directly regulates cell proliferation, cell polarity and cell fate determination during embryonic development and tissue homeostasis. Abrupt activation of the Wnt/β-catenin signaling pathway is linked to a high frequency of numerous tumors. We screened the chemical library to develop the new drug for lung cancer therapy as a target protein of Wnt-2 using a cell-based reporter gene assay which was used to measure the transcriptional activity of β-catenin-TCP/LEF in A549/Wnt2 cells stably expressed wnt2. Among the screened compounds, we investigated one compound, GDK-100017, which was reduced to below 50% of the luciferase activity at IC50 concentration of the cell proliferation. GDK-100017 reduced the β-catenin-TCP/LEF dependent transcriptional activity in a dose-dependent manner with an IC50 value of about 10μM and decreased cell proliferation about 25% and 50% of IC50 values at 24, 48 and 72h, respectively, however, did not affect to human embryonic pulmonary cell lines, L132 cells. It was down-regulated the expressions of Wnt/β-catenin signaling pathway target genes, such as E2F and cyclin D1 which play critical roles in proliferation. FACs analysis showed that GDK-100017 significantly induced the G1 phase arrest in A549 cell lines and this G1 arrest seemed to correlate with inhibition of E2F and cyclin D1 expression. Altogether, these results showed that GDK-100017 possesses potential anti-cancer activity against lung cancer cells by inhibiting their proliferation and inducing cell cycle G1 arrest partly via the inhibition of Wnt/β-catenin pathway.

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The mitochondrial toxicity in vitro has been hampered by the reliance immortalised cell lines have on glycolysis for ATP production. Therefore, compounds that cause impairment of normal mitochondrial function can go undetected as blockage of oxidative phosphorylation does not lead to ATP depletion. The replacement of glucose by galactose within tissue culture media forces cells to become more reliant on oxidative phosphorylation, and therefore more sensitive to mitochondrial poisons. HepG2 cells cultured in glucose or galactose containing media were exposed to 8 mitochondrial toxins with known effects on specific complexes of the electron transport chain (ETC) and 11 compounds with no effect on mitochondrial function for 24 hours in a 96-well plate format. Intracellular ATP was measured and expressed relative to control levels. A ratio of the IC50 values observed in the glucose versus galactose cells was calculated to distinguish mitochondrial toxins (IC50 ratio > 2.5) from compounds that caused gross cytotoxicity or non-cytotoxic compounds (IC50 ratio < 2.5). The sensitivity of the screen was calculated as 75% with 100% specificity. Routine screening of AstraZeneca compounds revealed a hit rate (IC50 ratio > 2.5) of 7% across 1617 compounds. A subset of 29 compounds, 17 active in the mitochondrial toxicity screen and 12 inactive, were screened to identify inhibitors of complex I. 11/17 of the active compounds from the screen were Complex I inhibitors (inhibition > 50%) and 10/12 of the inactive compounds from the screen were inactive at this complex. Overall, the combination of the mitochondrial toxicity screen and the complex I inhibition assay are useful for detecting risk of mitochondrial toxicity in the early stages of drug discovery.

Cytokine production and expansion of T lymphocytes following antigen exposure are key components of the adaptive immune response. Monitoring compound-related effects on T-cell activation and proliferation may provide important information on the immunomodulatory potential of a compound. The purpose of this study was to develop methods to monitor antigen-specific T-cell responses in cynomolgus monkeys using peripheral blood mononuclear cells (PBMC). Two cynomolgus monkeys (1/sex) were immunized with hepatitis B specific antigen (HBsAg) (Engerix-B, GlaxoSmithKline) at 10 μg/animal intramuscularly on days 1 and 15. One male monkey was used as a non-immunized control. Beginning on day 26 and every week for 6 consecutive weeks, HBsAg-specific T-cell responses were monitored using flow cytometric evaluation of intracellular cytokine staining and ex vivo T-cell proliferation. After priming with hepatitis B antigen for 5 hours followed by co-incubation with Brefeldin A overnight, cytokines (IL-2, TNF-alpha, IFN-gamma) were detected mainly in CD3+CD4+ T-cells via intracellular cytokine staining procedure. Significant antigen-specific proliferation was detected for both CD3+CD4+ and CD3+CD8+ T-cells when stimulated with hepatitis B antigen for 96 hours. The percentage of CD3+CD4+IL-2+ cells correlated positively with proliferation for CD3+CD4+ and CD3+CD8+ T-cells (evaluated in a subsequent study). The results demonstrate that intracellular cytokine staining and ex vivo proliferation may be used to detect antigen-specific T-cell responses following hepatitis B vaccination in cynomolgus monkeys.

Molindone is an antipsychotic drug developed for treatment of schizophrenia. A 3-month rat study was conducted to evaluate toxicity and prolactin-mediated effects of molindone. Wistar rats (10/sex/group) were treated daily via oral gavage with molindone at 0 (control), 5, 20, and 60 mg/kg/day. Plasma samples for toxicokinetics and prolactin were obtained up to 6 timepoints over a 24-hr period postdose using 3 general anesthetics on Days 0 and 85. In males, there was a dose-dependent decrease in body weight gain of up to 35% that correlated with decrease in food consumption. The plasma concentrations of molindone were more than dose proportional with a 12-fold increase in dose resulting in a 49-fold increase in exposure. The exposure in females was generally higher than in males at Tmax between 0.5 to 1 hr. The prolactin concentrations on Days 0 and 85 peaked up to 265 ng/mL within 0.5 - 1 hr postdose compared to maximum predose value of 40 ng/mL. Historically, molindone-treated rats showed a dose dependent, pharmacologically mediated, mostly rodent specific changes known for this class of drugs, including increase in mammary hypertrophy and retention and enlargement of corpora lutea, disruption of estrus cycle, mucification of epithelia of reproductive tract, follicular atresia and uterine atrophy in females, and inflammation of prostate in males. There were no other major signs of general toxicity on clinical chemistry or anatomic pathology. Based on the known role of prolactin on hormonal homeostasis and reproductive physiology of rodents, the changes seen in this study demonstrate the pharmacologically mediated effects of molindone. Also these changes seem to be species-specific similar to those observed for other antipsychotics in this class. Since such changes have not been reported in humans at therapeutic doses (15 to 225 mg/day) with the use of molindone for many years, it is concluded that the changes reported in this study do not pose any significant risk.

Leflunomide (Arava®) is an oral disease modifying anti-rheumatic drug. While its safety profile in the clinic has been demonstrated it carries a black box warning for idiosyncratic liver toxicity, the mechanism of which is unknown. The active metabolite of leflunomide, teriflunomide, is being considered as a treatment for multiple sclerosis. It is not known if teriflunomide, leflunomide, or other minor metabolites are responsible for the hepatotoxicity. Recently multiple reports have been published establishing the important role of the mitochondria in idiosyncratic liver toxicity. We investigated the abilities of these two drugs to impair oxygen consumption in isolated mitochondria, and to cause cytotoxicity in various cell models. Leflunomide was found to be 10-fold more potent inhibitor of mitochondrial respiration and generation of ROS than teriflunomide. We also demonstrate that the P450 inhibitor SKF-525a potentiates the inhibitory effect of teriflunomide on mitochondrial respiration and the cytotoxicity of both compounds in isolated rat hepatocytes. However, metabolism studies with both compounds showed that SKF-525a has no effect on the metabolism of teriflunomide in rat hepatocytes, leading us to conclude that SKF-525 exerts its effect by potentiating the inhibitory effect on mitochondrial respiration. We further demonstrated the importance of mitochondrial respiration in the cytotoxicity of both compounds by showing that HepG2 cells reliant on oxidative phosphorylation are more susceptible to drug induced cytotoxicity, while HepG2 cells grown in glucose rich cell culture media are protected. In addition, Rho0 HeLa cells, which lack mitochondrial DNA, are found to be less sensitive to the cytotoxic effects of leflunomide than the wild-type HeLa cells. In conclusion, if mitochondrial toxicity is involved in the hepatotoxicity observed with leflunomide treatment, it is reasonable to believe that teriflunomide will have a better hepatic safety profile than leflunomide.
non compartmental analysis. Comparison of the two formulations showed that both formulations had similar Tmax (Dextran 10/insulin: 15 min, Exubera: 10 minutes), and Cmax (Dextran 10/insulin: 115 μU/mL, Exubera: 114 μU/mL). However, after dose normalization, the Dextran 10/insulin formulation resulted in a 13% higher relative bioavailability (determined from AUC0-last).

Latanoprost is used for the treatment of increased intraocular pressure to prevent the progression of glaucoma. Since the lack of compliance with topical ocular dosing may compromise efficacy, alternate methods of delivery are being sought. A 9-month study was conducted to assess the safety and tolerability of a latanoprost-containing biodegradable device. Dutch-belted rabbits were implanted subconjunctivally with up to 5 placebo or drug-containing devices containing from 50 to 190 μg of latanoprost per device. Study assessment consisted of irritation scoring, clinical signs, ophthalmic exams, electroretinography, and ocular histology of cohorts at 3 and 9 months post implantation. The implants were well tolerated, with the most severe clinical signs and irritation observed in the first few days of the study associated with the implantation procedure. Mild conjunctival congestion persisted through week 13 of the study and tended to correlate with the number of devices and presence of drug. Ophthalmic exams revealed no effects beyond the ocular surface irritation, including no effects on intraocular pressure, corneal thickness, or ERG parameters. Microscopically, implants at the 3-month necropsy were associated with cavities (containing the implants), fibrous encapsulation, and an infiltrate of macrophages, sometimes as multinucleate cells, into the implant cavity. Drug containing implants were often associated with additional inflammatory cell infiltrates within the implant subconjunctival cavities and adjacent to the implant sites. At the 9-month necropsy, neutrophils were no longer common among the inflammatory cell infiltrates, most implants were fragmented and disintegrating, and fibrovascular proliferation was present within implant luminal remnants. None of the findings were considered adverse. Overall, the study supports the safety of the latanoprost-containing subconjunctival device.

Lack of nephrotoxicity and myelosuppression following CMX001 administration in humans, rodents, and monkeys.

Introduction: CMX001 is an oral Lipid-Amantiviral-Conjugal (LAC) that delivers high intracellular levels of the active antiviral agent cidofovir-diphosphate. CMX001 is in development for prevention and treatment of CMV and adenovirus in transplant recipients and as a therapeutic countermeasure for smallpox. CMX001, a phospholipid covalently linked to cidofovir (CDV), was designed to take advantage of the broad-spectrum antiviral activity of CDV while eliminating the toxicities seen with MEKi. The Ras/Raf/MEK/ERK mitogen-activated protein kinase pathway mediates cellular responses to growth factors, cytokines, and growth-promoting oncogenes. It has been repeatedly substituted for propylene glycol or glycerin, liquids of similar appearance, in pharmaceutical preparations. Since diethylene glycol became commercially available in the late 1920’s, it has been repeatedly substituted for propylene glycol. Glycolic acid, with properties of similar appearance, in pharmaceutical preparations. This is done because diethylene glycol is much cheaper than either of the safe drug solvents. Many people have died after ingesting diethylene glycol contaminated pharmaceuticals. The countries where these deaths occurred are usually resource poor and cannot afford or do not have the technology for the expensive tests needed to determine if their raw ingredients are pure. Our objective is designing a simple and inexpensive enzyme-linked spectrophotometric assay to test for contamination of glycerin and propylene glycol. The assay measures the generation of NADH by the action of glycerol dehydrogenase (EC 1.1.1.6) employing the tetrazolium dye WST-1 (Dojindo Molecular Technologies, INC) to enhance sensitivity. Propylene glycol and glycerin are substrates for glycerol dehydrogenase and diethylene glycol is not. Thus solutions that are contaminated with diethylene glycol will show less absorbance. Linear absorbance versus concentration plots for propylene glycol (R2=0.9941) and glycerin (R2=0.9904) were generated. The absorbance with either substrate was diminished in a concentration dependent manner by added diethylene glycol. A preliminary estimate of the limit of detection for diethylene glycol contamination of propylene glycol was 6%. The values generated by the spectrophotometric assay were comparable to results obtained by gas chromatograph-mass spectroscopy determination using the same samples. The spectrophotometric assay described here has utility for alerting to potential diethylene glycol contamination of ingredients used in the fabrication pharmaceuticals.

A preclinical model for MEK inhibitor-induced retinopathy.

The Ras/Raf/MEK/ERK mitogen-activated protein kinase pathway mediates cellular responses to growth signals and is frequently activated in cancer. Several MEK inhibitors (MEKi) are currently in development. The most common dose-limiting toxicities seen with MEKi are: rash, diarrhea, nausea, vomiting, and less frequently but more troubling, central sensory retinopathy (CSR) and retinal vein occlusion (RVO). CSR is characterized by leakage of fluid under the neural retina of the mac-
ula resulting in the formation of a “blister” with detachment of the neural retina from the pigmented epithelium (PRE). This results in decreased visual acuity that may persist after the fluid has subsided. Similar findings have not been seen in any preclinical species with any MEKi tested to date making it difficult to select safer compounds for testing in humans. Using the Brown Norway Rat we assessed the ability of various MEKi to decrease the expression of MEK responsive RNAs (DUSP6 and Spry4) in the neural retina and PRE and correlated these effects with increased thickness of the photoreceptor layer as measured by Optical Coherence Tomography (OCT), free plasma concentrations and melanin binding. Toxicogenomic analyses also revealed that MEK inhibitors significantly decreased the expression of Tight-Junction mRNAs (Ocludins, Claudins and Junctional Adhesion Molecule) and related scaffold proteins (ZO-1, Actin, Rho PLA51, PAR6 and Cdc42) in the PRE. The data suggest that MEK alters the permeability PRE resulting in retinal fluid imbalance that causes CSR. Potential strategies to prevent MEKi induced CSR and the potential cause of RVO will also be discussed.

471 LONG-TERM EFFECTS OF PRENATAL ATRAZINE EXPOSURE ON PHYSIOLOGY, BODY COMPOSITION, AND STRESS REACTIVITY IN RATS.

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Low birth weight in humans is associated with increased risk of coronary heart disease, hypertension, and diabetes in adulthood. Experimental studies have also reported that undernutrition, stress or exposure to glucocorticoids during pregnancy is associated with hypertension, glucose intolerance, obesity, and altered stress reactivity. The mechanism(s) of these effects may be due to increased fetal exposure to maternal glucocorticoids. We hypothesized that environmental contaminants that increase maternal corticosterone (CORT) levels would elicit similar effects in offspring. Atrazine, a widely used herbicide, increases CORT in female rats. We exposed pregnant rats to 125 mg/kg/d atrazine, 0.1 mg/kg/d dexamethasone (DEX), or methyl cellulose vehicle from gestational day 14-20. Offspring were divided into 3 test groups: adult offspring were surgically implanted with radio transmitters for recording blood pressure (BP), core temperature, heart rate (HR), and activity during and without 1h restraint for 4 d (group 1); body composition was measured by magnetic resonance (Bruker) to determine percent body fat (group 2); and stress reactivity was measured by determining serum CORT levels prior to, during, and following 1h restraint (group 3). Baseline BP was increased in atrazine and DEX-treated animals, but differences were minimal during restraint. HR and temperature decreased daily during restraint, suggesting adaptation, but BP was elevated on subsequent days. Body fat was increased and lean mass decreased in DEX-treated female offspring. Prenatal atrazine treatment altered the adult offspring stress response to restraint: CORT levels were increased and did not return to control levels 2 h following restraint. These data indicate that exposure to chemicals that increase maternal CORT levels may lead to similar adult diseases as observed in maternal stress and undernutrition studies. Disclaimer: This is an abstraction of a proposed presentation and does not necessarily reflect EPA policy.
fetal testes were resistant to phthalate-induced suppression of steroidogenic gene expression across a range of doses. Based on these findings, we conclude that the human fetal tests is resistant to phthalate-induced androgen suppression, and developmental phthalate exposure is an unlikely contributor to the anti-androgenic related effects of TDS, namely hypospadias and cryptorchidism.

473 THE EFFECTS OF ENDOCRINE DISRUPTION ON THE DEVELOPING HUMAN FETAL PROSTATE.

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Prostate cancer is the most commonly diagnosed cancer and is the second highest cancer-related cause of death in men. This high incidence has led to speculation about the fetal origins of this adult disease and its etiology. Exposure to exogenous estrogens in pregnant women has been postulated to disturb the normal development of the human fetal prostate by disrupting the natural hormonal balance. Previous studies demonstrate that early life exposure to estrogens in rats can cause some degree of epithelial and stromal hyperplasia, inflammation, and prostatic intraepithelial neoplasia (PIN) lesions. The present study used a xenograft model to characterize the differentiation of human fetal prostate implants (gestation 12-22 weeks) exposed to either corn oil (control) or 250 ug/kg/body weight of 17β-estradiol 3-benzoate during an early acute exposure, as well as an additional later life exposure post-transplant. This xenograft model uses the renal subcapsular space as the site of implantation, thereby allowing for proper vascularization and growth of the implant. Characterization of the model included the expression of key immunohistochemical markers responsible for stromal and epithelial maturation, neuroendocrine cells, hormone receptors, cellular proliferation as well as apoptosis. As expected, the prostate implants grew and matured as seen in 7, 14, 30, 90, 200 and 400 days post-transplant xenografting. Interestingly, the human prostate xenografts exhibited marked differences in response to estrogen exposure compared to their endogenous rat prostate counterparts. Human prostate xenografts at 200 days post-transplant demonstrate basal cell hyperplasia shown by p63 staining, while the endogenous rat prostate exhibited atypical hyperplasia and the presence of cellular debris following estrogen exposure. This unique xenograft model provides insight on the growth and development of the human fetal prostate following developmental endocrine disruption.

474 GLUTAMATE CYSTEINE LIGASE MODIFIER SUBUNIT (GCLM) NULL MALE MICE ARE MORE SENSITIVE TO THE REPRODUCTIVE TOXICITY OF PRENATAL BENZO(A)PYRENE.

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Benzo-a-pyrene (BaP), a ubiquitous environmental pollutant, is a testicular toxicant in fetus and adult. The tripeptide glutathione (GSH) is important in Phase II biotransformation of BaP and a major antioxidant. Mice null for the gene Gclm, which codes for the rate-limiting enzyme in GSH synthesis, have decreased GSH concentrations. We hypothesized that male Gclm-/- mice are more sensitive to the in utero reproductive toxicity of BaP than Gclm+/+ males. Female Gclm-/- mice were mated with Gclm+/+ males and were treated orally with 0 or 10 mg/kg/day BaP for Experiment 1 and 0 or 2 mg/kg/day BaP in Experiment 2 from gestation day 7-16. Male offspring were euthanized at 10 wks. In Experiment 1, tests weights, testicular sperm head counts, epididymal sperm counts, and epididymal sperm motility were significantly decreased by BaP exposure, and the effects of BaP were significantly greater in Gclm-/- males than Gclm+/+ males. In Experiment 2, testicular and epididymal sperm counts were slightly, significantly decreased by prenatal BaP exposure. There were inconsistent effects of Gclm genotype alone on cauda epididymal sperm counts and motility, with no effect in Experiment 1 and lower counts and motility in Gclm-/- males in Experiment 2. Prenatal BaP treatment increased the percentage of vacuolated seminiferous tubules in a dose-dependent manner. Cauda epididymal sperm morphology was assessed in Experiment 2: Percentages of sperm with abnormal heads and cytoplasmic droplets decreased with prenatal BaP exposure, and Gclm-/- males had increased percentages of sperm with abnormal heads. Serum testosterone was unaffected. These results show no effects of Gclm deletion alone on testicular spermatogenesis and inconsistent epididymal effects, but show increased sensitivity of Gclm-/- males to the transplacental testicular toxicity of BaP.

475 EFFECTS OF THE SMOKELESS TOBACCO PRODUCT, GUTKHA, ON REPRODUCTIVE AND DEVELOPMENTAL PARAMETERS IN PREGNANT MICE.

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Gutkha, an extremely popular herbal concoction made and sold throughout India, is exported worldwide. The product, a combination of tobacco, lime, betel nut, flavored, and catechu, is highly addictive and used by adults and children as a mild psychoactive drug. To assess the effects of Gutkha usage on gestational, developmental, and neonatal growth parameters, pregnant B6C3F1 mice were exposed daily to 21 mg of a water-soluble Gutkha solution beginning on gestational day (GD) 2 until parturition. The main objectives of this study were to: 1) develop an oral route of Gutkha administration that yielded relevant levels of serum and/or tissue cotinine; and, 2) determine whether Gutkha usage during pregnancy affected gestational outcomes. Female mice, paired with males for 2-4 nights prior to exposure, were painted with the Gutkha solution using a natural bristle paintbrush to coat the tongue and oral mucosa. Serum cotinine levels in the dams were measured weekly and ranged from 24-44 ng/ml. Cotinine was found in the amniotic fluid on GD 15 (16-22 ng/ml), and liver cotinine levels on the same GD were ~60% of those measured in serum (21-22 ng/ml). Dams were weighed daily throughout pregnancy; offspring were weighed from birth until weaning. Results demonstrated that Gutkha-exposed dams had a significantly longer duration of gestation than their control counterparts and gave birth on average 1.6 days later than control dams (p<0.05). In contrast, no effects of Gutkha were observed on pregnancy incidence, maternal weight gain, litter size, pup sex ratio, or offspring weight gain. In conclusion, a pregnant mouse model and relevant route of exposure has been developed for studying the toxic effects of smokeless tobacco. Findings from these studies demonstrate that nicotine, as its longer-lived metabolite cotinine, can reach the amniotic fluid and thus pose potential health risks to the fetus and/or growing neonate. Supported by NYU Cancer Institute.

476 ALZHEIMER’S DISEASE: TAU AND EARLY LIFE EXPOSURE TO PB IN PRIMATES.

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Late onset Alzheimer Disease (LOAD) constitutes the majority of AD cases (~90%) that have no clear genetic association. The differential susceptibility and course of illness, as well as the late age onset of the disease suggest that epigenetic and environmental components play a role in its etiology. Published reports from our lab showed that lead exposure occurring during brain development pre-determined the expression of AD-related genes such as the amyloid precursor protein (APP) later in life, influencing the course of amyloidogenesis and oxidative DNA damage via a process that involved DNA methylation and the transcription factor Sp1 (Basile et al. 2005; Wu et al. 2006; Bihaqi et al. 2011). In addition to an accumulation of β-amyloid (Aβ), deposition of another protein called tau in its hyperphosphorylated form is characteristic of the pathology of AD. Since Sp1 binding sites are present in the promoters of genes of APP as well as in tau, we hypothesized that early Pb exposure will also predetermine the expression of tau late in life. To test this hypothesis, we studied the brains of Cynomolgus monkeys, which were orally dosed in 1980-81 with 1.5 mg/kg/day of Pb- acetate from birth to 400 days of age and vehicle thereafter. The monkeys were sacrificed 23 years later. Here we present data on the protein expression of Tau46, Phospho tau (pSer199), cyclin dependent kinase-5 (cdk5) and F35/P25 in primate cerebral cortex that validate our hypotheses.

477 NEONATAL INFLAMMATION CONTRIBUTES TO FIBROTIC LUNG DISEASE IN ADULT MICE.

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Maternally-derived inflammation contributes to prematurity and low birth weight. Preterm infants often develop respiratory insufficiency and are exposed to hyperoxia which elicits additional inflammatory responses. The long-term pulmonary consequences of multiple exposures during the neonatal period have not been ex-
tensively explored. In the present study, we tested the hypothesis that exposure to maternal inflammatory mediators neonatal hyperoxia alters lung physiology in the offspring at adulthood. Pregnant C3H/HeN mice at embryonic day (E)16 were injected with LPS (80 μg/kg) or saline (i.p.). Offspring were placed in room air (RA) or 85% O2 for 14 d and subsequently raised in RA. Pulmonary function tests (PFT), microCTs, and histological analyses were performed in the offspring at 8 wks and molecular analyses between E18 and 2 wks of age. PFT indicated decreased compliance and increased elastance in LPS/O2 mice. MicroCTs showed hyper-expanded lungs including patchy centrilobular areas of emphysema and higher tissue densities in the LPS/O2 group. Histological analyses revealed decreased alveolar numbers and increased lung collagen I and III protein levels (4- and 20-fold greater than control, respectively). In fetal lung tissues, IL-1β, TGF-β, and collagen I mRNA were increased on E18, following maternal LPS injection. Elevated levels of TNF-α, MCP-1, TGF-β, collagen I and collagen III mRNA and protein levels were sustained throughout hyperoxic exposure only in pups born to LPS injected dams. Early dysregulation of miR-29 expression coincided with changes in lung inflammation and proliferation. In mice, systemic maternal inflammation followed by neonatal hyperoxic exposure causes fibrotic lung disease in the offspring. These findings support the novel hypothesis that neonatal exposures contribute to the pathogenesis of adult pulmonary disease and suggest a need for similar studies in ex-preterm infants.

**478 ALTERATIONS IN NEUROBEHAVIOR AND HIPPOCAMPAL MORPHOLOGY FOLLOWING DEVELOPMENTAL EXPOSURE TO 3,3′, 4,4′-TETRACHLOROAZOBENZENE IN SPRAGUE DAWLEY RATS.**

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A TRANSCRIPTOMICS APPROACH USING HBCD TO PRIORITIZE CHEMICALS AND MIXTURES FOR DEVELOPMENTAL NEUROTOXICITY RISK ASSESSMENT.

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Omic presents a promising tool to identify mechanistic pathways associated with developmental neurotoxicity (DNT), with which to precise chemicals/mixtures with limited toxicity data. This approach can be used to mask low-dose toxicity not assessable by conventional testing, identify vulnerable developmental periods, test across species, and validate high throughput in vitro assays. Using this approach, we evaluated short-term disruptions to the developmental profile of genes in the hippocampus, a brain region involved in learning and memory, after exposure to hexabromocyclododecane (HBCD). HBCD is a flame-retardant mixture of 3 isomers (α, β, γ); γ dominates the mixture while α dominates in human blood/breast milk. In mice, infantile exposure to HBCD disrupts adult neurobehavioral function. Using a similar model, we examined hippocampal gene transcription following prenatal HBCD exposure on postnatal day 10 to the mixture, α, β or γ. Transcriptomic profiles differed between α, β, and the mixture (p-value <0.05 and fold change >1.5). α had the most robust changes in gene expression number, magnitude, and enrichment of canonical signaling pathways (α, 38; mixture, 18; and γ, 11 pathways). A top Ingenuity pathway identified as altered by all exposures was long-term potentiation (α, p=0.002; mixture, p=0.03; and γ, p=0.04), with an overall depression/delay in function. These data suggest developmental exposure to HBCD can alter the timing and possible structure of synaptogenesis, underlying the previously reported deficits in learning and memory. The different profiles generated with each compound suggest the necessity to assess the effects from the isomers as well as the mixture. We demonstrate the utility of an omics strategy to capture information on various aspects of brain development that can be altered with environmental exposures and to provide focus for further studies in determining possible modes of action. Disclaimer: Views are of the authors and do not necessarily represent views and/or policies of US EPA or NCI/NIEHS.

**480 PRENATAL CIGARETTE SMOKE EXPOSURE REPRODUCES FEATURES OF ADHD.**

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Attention-deficit hyperactivity disorder (ADHD) is a complex disorder with significant genetic contributions and numerous co-morbid conditions such as conduct disorder and oppositional defiant disorder. However, no single gene has been linked to a significant percentage of cases, suggesting that environmental factors or gene-environment interactions may play a role in ADHD of the unique environmental factors that have been found in epidemiological studies to increase risk of ADHD, maternal smoking has been the most extensively studied. However, the mechanism(s) by which maternal smoking leads to increased risk of ADHD are not clear. Here, we utilized an animal model of maternal smoking to determine the effects of prenatal cigarette smoke (CS) exposure on neurobehavioral development. Pregnant B6C3F1 mice were exposed whole body to either filtered air or mainstream CS from gestation day (GD) 4 to parturition. Exposures were conducted 4 h/d and 5 d/wk, with each exposure producing the equivalent of smoking <1 pack of cigarettes per day. Pups were weaned at postnatal day (PND) 21 and behavior assessed on PND30 and again at 6 months of age. Male, but not female, offspring of CS-exposed dams demonstrated a significant increase in locomotor activity (~40%) that was ameliorated by methylphenidate treatment. Additionally, male offspring exhibited increased aggression, as evidenced by a 125% increase in the number of fights in a resident intruder task. These behavioral abnormalities were accompanied by a 37% decrease in striatal dopamine and a 46% reduction in brain-derived neurotrophic factor (BDNF) mRNA. Taken in concert, these data demonstrate that prenatal exposure to CS produces neurobehavioral alterations in mice that are similar to those observed in epidemiological studies and suggest a role for dopaminergic and neurotrophic alterations in these effects. Supported in part by R01ES051991; ES050602; IFSH; ES000260.

**481 PRENATAL TCDD AND POSTNATAL LUPUS IN EARLY GERIATRIC C57BL/6 MICE.**

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We recently reported an autoimmune profile in 24-week-old C57BL/6 mice that received a 2.5 or 5.0 μg/kg mid-gestation dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Clinical signs were consistent with a lupus-like syndrome and included: increased autoantibody levels, renal IgG and C3 immune complex deposition with associated inflammation, and increased peripheral Vβ+ T cells. No studies currently exist following the progression of such disease into middle or advanced ages, when human autoimmune disease manifests. Therefore in the present study, lettermates of mice from the previous 24 week prenatal TCDD study were allowed to age to 48 weeks, considered early geriatric in mice. Similarities and differences in the disease profile based on age and sex were observed. Peripheral autoimmune reactive T cells were increased only to 48 weeks, in contrast to males only to 24 weeks. Activated T cells from 48-week-old prenatal TCDD females over-produced the pro-inflammatory cytokine IFN-γ while males over-produced IL-10, effects again not seen at 24 weeks. Splenic transitional-2 B cells (CD21intCD24hi) were increased in males while transitional-1 B cells (CD21neg CD24neg) were increased in females at 48 weeks. Autoantibodies to cardiolipin and CD138+ spleen plasma cells were significantly increased in the aged males but not females. Anti-IgG and anti-C3 immune complex renal deposition were also significantly increased in the prenatal TCDD males but not females. These selective changes in the
Aged male mice may be noteworthy, in that the prevalence of SLE in humans shifts dramatically upward with aging. The collective findings suggest that prenatal TCDD permanently biases the postnatal immune response in aged C57BL/6 mice toward a sustained autoimmunity.

**Impact of Early Life BPA on Murine Cardiac Structure/Function.**

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Rationale: Bisphenol A (BPA), an endocrine disruptor used in the plastics industry, leaches out into the environment. Adult exposure is about 0.5 μg/kg/day with exposure in children about 10-fold higher. BPA can bind to estrogen receptors known to be present in fetal cardiomyocytes. We propose that BPA epigenetically reprograms early cardiac development to alter cardiac structure/function, DNA methylation and expression of proteins involved in calcium homeostasis.

Methods: BPA (BPA 0.5, 5, 200 μg/kg/day) or vehicle (VEH) was given orally to pregnant C57Bl/6 mice starting on gestation day 11. BPA200 pups received regular water at weaning. Cardiac function was measured by tail cuff blood pressure, echocardiography and electrocardiography. Physical parameters were measured monthly until 4M. Organ weights were collected at euthanasia. Protein expression was measured by immunoblotting.

Results: We report the impact of BPA on adult male progeny. Prostate, but not testes or seminal vesicle, weight was 1.5-fold increased in BPA5.0 mice. Kidney and abdominal fat was 2-fold increased in BPA5.0 mice. BPA200 had reduced blood pressure suggesting some hypotension. BPA5 had a longer PR interval suggesting a reduction in atria function. Echocardiography showed an increase in relative wall thickness in all BPA treated males suggesting cardiac hypertrophy. Fractional shortening, Vcf, pulmonary and ascending aorta VTI and cardiac output were similar suggesting no impact on ventricile function. SERCA2a and CASQ2 were increased >2-fold with no change in PLB or phosphorylated PLB at all BPA doses suggesting an increased ability to restore calcium and store calcium in the SER. PLB, LTCC, NCX1 expression was unaffected. DNMT3a was 2-fold increased at all BPA doses and DNMT3b was reduced suggesting altered DNA methylation activity.

Conclusions: Differences observed in cardiac function enable us to conclude that early life exposure to BPA, at BPA levels similar to those of childhood exposure, alters cardiac structure/function as well as cardiac gene expression in adult progeny, likely via changes in DNA methylation.

**Prediction of Drug-Induced Liver Injury by High-Content Analysis System Using HepG2 Cells: Detection of Reactive Oxygen Species.**

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Drug-induced liver injury (DILI) is a major reason for drug withdrawals from the market. It has been demonstrated that reactive oxygen species (ROS) are one of the primary causes of DILI. In this study, we examined the production of ROS (superoxide and peroxide) to predict DILI using a high content analysis system, namely, a high-throughput cell-based assay consisting of fluorescence microscopy and imaging data informatics. HepG2 cells from a human hepatoma cell line were seeded onto a 384-well plate and treated with test compounds for 1, 3, and 24 hr, and then triple-stained with dihydroethidium, CM-H2DCFDA, and Hoechst 33342 for detection of superoxide, peroxide, and cell loss, respectively. Then, the cells were analyzed by ArrayScan VTI and minimum effective concentrations for ROS production were calculated. At first, 7 compounds that induced oxidative stress were used to decide criteria for judgment in the multiplexed assay. Four of 7 compounds, hydrogen peroxide, menadione, L-buthionine sulfoximine, and blomycin, induced superoxide and/or peroxide at non-cytotoxic concentrations after 1, 3, and/or 24 hr-treatment. The remaining 3 compounds, mitomycin C, doxorubicin, and genticam, failed to detect superoxide and peroxide up to cytotoxic concentrations after 24 hr-treatment in HepG2 cells. Secondary, 9 hepatotoxicants and 6 non-hepatotoxins were tested to investigate the utility of the multiplexed assay as a screening system for hepatotoxic potential. As a result, all 9 hepatotoxicants induced superoxide and/or peroxide at non-cytotoxic concentrations (sensitivity = 100%), and 4 of 6 non-hepatotoxicants did not induce superoxide and peroxide up to cytotoxic concentrations (specificity = 66.7%). ROS5 production pattern, species and reactive time-point were revealed to be unique in some compounds. It is concluded that this high throughput multiplexed assay is a powerful tool to predict the DILI potential in the early phase of drug discovery.

**Enhancement of Proliferation in a Rat Hepatocyte Coculture Model After Mitogenic Stimulation.**


Primary mouse and rat hepatocyte cultures have long been the gold standard for assessment of cellular changes following chemical exposure. While helpful for assessing proliferative and responses in vitro, these cultures are limited to 1 or 2 days of incubation. Our motivation was to test whether extending the culture time beyond 3 days using micropatterned primary rat cultures coupled with quantitative high content imaging (HCI) technology could provide a more accurate assessment of the cell proliferative response after mitogenic stimulation. The HepatoPac® 96-well coculture system utilizes primary Sprague-Dawley hepatocytes seeded on micropatterned islands of collagen, surrounded by non-parenchymal murine fibroblasts. Following 8 days of stabilization, HepatoPac® cultures were incubated for 3, 5, and 7 days in serum-free media containing three mitogenic concentrations of Wyleh 14, 643 (WY) and Phenobarbital (PB) followed by parallel imaging analysis at 2 laboratories for Ki67 signal (a proliferation marker present in G1, S, G2, and mitosis, but not in G0). PB results showed an enhancement of Ki67 signal reported as % of control: 153%, 146%, and 83% at 3, 5, and 7 days respectively. WY results showed a similar trend: 140%, 108%, and 111% at 3, 5, and 7 days respectively. These results show an improvement of proliferative response with this hepatocyte model when compared to previous experiments using mono-layered primary rat cultures that produced a 5-10% hepatocyte proliferative response when held for 48hrs. These experiments suggest that the cellular environment & extended incubation capacity provided in the micropatterned cultures is a useful feature when testing compounds for mitogenic activity. (This abstract does not reflect US EPA policy and mention of trade names is not an endorsement for any product).

**Use of the HepaRG Cell Line to Assess Potential Human Hepatotoxicity of Toxcast Chemicals.**

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The HepaRG cell line is a promising model system for predicting human hepatotoxicity in part because of the greater capacity to metabolize chemicals compared to other hepatocyte models. We hypothesized that this cell line would be a relevant model for toxicity testing of industrial chemicals. Gene expression profiles were used to predict changes in xenobiotic-activated transcription factors. Approximately 20 chemicals that have been evaluated in high throughput assays as part of the ToxCast screening program are under investigation. Initial studies examined the gene expression response to the fungicide vinclozolin, a rodent liver carcinogen. Three separate preparations of differentiated HepaRG cells were exposed for 72 hr with either DMSO or one of six concentrations of vinclozolin ranging from 7.8-250 μM. RNA expression was analyzed using Illumina Human HT-12v4 bead chips. The gene expression data were normalized by Illumina GenomeStudio, and differentially expressed genes were identified by CyberT. The doses of 250, 125 and 63 μM resulted in 404, 258, and 65 genes altered, respectively. No genes were significantly altered at doses below 63 μM. Ingenuity Pathways Analysis (IPA) indicated that a number of pathways were altered including metabolism of androgen/estrogen, xenobiotics and fatty acids. Genes associated with dose-dependent AHR (CYP1A1, CYP1A2, CYP1B1) and PPARα (ACADM, ACDVL1, CPT1A, ECH1) activation were identified. These findings overlap with changes observed after vinclozolin exposure in cell models as part of the ToxCast screening program including HepG2 transcription factor screening and nuclear receptor marker gene evaluation in human primary hepatocytes. Future research with additional ToxCast chemicals will allow more comprehensive baseline characterization of the HepaRG model and help to elucidate mode of action. (This abstract does not reflect EPA policy).
**486 A MULTIPLEX, AUTOMATED APPROACH TO SCREEN FOR MITOTOXICITY IN HUMAN HEPATOCYTES AND HEPG2 CELLS.**

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Many drugs and environmental chemicals have been implicated in mitochondrial toxicity resulting in myopathies, hepatotoxicities, peripheral neuropathies and cardiovascular disorders. The mechanisms of mitotoxicities are complex due to multiple modes of action such as modulating mitochondrial replication and disruption of electron chain transport, and by organ-specific susceptibility. For example, the mitotostatin preferentially targets the liver over muscle due to metabolic activation in hepatocytes. Therefore, multiple approaches are needed to assess a chemical's mitochondrial liabilities in specific cellular systems. Herein, we present one approach to utilize two markers of cellular viability in an automated multiplexed format for high-throughput screening to monitor hepatotoxicity that can discriminate primary mitotoxicity from general cytotoxicity. Primary human hepatocytes and HepG2 cells were used in a 384-well suspension culture format in serum-free, glucose-free galactoside-containing medium for up to 6 hour exposure with cytotoxins in a 11-point response curve. ATP Detection Reagent and Cytotoxicity Reagent were used to measure ATP levels and cell membrane integrity, respectively. A mitotoxic effect was defined as a reduction of ATP relative to untreated control, with no or minor changes in cell membrane integrity. CCCP and antimycin were used as model compounds to disrupt mitochondrial activity. Digitonin cytotoxicity was caused by solubilizing the cell membrane, resulting in decreased ATP and loss of cell membrane integrity. Staurosporine and tamoxifen were also used as cytotoxins to validate the system. Similar results were observed between HepG2 and human hepatocytes except for antimycin where a Crabtree effect was implicated for the difference in its potency. In summary, the multiplex system provided a robust and informative platform to screen drugs and chemicals for hepatic mitotoxicity by measuring ATP and cell membrane integrity.

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**488 AN RNA INTERFERENCE-BASED SCREENING IDENTIFIES THE SIGNALING NETWORK THAT DETERMINES DICLOFENAC-INDUCED LIVER CELL DEATH.**


Drug-induced liver injuries (DILI) are the leading cause of acute liver failure. DILI often involves a synergy between chemical-induced cell injury stress and immune-mediated cytokine signaling responses. So far the underlying signaling mechanisms in this interaction are unknown. We therefore applied a siRNA screening approach targeting all kinases and (de)ubiquitinas as well as cytokine signaling components. As a model we used diclofenac, a clinical relevant drug that can cause idiosyncratic DILI. Diclofenac-induced cell death of HepG2 cells is enhanced by the cytokine TNF-α and related to perturbation of the anti-apoptotic NF-κB pathway and activation of the pro-apoptotic caspase-8 pathway. Here we used a high-throughput siRNA screening approach to identify key signaling players in diclofenac/TNF-α-induced HepG2 cell death. HepG2 cells were transfected with Dharmacon smartpool siRNAs and thereafter treated with diclofenac for 8 hours followed by TNF-α challenge for 16 hours in the presence of annexin V-Alexa633. Cell death was assessed by measuring the relative annexin V fluorescence intensity per cell area at 8 and 24 hours. In total, 1596 genes were targeted. After automated image analysis 94 hits were selected with a Z-score cut-off of 2.0. The hits include those that increase or decrease diclofenac/TNF-α-induced cell death after 24h, that increase cell death induced by diclofenac alone at 8 hours, and knockdowns that cause cell death by TNF-α exposure at 24 hours. A deconvolution screen was then performed using 4 single siRNAs per target gene by our live apoptosis assay to assess the previously identified hits. This screen revealed key signaling molecules that define the sensitivity for diclofenac-induced hepatocyte killing. These genes are likely candidates in the predisposition for DILI in the patient population.

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**487 AN IMAGING-BASED RNA-INTERFERENCE SCREEN IDENTIFIES MODULATORS OF TNF-MEDIATED NF-κB SIGNALING IN DRUG-INDUCED LIVER INJURY.**


Tumor necrosis factor-α (TNFα) is a cytokine that plays a dual role in the liver: TNFα can induce hepatocyte proliferation and liver regeneration, but it also affects cell death. TNFα has been implicated in various liver diseases as well as in drug-induced liver injuries (DILI). Thus, TNFα signaling enhances the diclofenac-induced cell killing of hepatocytes. The protective effect of TNFα is mediated by the timely nuclear translocation of NF-κB to activate its target gene transcription. Diclofenac negatively affects the frequency and amplitude of NF-κB oscillation in liver cells, thereby promoting synergistic pro-apoptotic responses. Here we performed a functional genomics screen to identify kinases and (de)ubiquitinases that are involved in the deregulation of the TNFα-induced NF-κB oscillatory responses caused by diclofenac. We used HepG2 cells expressing GFP-tagged p65/RelA (NF-κB subunit) in 96-well plates under control and drug-treatment (diclofenac) conditions and followed the GFP-p65 oscillation of ~200 cells per well for 6 hours using automated confocal laser scanning microscopy. The cell-specific NF-κB responses within a knockdown population were mapped using automated ImageJ-based algorithms. This work firstly uncovered distinct cell behaviors and population dynamics in the context of hepatotoxicant treatment in association with increased sensitivity towards apoptosis. Using a robust statistical analysis including several parameters, we selected 127 hits out of the 1596 genes screened. A validation deconvolution screen was then performed to confirm the functional role of the identified kinases and (de)ubiquitinases controlling the NF-κB response. Pathway analysis of the hits identified indicates that the activity of the NF-κB response is intricately linked to various stress responses, which, upon drug toxicity conditions, modulate the activity of the protective abilities of the NF-κB response. The identified candidate genes may play a critical role in modulating DILI responses.

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**489 A HIGH-CONTENT IMAGING-BASED IN VITRO 3D MODEL FOR ASSESSMENT OF DRUG-INDUCED LIVER INJURY.**

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We have developed a 3D HepG2 cell culture model for the assessment of hepatotoxicity. HepG2 cell-based monolayer models are widely used for studying liver toxicity of xenobiotics. It is well anticipated that under these conditions HepG2 cells have low expression of metabolic enzymes and drug transporters, which will compromise the validity of high-throughput safety assessment studies using these cells. Here we describe a 3D in vitro model using HepG2 cells. This model induces robust morphological and functional differentiation, with a strong induction of metabolic enzymes and transporters, many of which are poorly expressed in monolayer cultures. Unlike primary hepatocytes in 2D culture, the metabolic competence of HepG2 in 3D could be retained for up to 4 weeks, making chronic exposures feasible. The assay we have developed is implemented in a 384 well format for low cost and increased throughput. We combine conventional toxicity readouts with automated high-content imaging to measure expression of fluorescent stress reporters, such as KEAP1/Nrf2 activation, combined with other fluorescent labels for cell toxicity. This assay represents a novel and more physiologically relevant method for predicting drug-induced liver injury.

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**490 TOXICITY PATHWAY ANALYSIS OF HEPATOTOXIC COMPOUNDS IN PRIMARY HUMAN HEPATOCYTES IN 2D AND 3D-CULTURE.**

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Hepatotoxicity is the most common reason for a drug to be withdrawn from the market and the most common cause of drug attrition. Since it is increasingly important to identify hepatotoxic liabilities earlier in drug discovery, in vitro evaluation screening systems play an important role in the early phase of pharmaceutical development. They are also important to clarify the mechanisms of toxicity observed during development.
In this study we describe the suitability of human hepatocytes in 2D- and 3D-con-figurations for lead optimization screening. The scaffold-free 3D liver microtissues are produced by gravity-enforced self-assembly in hanging drops. They are com-posed of primary human hepatocytes in coculture with nonparenchymal cells, in-cluding Kupffer macrophages. This highly organotypic culture is characterized by extensive cell-cell contacts, distinct morphology between cell-types and higher he-patocyte functionality than in 2D-cultures. Since liver microtissues are also stable and viable over several weeks in culture, this allows chronic repeat dose exposures to analyse potential hepatotoxic liabilities.

Due to uniform production and standardisation of the microtissue highly reproducible data is generated. Here it is shown for the first time, that liver microtissues are suitable for compound screening. Known compounds that result in bioactivation, mitochondrial impairment, steatosis and oxidative stress are assessed using a range of biomarkers including ATP content, GSH depletion and lipid accumulation. Many new in vitro cell models are being created to help understand and improve our ability to predict hepatotoxicity early in the drug discovery process; we show the potential of human hepatocyte microtissues as a promising alternative to 2D cultures.

Liver Injury remains a major reason for late stage drug attrition. Therefore the phar-maceutical industry aims at developing predictive assays that can be deployed early in the drug discovery process when SAR approaches are still feasible. Xu et al., 2008 have shown that drug induced liver injury can be predicted to some extent using primary human hepatocytes in sandwich cultures and high content imaging of key endpoints of injury (i.e. mitochondrial membrane potential, reactive oxygen species, lipid accumulation and nuclear stain). The sensitivity of this assay was approxi-mately 50% with a 0-5% false positive rate. While high content imaging using sandwich cultures represents a key advance in the field, the sensitivity of the assay needs to be further improved. One can hypothesize that the low sensitivity could be due to the fact that the assay was only performed at 24 hours and that longer incu-bation with compounds could improve outcomes. However, the requirement would be that the hepatocyte culture would stay viable and metabolically compre-hensible for an extended period of time. In addition, full phase I and II enzymes should be expressed at levels comparable to those found in vivo and transporters should be functional. Here, we use micropatterned human hepatocyte cocultures (Khetani and Bhatia, Nature Biotechnology 2008), also called HepatoPac™, to detect drug induced liver injury for compounds that were previously missed utilizing the Xu assay. We show that in comparison to conventional sandwich cultures, HepatoPac was able to accurately identify 10 toxic compounds (True Positives) without in-creasing the false positive rate for truly negative compounds. In addition, HepatoPac was able to identify 14 of 25 toxic compounds that were missed previ-ously in the Xu assay and reported as false negatives. In summary, this method shows superiority over the conventional sandwich culture method.

In this study, 12 compounds with known hepatotoxicities were used in an in vitro microtissue model to assess for lead optimization potential. Using high content screening, we were able to score liver abnormalities and fibrosis in zebrafish larvae (trichrome stain) and fibrosis (trichrome stain). For some compounds (e.g., nefazodone), histological alterations were only detected at concentrations that had been scored for subtle detection and delineation between different mechanisms of liver toxicity.

Here it is shown for the first time, that liver microtissues are suitable for compound screening. Known compounds that result in bioactivation, mitochondrial impairment, steatosis and oxidative stress are assessed using a range of biomarkers including ATP content, GSH depletion and lipid accumulation. Many new in vitro cell models are being created to help understand and improve our ability to predict hepatotoxicity early in the drug discovery process; we show the potential of human hepatocyte microtissues as a promising alternative to 2D cultures.
495 INHIBITION OF THE HEPATIC BILE ACID TRANSPORTER MRP4: A POTENTIAL RISK FACTOR FOR CHOLESTATIC DRUG-INDUCED LIVER INJURY.

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Inhibition of the bile salt export pump (BSEP) leading to increased hepatocellular concentrations of bile acids has been proposed to contribute to the development of drug-induced liver injury (DILI). MRP4 is responsible for the hepatic basolateral efflux of xenobiotics and bile acids, especially when biliary excretion of bile acids is compromised. Inhibition of MRP4 function may result in accumulation of toxic species and deleterious consequences. In the present study, inhibition of MRP4-mediated transport by cholestatic and non-cholestatic compounds was investigated to obtain further insights into potential risk factors for DILI. Membrane vesicles prepared from HEK293T cells transfected with human MRP4 or empty vector were used to determine the inhibitory effect of test compounds at 100 μM on MRP4-mediated uptake of the probe substrate 3H-dehydroepiandrosteronesulfate (DHEAS; 2 μM) in the presence of ATP and glutathione. Test compounds consisted of 16 cholestatic (4 BSEP inhibitors; 12 non-BSEP inhibitors) and 15 non-cholestatic (4 BSEP inhibitors; 11 non-BSEP inhibitors) drugs. Test compounds that inhibited MRP4-mediated DHEAS transport >30% were defined as MRP4 inhibitors. A χ²-test was used to determine whether MRP4 inhibition was related to the cholestatic properties of the test compounds. Inhibition of MRP4-mediated DHEAS transport was observed for 12/31 compounds (39%); 9/16 compounds associated with cholestatic liver injury or jaundice in humans (56%), but only 3/15 non-cholestatic compounds (20%), inhibited MRP4 (p<0.05). BSEP inhibition is a known susceptibility factor for DILI. Therefore, compounds that were not BSEP inhibitors were analyzed further: 6/12 cholestatic compounds (50%), but only 1/11 (9%) non-cholestatic compounds inhibited MRP4 (p<0.05). These results suggest that inhibition of MRP4 might result in hepatic accumulation of toxic compounds and contribute to the development of cholestatic DILI. Morgan et al., Toxicol Sci., 2010 Dec; 118(2):485-500. Supported by NIH GM41935 and DFG Ko4186/1-1.

496 POTENTIAL ROLE OF FASL EXPRESSION AND SIGNALING IN THE INDUCTION OF HEPATOTOXICITY BY AZT.

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The induction of Highly Active Antiretroviral Therapy (HAART) in the mid-1990s has led to a significant increase in the life expectancy of patients with HIV. However, hepatotoxicity due to HAART is also common, and up to 30% of patients on HAART experience World Health Organization grade 3 liver enzyme elevations. Furthermore, hepatotoxicity from antiretroviral drugs leads to adverse patient outcomes either from fulminant hepatitis or jaundice in humans (56%), but only 3/15 non-cholestatic compounds (20%), inhibited MRP4 (p<0.05). BSEP inhibition is a known susceptibility factor for DILI. Therefore, compounds that were not BSEP inhibitors were analyzed further: 6/12 cholestatic compounds (50%), but only 1/11 (9%) non-cholestatic compounds inhibited MRP4 (p<0.05). These results suggest that inhibition of MRP4 might result in hepatic accumulation of toxic compounds and contribute to the development of cholestatic DILI. Morgan et al., Toxicol Sci., 2010 Dec; 118(2):485-500. Supported by NIH GM41935 and DFG Ko4186/1-1.

497 ACCELERATED ETHANOL CLEARANCE AND RESISTANCE TO ALCOHOL-INDUCED STEATOSIS IN GLUTATHIONE-DEFICIENT MICE.

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Alcoholic liver disease (ALD) is one of the leading lifestyle-related causes of illness and death in the United States. Liver is the primary alcohol-metabolizing organ, where ethanol is first metabolized to acetaldehyde by alcohol dehydrogenases and CYP2E1 and then to acetic acid by aldehyde dehydrogenases (ALDH). While the molecular mechanisms underlying the pathogenesis of ALD are not completely understood, oxidative stress associated with ethanol metabolisms has been implicated in this process. The aim of this study was to elucidate the role of antioxidant glutathione (GSH) in ethanol metabolism and alcohol-induced hepatotoxicity by using Gclm(−/−) transgenic mice that exhibit 10-20% of normal tissue GSH levels. Following a single injection of ethanol (i.p., 5 g/kg), circulating ethanol and acetaldehyde were measured in Gclm(−/−) and Gclm(+/+) mice at 1, 3 and 24 hour. Gclm(−/−) mice revealed a faster clearance of acetaldehyde and a to lesser extend ethanol. After 6 weeks of feeding with a liquid diet containing ethanol up to 6%, both Gclm(−/−) and Gclm(+/+) mice showed normal liver weight and plasma ALT levels. Histologically, simple steatosis was observed in 60% of ethanol-fed Gclm(−/−) mice, but was absent in all ethanol-fed Gclm(+/+) mice. Hepatic gene and protein expression analyses revealed suppressed lipid metabolism but induced ethanol metabolism and stress-response pathways in Gclm(−/−) mice compared to wild-type animals. Collectively our results suggest a potential role of GSH in fine tuning of the metabolic and stress response in ethanol-induced liver injuries. Supported in part by NIH grants R01 EY011490 and R21AA017754.

498 HEPATIC BILE ACID COMPOSITION IN PROGRESSIVE HUMAN NONALCOHOLIC FATTY LIVER DISEASE.

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Individual bile acids exhibit either hepatoprotective or toxic roles within the liver due to their diverse chemical properties. Alteration of the bile acid profile can have a profound involvement in the development of drug induced liver injury and hepatotoxicity. Chronic liver diseases such as nonalcoholic fatty liver disease (NAFLD) may alter the bile acid profile predisposing individuals to hepatic injury. NAFLD originates as steatosis and may progress to non-alcoholic steatohepatitis (NASH), which is characterized by increased oxidative stress, inflammation, and fibrosis. The purpose of this study was to analyze the transcriptomic and metabolomic alterations of the classical and alternative bile acid synthetic pathways within the context of progressive human NAFLD. Human livers classified as normal, steatosis and NASH were prepared for analysis using Affymetrix GeneChip Human 1.0 ST arrays as well as LC-MS and NMR metabolomics methodologies. In general, transcriptional changes in the expression of nuclear receptors, transporters, and metabolizing enzymes of the bile acid synthetic pathway appear to drive alterations in the bile acid metabolomic profiles of NASH patients. Increased expression of the tauine conjugation enzyme bile acid-Coenzyme A amino acid N-acyltransferase (BAAT) and CYP7B1 were observed in NASH livers resulting in metabolic increases of tauine and taurine conjugated bile acids such as taurocholic acid (TCA) and taurodeoxycholic acid (TDCA). Concurrent decreases of the more hepatotoxic bile acids were observed including cholic acid (CA), deoxycholic acid (DCA) and glycocholic acid (GCA). These findings reveal a shift toward the alternative (CA) and more hepatotoxic bile acid synthesis characterized by increased taurine conjugation which may be indicative of an initiation of hepatoprotective mechanisms in NASH patients.

499 TRANSPLACENTAL ARSENIC EXPOSURE INDUCES GENDER-SPECIFIC GENE EXPRESSION CHANGES IN FATTY ACID METABOLISM IN C57BL/6 MOUSE LIVER.

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Arsenic contamination in ground water is present in many places around the world. Several regions, including southeast Asia and northern New England, have arsenic concentrations in water sources that measure well above the 10 ppb action level set.
by the EPA and WHO. Many diseases have been linked to arsenic exposure includ- ing cancer and diabetes. Investigations of in utero exposure to arsenic have shown alterations in DNA regulatory elements, although the mechanisms of toxicity are still poorly defined. To address the effects of arsenic exposure on their offspring, female C57BL/6 mice were exposed to 0 to 500 ppb sodium arsenite in drinking water, from 4 days prior to mating to 21 days post partum. Livers from offspring were collected and gene expression was measured using a fatty acid metabolism target array (SABiosciences). Gender differences were detected, with altered expression in 14 of 84 genes in females and 40 of 84 in males. Gene targets focused on pathways including the catabolism, transport and biosynthesis of fatty acids, triglycerol carabolism and ketogenesis. Nearly half of the genes with altered expression are regulated by peroxisome proliferator-activated receptor (PPAR), a tran- scription factor involved in glucose regulation and adipose tissue differentiation. Pparγ has been previously identified as a target of acute arsenic exposure in mouse fibroblasts (NIH 3T3), showing altered gene expression and protein function. The investigation into the transcriptional regulation of the genes identified in the array is ongoing, focused on the impact of maternal arsenic exposure on Pparγ gene and protein expression following chronic arsenic exposure. This study will help con- tribute to our understanding of how early-life arsenic exposure may influence cru- cial regulatory mechanisms involved in lipid metabolism, and impact the develop- ment of metabolic diseases in adults.

**500 ACETALDEHYDE DEHYDROGENASE 2 (ALDH2) ACTIVATION PROTECTS HEPATOCYTES FROM MITOCHONDRIAL DAMAGE AND DEATH CAUSED BY 4-HYDROXYNENAL.**

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ALDH2 is a mitochondrial enzyme that oxidizes acetaldehyde to acetate. Lipid aldehydes (e.g., 4-HNE) are also substrates for ALDH2, and it has been hypothesized that this enzyme may also protect against oxidative stress. Indeed, recent studies by this group indicate that activating ALDH2 protects against oxida- tive stress and damage to the liver. The purpose of the current study was to build on these findings and to assess the mechanistic effect of activating ALDH2 using high throughput screening (HTS) and microarray (HCS) in vitro. Primary hepatocytes were isolated from C57BL/6J mice and seeded on 96 well plates. Some mice were administered EtOH (6 g/kg/d i.g. for 3 d) prior to isolation, or hepatocytes were exposed to Alda-1 (1.5 μM) in culture, to acti- vate ALDH2. Cells were exposed to 4-HNE (0-1000 μM) for 0-24 h. Cell toxicity was measured via automated quantitative multi-probe fluorescence microscopy, using a Cellomics Array Scan VTI HCS reader. This technique enabled monitoring of live cells for multiple fluorescent markers of processes that are involved in the pathogenesis of toxicity, including: inner mitochondrial membrane potential (TMRR), membrane permeability (TOTO-3), and nuclear number and size (Hoechst). Additionally, the Seahorse Biosciences XF-96 was employed to assess mitochondrial function of the primary hepatocytes. 4-HNE exposure caused cyto- toxicity and cell death time- and dose-dependently. Mitochondrial dysfunction was observed to precede cell death after 4-HNE exposure. Activating ALDH2 signifi- cantly protected against 4-HNE toxicity. Taken together, these data suggest that ac- tivating ALDH2 protects hepatocytes from 4-HNE-induced mitochondrial damage and cell death. These results lend further support to the idea that ALDH2 activity (high or low) may significantly impact the severity and response to liver injury and liver diseases. Supported in part by NIAAA

**501 MOLECULAR MARKERS OF MOUSE HEPATOCARCINOMA THAT IDENTIFY THE INITIATING ONCOGENE(S).**

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We are searching for molecular markers that can distinguish between different he- patocellular carcinomas (HCC) forms of HCC by virtue of their distinct initiating oncogene alterations. Only if the initiating cause is known can pathway-targeted therapy be em- ployed. In this study we evaluate patterns of gene expression in mouse liver tumors that are caused by known oncogene(s): c-myc, mutant H ras, mutant β-catenin, and/or TGFα. We employ an inducible transgene system coupled with an hepatocyte transplantation assay to generate focal HCCs growing in an otherwise non-neo- plastic liver. For each tumor-initiating genotype, we compare microarray expression profiles to identify specific and unique patterns of gene expression. In this model, the incidence and latency of HCC progression varies depending on the initiating oncogene(s), indicating that the original genetic changes have different effects on the development of aggressiveness of the disease. For these oncogene combinations, we identified a panel of genes whose collective expression patterns allowed us to discriminate among the groups. Using the “leave one out” method, we found that an observer without knowledge of sample identity could correctly identify a tumor’s initiating genetic changes by examining the expression profile of this panel of genes. Our findings suggest that we can identify molecular (gene expression) markers that can assign mouse liver tumors to appropriate oncogenic initiating groups.

**502 ACTIVATION OF SIRTUIN 1 PATHWAY TO REVERSE DRUG TRANSPORTER AND NUCLEAR RECEPTOR PATHWAYS IN FATTY LIVER.**

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Nuclear receptors (NR), CAR, PXR, FXR, PPAR and Nr5f2 are transcriptional regul- ators of transporter expression in liver in coordination with Phase-I and -II bio- transformation enzymes. NAFLD markedly alters hepatic transporter expression in mouse. Caloric restriction (CR) is a common regimen to treat NAFLD and proven model to activate Sirtuin1 deacetylase activity in liver conferring protection against steatosis. Purpose of this study was to expand on currently negligible information to evaluate whether CR affects hepatic transporter expression in livers from lean mice and “reverse” transporter expression changes observed with steatosis. Adult lean (C57BL/6) and obese (ob/ob, OB) mice were fed ad libitum or placed on a 40% (kCal) reduced diet. CR decreased hepatic triglyceride levels in both mice, but not to the same degree in livers from OB mice. CR decreased Oatp1a1; Oatp1b2 (~90%) as well as Bsep, Btcp (~40%) mRNA and protein expression in lean but not OB mice. CR increased Abcc2 mRNA expression by ~2 fold in OB livers only. However, protein expression increased in both lean and OB mice, reversing basal downregulation of Abcc2 in OB mice to a level comparable to lean fed controls. Steatotic livers from OB mice show increased Abcc1, 3, 4 and 5 mRNA expression. CR decreased Abcc3 protein expression in lean mice but increased it in OB livers. CR did not alter Abcc1, 4 and 5 mRNA expression in lean mice but significantly decreased their expression in OB mouse livers. Abcc4 protein expression increased in lean and OB mice upon CR. In steatotic livers from OB mice; AhR, PXR, Nfkb, Nr5f2, CREB, CAR and PPAR binding to respective RE increased by 147- 275% but FXR binding decreased by 60%. This increased binding in hepatic nu- clear fractions from OB mice was reversed by CR and mirrored by respective target gene expression. In summary, CR can “reverse” expression of some transporters in liver, but not all. Overall, these data indicate a potential to restore hepatic disposi- tion changes associated with obesity through CR. (KEIS013782A, RES016042A)

**503 HTS FOR SMALL MOLECULES CAPABLE OF MODULATING THE CELLULAR PHENOTYPES OF HUMAN iPSC-DERIVED HEPATOCYTES.**

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The liver is the major drug metabolizing organ and a frequent site of drug induced toxicity. In drug discovery, robust in vitro methods are needed for modeling liver function. Current methods employing primary human hepatocyte cultures and cell lines have well-documented shortcomings, namely donor to donor variability and functional instability. Although pluriptotent stem cell derived tissues hold promise to address these problems, thus far most reports on human induced pluripotent stem cell (hiPSC)-derived hepatocytes indicate a higher similarity to fetal tissues than adult in many aspects including metabolic activity, which could make their ex- trapolation to the adult situation difficult. To address these shortcomings, human iPSC-derived hepatocytes were exposed to a library of small molecules to identify compounds that resemble functionality of an adult liver. High-throughput, mi- crofluidic qRT-PCR was used to examine the expression of 26 genes that span a spectrum of hepatocyte functions that were either low or exhibited an immature phenotype in hiPSC-derived hepatocytes when compared to adult primary human hepatocytes. During the primary screen, multiple compounds significantly in- creased maturation-associated genes. These hits were confirmed in a secondary screen, and the ability for a sustained response was quantified. These data suggest it may be possible to modulate the maturation of stem cell-derived tissues, potentially generating even more relevant models for drug discovery and safety platforms.
504 PHYSIOLOGICAL HEMODYNAMIC FLOW AND TRANSPORT ARE NECESSARY FOR RETENTION OF PRIMARY HEPATOCYTE DRUG METABOLISM AND TOXICITY INDICES.

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Static primary hepatocyte cultures, used in drug metabolism and toxicity testing, experience non-physiological drug exposure profiles due to design limitations, and lose their metabolic phenotype over time, possibly contributing to poor in vitro in vivo correlations. We developed a system using rat hepatocytes plated in a perfused transwell device exposed to hemodynamics matching physiological liver blood flow. Hepatocytes in this system demonstrated polarized morphology, retention of differentiation markers (hepatocyte nuclear factor 4-α and E-cadherin) and significantly higher levels of liver function compared to static cultures over 2 weeks (Albumin =74.4 ± 10.04 vs. 9.8 ± 2.32 μg/106 cells/day, Urea =698.93 ± 61.62 vs. 102.17 ± 32.85 μg/106 cells/day, n=3). Gene expression of Cytochrome (Cyp) enzymes was significantly higher (n=10, Fold changes over static cultures: Cyp1A1 = 60.5 ± 11.6, Cyp1A2 = 69.2 ± 36.6, Cyp2C = 20.4 ± 5.6, Cyp2D = 12.5 ± 5.3, Cyp3A = 10.4 ± 3.3). This translated into higher levels of basal enzyme activity (n=3, Fold changes over static: Cyp1A1 = 11.5 ± 4.9, Cyp2C = 7.5 ± 3.7, Cyp3A = 4.4 ± 0.9) and higher inducibility by 3-methyl cholanthrene and dexamethasone than static cultures (Fold changes over DMSO controls: Cyp1A1 = 66.9 ± 5.1 vs. 25.4 ± 3.2, and Cyp3A = 29.6 ± 1.9 vs. 12.9 ± 0.3). Hepatocytes under hemodynamics responded to classical Cyp inducers like dexamethasone at concentrations that matched plasma concentrations of G/606 rat studies. Efflux transporter proteins, localized along the canalicular membranes, exhibited substrate transport into bile canaliculi. The retention of in vitro like hepatocyte phenotype and metabolic functions coupled with drug response at more physiological concentrations potentially makes this system a more predictive tool for drug metabolism and toxicity.

505 HEPATIC TOXICITY OF VINCOLOZIN IN ADULT MALE RATS.

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Vincolozin (V) is a fungicide widely used in plants, fruits and vegetables in United States, Europe and Mexico. The V is a well-characterized antiandrogenic fungicide and it is able to produce effects on the male reproductive system in different species. Little data are currently available regarding possible impacts of V on the liver. The objective of this work was to assess the effect of V on hepatic function. Male adult Wistar rats were administered 100 mg/kg V in corn oil for 7 days by gavage. Non-treated animals received only corn oil. Animals were sacrificed 24 h after last dose. Blood was extracted for analysis of reduced and oxidized glutathione, alanine aminotransferase (ALT) and aspartate aminotransferase (ASAT). The liver was removed and processed to obtain microsomes for the determination of glycogen, reduced and oxidized glutathione, and lipid peroxidation. Significant effect of V on weight of animals was observed. V administration significantly increased the liver weight, this effect was accompanied by an increase by 53% of total cytochrome P450 (CYP) content and a decrease of 35% of glycogen content and both reduced (60%) and oxidized (85%) glutathione. ALT serum activity decreased 19%, whereas both reduced and oxidized glutathione serum levels also decreased 26% and 26%, with respect to the non-treated group. Liver lipid peroxidation and AP were not affected by V. These results suggest that V affects hepatic function mainly the glutathione-dependent antioxidant system, CYP-dependent metabolism of xenobiotics and carbohydrates metabolism. In addition, they indicate that liver is another target organ of this fungicide. Determination of toxicity in organs or systems will provide further insight into the relationship between toxicity and V exposure.

506 A PROVISIONAL REGULATORY NETWORK OF PPARα-MEDIATED TRANSCRIPTION IN PRIMARY HUMAN HEPATOCYTES.

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Activation of the PPARα nuclear receptor in liver parenchymal cells results in coordinated events leading to downstream alterations in gene expression: (i) phosphorylation of PPARα in the cytosol; (ii) translocation of PPARα to the nucleus; (iii) heterodimerization with RXRα; (iv) binding of the heterodimer to DNA-response elements (PPREs) in the promoters of target genes; and (v) alterations in binding of co-activators and co-repressors. We used a combination of microarray-based gene expression data, regulatory interactions inferred from protein-DNA transcription factor (TF) arrays and ChIP-on-chip results to develop a comprehensive picture of PPARα-mediated transcriptional regulation in primary human hepatocytes exposed to GW7647 (GW). Cells were treated for up to 72 hours with concentrations of GW between 0.001 and 10 μM. We identified several TFs involved in the regulatory cascade, leading to a clearer picture of the hierarchical organization of the PPARα response network as well as concentration- and time-dependent transitions in the state of the network. A small subset of 183 genes was upregulated by GW in a time- and concentration-dependent manner. Additionally, only a limited number of these genes were direct gene targets of PPARα. The remaining genes were likely regulated by cross-talk at the transcriptional level or by downstream activation of other pathways in the cascade, including ERα, HNF4α, STAT1, ETS1 and Sp1. The inferred response network, in combination with an on-going study with rat hepatocytes, will help guide quantitative computational models of the PPARα pathway. More generally, this approach of network mapping and modeling can lead to more accurate dose-response evaluation of receptor-mediated cellular processes.

507 INHALATION OF LEAD ACETATE AND LIVER DAMAGE: MORPHOFUNCTIONAL STUDY IN A MOUSE MODEL.

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Lead (Pb) is an element that plays an unknown biological role, and it is highly toxic even in low concentrations. However, the main emission sources of this metal are lead-anti-kick fuels and cigarettes, which are in daily use in society. The main route of exposure to Pb occurs through the respiratory tract. Once in blood, lead is distributed in the body, and 33% is accumulated in the liver. Since this organ is a very active metabolic tissue that stores and biosynthesizes nutrients, the alteration of these activities could be expected after the exposure. We decided to explore the morpho-functional repercussion of inhaled Pb in the liver determined histologically and by determining the serum concentration of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). A group of thirty male mice (CD1 strain) were exposed to (0.1M) lead acetate for one hour twice a week for 4 weeks. The control group inhaled saline, following the same protocol. Inhalation was carried out according to Fortoul et al., (2005). Each week, three animals were sacrificed from each group; the liver was processed for histological evaluation (H & E and Mason). One month after the exposure, a blood sample was drawn for measuring AST and ALT as indicators of liver damage.

An inflammatory infiltrate with neutrophils and lymphocytes was observed since the first week in the exposed mice and the presence of numerous granulomas was notorious in the same group. Masson stain did not show increase in fibrotic tissue. A significant increase in AST was detected.

Our results indicate that inhaled lead induces acute liver damage with unspecified changes. There was only other report with an inhaled metal mixture that reports necrosis and periporal infiltrate (Mani et al., 2007). Pb is still in the air in some cities atmosphere, and the literature is scarce about this topic. Further analysis is needed to evaluate the impact that inhaled Pb has in organs such as the liver.

508 DNA-PROTEIN CROSSLINKING MEDIATED BY DEHYDROMONOCROTALINE IN VITRO.

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Pyrrolizidine alkaloids (PAs) occur in a wide variety of plant species and have been identified in over 6,000 plants. Exposures to some PAs can cause massive hepatotoxicity, including hemorrhagic necrosis, hepatic megalocytosis and veno-occlusion, liver cirrhosis, nodular hyperplasia, and hepatic carcinomas. Hepatotoxicity induction by PAs has been suggested to be mediated by cytochrome P450 enzymes, through metabolic activation and reactions of electrophilic pyrrole metabolites. Monocrotaline is one of hepatotoxic PAs. Its reactive metabolite dehydromonocrotaline has been reported to cause DNA modification and protein covalent binding. Since dehydromonocrotaline molecule has two electrophilic centers which can react with nucleophiles, we speculated that the reactive metabolite may induce DNA-protein crosslinking. In the present study, we investigated the DNA-protein crosslinking mediated by dehydromonocrotaline using a plasmid DNA and BSA as model biomolecules. We found the exposure of a mixture of the DNA and BSA to
dehydromonocrotaline caused DNA-protein crosslinking. The degree of the crosslinking was concentration-dependent. In addition, we observed that DNA-protein crosslinking induced by dehydromonocrotaline was protected by the presence of glutathione. We hypothesize that DNA-protein crosslinking may be involved in monocrotaline-induced hepatotoxicity.

**509 LACK OF ORGANIC ANION TRANSPORTING POLYPEPTIDE 1A1 EXACERBATES CHOLESTATIC INJURY IN MICE.**

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Organic anion transporting polypeptide 1a1 (Oatp1a1) is predominantly expressed in livers of mice, and is thought to transport BAs from blood into liver. Because Oatp1a1 expression is markedly decreased in livers of bile-duct ligated (BDL) mice, we hypothesized that lack of Oatp1a1 will reduce the hepatic uptake of BAs and thus protect against cholestatic liver injury. To evaluate our hypothesis, we performed BDL surgeries in both WT and Oatp1a1-null mice. Surprisingly, all Oatp1a1-null mice died within four days after BDL, whereas all wild-type (WT) mice survived. Even one day after BDL, Oatp1a1-null mice had higher serum ALT and more severe liver injury than WT mice. Oatp1a1-null BDL mice had higher total BA concentration in livers than WT BDL mice, suggesting that lack of Oatp1a1 does not prevent BA accumulation in liver. Secondary BAs, which are produced by in testicular bacteria, dramatically increased in serum of Oatp1a1-null BDL mice, but not in WT BDL mice. In addition, Oatp1a1-null BDL mice have higher secondary BAs in livers than WT BDL mice. The increased secondary BAs in serum and livers of Oatp1a1-null BDL mice is not due to altered BA transport or BA conjugation in liver, because Oatp1a1-null BDL mice had similar mRNA of basolateral BA-uptake (Ntcp and Oatp1b2) and BA-efflux (Mrp3, Mrp4, and Oatpβ/β) transporters, as well as similar BA-conjugation enzymes (Fap5 and Baat) in livers than WT BDL mice. Transcription factors such as FXR, PXR, PPARα, and Nrfl2 play important roles in the cytoprotective response to cholestasis. One day after BDL, the mRNA expression of SHP, Cyp3a11, Cyp4a14, and Cyp4b1, which are target genes of FXR, PXR, PPARα, and Nrfl2, respectively, were increased in WT mice, but not in Oatp1a1-null mice. In conclusion, Oatp1a1 plays a unique role in the adaptive response to liver injury during obstructive cholestasis, and lack of Oatp1a1 exacerbates cholestatic liver injury in mice. (Supported by NIH grants DK-081461, ES009649, and ES-019487).

**510 BILE ACID REGULATION OF GENE EXPRESSION IN HUMAN PRIMARY HEPATOCYTE CULTURES.**

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Bile acids (BAs) are known to regulate their own homeostasis. This study was initiated to examine the effects of cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), lithocholic acid (LCA) and ursodeoxycholic acid (UDCA) on BA-synthetic enzymes and transporters in human primary hepatocyte cultures from 6 donors. Human hepatocyte cultures were obtained from the University of Pittsburgh, through the Liver Tissue Cell Distribution System. Hepatocytes were plated in 12-well collagen-coated dishes, and overlaid with Matrigel in supplemented William’s E media. Following a minimum 48-h recovery period, hepatocytes were treated with the 5 individual BAs with final concentrations of 10, 30, and 100 μM for 48 h. Total RNA was extracted from the hepatocytes with RNAzol Bee reagent, and reverse-transcribed with High Capacity RT kits, and the expression of genes of interest was determined by real-time PCR. All 5 BAs dramatically suppressed CYP7A1, the rate-limiting enzyme in BA synthesis, and dramatically increased the expression of FGF19, a major BA signaling molecule. The nuclear receptor farnesoid X receptor (FXR) and small heterodimer partner (SHP) were also upregulated, but to a lesser extent. The mRNA of the bile salt efflux pump (BSEP), as well as Ostα and Ostβ were significantly increased by BAs, whereas the sodium taurocholate co-transporting polypeptide (NTCP), a major BA uptake transporter, was slightly decreased. Of the 5 BA examined, UDCA was the least effective in producing gene expression changes. Among the 6 hepatocyte donors, marked individual variations in BA-regulated gene expression were noted, but the alterations were similar. In summary, primary human hepatocyte cultures responded similarly to intact mice, indicating similarities in the regulation of BAs in mice and humans (Supported by NIH grants DK-81461 and ES019487).

**511 COMPARISON OF NOVEL LUCIFERIN-1A2 SUBSTRATE AND PHENACETIN FOR MEASURING CYP1A2 INDUCTION IN HUMAN HEPATOCYTES.**

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Induction of drug metabolizing enzymes is one critical component in assessing drug-drug interactions. The process is controlled through drugs binding to receptors which regulate gene transcription. One mechanism entails the aryl hydrocarbon receptor (AhR), a transcription factor responsible for the up-regulation of phase I and phase II enzymes such as CYP1A1/2 and UGT1A1. The most common method to determine a drug’s induction potential through AhR-dependent pathway is to measure the activity of CYP1A2 in hepatocytes using phenacetin as a substrate after exposure to the drug and compare it to the basal rate. The quantification of the metabolism of phenacetin requires bioanalytical methods like UPLC/MS/MS which demands special equipment and expertise. In contrast, the P450-GloTM assay with the luminescence CYP1A2 substrate Luciferin-1A2 (6-methoxybenzo[d]thiazole-2-carbonitrile) is a simple and effective method to measure CYP1A2 activity requiring a plate reader and minimal expertise which will expedite data acquisition and simplify workflow. Herein, we describe the comparison of phenacetin and Luciferin-1A2 substrates for CYP specific and to determine induction of CYP1A2 in human hepatocytes using omeprazole as the inducer. The induction ratios were similar for most of the 16 donors tested for both lytic and non-lytic P450-GloTM Luciferin-1A2 substrate methods as compared to phenacetin with significant correlation found (P value < 0.0001, R2 of 0.7). This new CYP1A2 substrate is an alternative for measuring CYP1A2 activity to traditional probe substrates that require HPLC or MS methods.

**512 ALTERATION OF GENE EXPRESSION OF HEPATIC PHASE 1 ENZYMES INVOLVED IN THE BIOTRANSFORMATION OF PESTICIDES DURING GESTATION IN A MURINE MODEL OF TYPE 1 DIABETES.**

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Previous data from our laboratory indicated that pregnancy in mice alters the hepatic expression levels of phase 1 enzymes involved in the biotransformation of numerous pesticides. These naturally-occurring changes may result in a higher susceptibility to neurotoxins and increase the risk of cognitive and behavioral deficits. The goal of the present study was to assess how diabetes and pregnancy interact to modify the level of transcription of phase 1 enzymes. Nulliparous C57BL/6 mice were administered streptozotocin (STZ, 50 mg/kg/day for up to 6 doses, ip) in order to induce the hyperglycemia for at least 2 weeks prior to overnight mating with normoglycemic male mice. Livers were collected from vehicle-treated and STZ-treated, pregnant (GD14) and non-pregnant mice. Total RNA was extracted for QPCR quantification of various cytochrome P450, carboxylesterase (Ces) isoenzymes and nuclear receptors that regulate expression of these enzymes. Three patterns of transcriptional regulation were observed: 1) Pregnancy and STZ treatment significantly decreased (p<0.05) the mRNA expression of Car (≥55%), Ahr (≥25%), Cyp1a2 (≥20%), Peroxodase 1 (Pon1) (≥40%), Ces1d (≥50%), Ces1g (≥20%), Ces3 (≥40%). 2) Pregnancy, but not STZ treatment, reduced levels of Pparγ (≥60%), Cyp2c37 (≥50%), Cyp2c50 (≥75%), Cyp2c54 (≥50%), Cyp3a11 (≥50%), and induced Cyp3a16 (300%), Cyp3a41a/b (100%) and Cyp3a44 (150%). 3) STZ treatment alone induced Ces2c (100%), Ces2e (≥50%) and reduced Ces1c (≥35%). The remaining genes, Pparγ, Cyp3a13, Cyp2b10, Cyp2d22, Ces1e, and Ces2b, were unchanged by either STZ treatment or pregnancy. These findings suggest that diabetes and pregnancy differentially regulate the expression of critical enzymes that detoxify pyrethroid and organophosphate pesticides, which could alter in utero exposure of developing animals. Supported by NIH ES015991, ES050622, DK080774, and ES020522.
transformation and local exposure. Because hepatocyte subcellular fractions are widely used in the hepatic metabolism prediction, subcellular fractions of a reconstructed human skin model (full thickness model of Episkin) were used to test the skin metabolism. This strategy comes within the scope of l’Oreal effort in the development of new in vitro alternative methods to animal testing. In this work, we compared metabolic stabilities (half-life time) of xenobiotics in the presence of cutaneous and hepatic subcellular fractions. Results confirmed that cutaneous subcellular fractions had a lower catalytic activity level than hepatic fractions for numerous compounds but that biotransformations could occur at the skin level for peculiar chemicals. For example, the cutaneous stability of Nicardipine which is a positive control used for probing Cytochrome P450-dependent monoxygenase activities, confirmed that skin is much less equipped of these enzymes than liver. However, a cutaneous specificity could be observed with N-acetylation of p-aminobenzenc acid and N-acetyl-p-phenylenediamine compared with the liver. In conclusion, although skin metabolism towards xenobiotics is weaker than that of the liver, results presented confirmed the interest in studying their cutaneous metabolism because skin can be active in term of metabolism for specific compounds and the site of a first pass effect for such compounds.

514 REGULATION OF HEPATIC CYTOCHROME P450 BY VINCLOZOLIN IN PREGNANT RATS.

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Vinclozolin (V) is a systemic fungicide used for agricultural settings. V is non-enzymatically hydrolyzed to M1 and M2 metabolites, as well it is efficiently metabolized in male rats to several products by cytochrome P450 (CYP) 2A, 2B and 3A subfamilies. There is no available information about the effect of V on liver CYP regulation in rats during pregnancy. V exposure affects the male reproductive system during gestational morphogenesis, sexual maturation and fertility. V, M1 and M2 have antiandrogenic properties by inhibiting competitively the androgen receptor. The objective of this study was to determine the effect of V exposure on the hepatic CYP regulation during pregnancy. Pregnant Wistar rats were administered 150 mg/kg/d V in corn oil from gestational days 14 to 21 by gavage. Animals were sacrificed two hours after last dose. The liver was removed and processed to obtain microsomes to determine the protein content of different CYP isofoms and their enzyme activities. V exposure significantly increased maternal liver relative weight but did not affect liver total CYP content. V-induced protein content of CYP1A1 (8.3-fold), 2B1 (11.7-fold) and 1A2 (10.2-fold), while a decrease of 80% of CYP3A2 activity, confirmed that skin is much less equipped of these enzymes than liver. These results suggest that V may modulate hepatic CYP expression at different periods. Prediction of their chronic toxic effects and risk evaluation for health represent a major challenge. The well-differentiated HepaRG cell line is thought to be a poten in vitro surrogate to investigate human hepatic effects of chronic exposure to toxicants. HepaRG cells, composed of both hepatocyte-like and biliary-like cells, present most of the metabolic performances of primary hepatocytes up to 4 weeks. In this study, we adapted the classical culture conditions of HepaRG cells in order to be closer to the permanent aggression of the human liver by contaminant agents. Cytotoxicity was evaluated using the MTT test after daily exposure for 1 or 2 weeks of the hepatocyte-like population to low doses of about 10 chemicals, including the mycotoxin aflatoxin B1, polycyclic aromatic hydrocarbons, plasticizers, pesticides and flame retardants. We first checked the stability of the differentiated HepaRG hepatocytes over this period using several genes, including CYP3A4, CYP2B6 and aldolase B, as markers of differentiation. mRNA expressions remained stable during the two weeks as did CYP3A4 protein level and activity. Moreover, the 24 h acute toxicity of each compound was similar when tested at the beginning or at the end of the 2 week-period, indicating that HepaRG hepatocytes kept all their metabolic functions. Chronic studies showed cumulative cytotoxic effects of most of the tested compounds with decreasing IC50 between 24 h and 7 days. Prolonging the treatment up to 14 days kept on lowering significantly IC50 of endosulfan (120, 60, 18 μM) and aflatoxin B1 (1.8, 0.4, 0.15 μM) both metabolized by CYP3A4, of benzo[a]pyrene (350, 10, 7 μM) and dimethylbenzanthracene (300, 18, 6 μM) metabolized by CYP1A1 and 1B, and to a lesser extent those of bisphenol A (105, 85, 77 μM), di(2-ethylhexyl) phthalate (300, 85, 60 μM) and tetrabromo bisphenol A (68, 37, 25 μM). Our results validate the hepatocyte-like cells as a suitable experimental model allowing toxicity evaluation of low doses after long-term exposure.

515 COMPARATIVE ASSESSMENT OF AUTOMATED ANALYSIS USING COMPUTER ASSISTED PATHOLOGY (CAPSTM) HISTOPATH™ SOFTWARE.


A proof of concept (POC) study was performed to assess the ability of CAPSTM (Computer Assisted Pathology System) HistoPath™ software to: 1) differentiate normal/non-normal in hepatocytes as a gold standard, and 2) interpret the hepatocyte-like cells as a suitable tool for assessing the severity of the change. Five groups (1 control / 4 treated groups) of 6 rats per group were used in the assessment. An additional 23 rats were used as historical controls. Slides were scanned (Aperio XT ScanScope) and resultant images analyzed using Histopath™ version 1.0.1. Slides were also independently analyzed by 3 Charles River pathologists (PT). CAPSTM normal/non-normal designations (83%) and non-normal reasons (86%) were similar to the PT rating, 77% to 93% and 71% to 100%, respectively. CAPSTM severity grade (rankings) were Mitotic Figures (MF) 100%, Vesculation (VES) 100%, Disrupted Tissue (DT) 97%.

516 CHRONIC TOXICITY OF ENVIRONMENTAL CONTAMINANTS IN HUMAN HEPAR HEPATOCYTES.


Humans are continuously exposed to low doses of contaminants for prolonged periods. Prediction of their chronic toxic effects and risk evaluation for health represent a major challenge. The well-differentiated HepaRG cell line is thought to be a potent in vitro surrogate to investigate human hepatic effects of chronic exposure to toxicants. HepaRG cells, composed of both hepatocyte-like and biliary-like cells, present most of the metabolic performances of primary hepatocytes up to 4 weeks. In this study, we adapted the classical culture conditions of HepaRG cells in order to be closer to the permanent aggression of the human liver by contaminant agents. Cytotoxicity was evaluated using the MTT test after daily exposure for 1 or 2 weeks of the hepatocyte-like population to low doses of about 10 chemicals, including the mycotoxin aflatoxin B1, polycyclic aromatic hydrocarbons, plasticizers, pesticides and flame retardants. We first checked the stability of the differentiated HepaRG hepatocytes over this period using several genes, including CYP3A4, CYP2B6 and aldolase B, as markers of differentiation. mRNA expressions remained stable during the two weeks as did CYP3A4 protein level and activity. Moreover, the 24 h acute toxicity of each compound was similar when tested at the beginning or at the end of the 2 week-period, indicating that HepaRG hepatocytes kept all their metabolic functions. Chronic studies showed cumulative cytotoxic effects of most of the tested compounds with decreasing IC50 between 24 h and 7 days. Prolonging the treatment up to 14 days kept on lowering significantly IC50 of endosulfan (120, 60, 18 μM) and aflatoxin B1 (1.8, 0.4, 0.15 μM) both metabolized by CYP3A4, of benzo[a]pyrene (350, 10, 7 μM) and dimethylbenzanthracene (300, 18, 6 μM) metabolized by CYP1A1 and 1B, and to a lesser extent those of bisphenol A (105, 85, 77 μM), di(2-ethylhexyl) phthalate (300, 85, 60 μM) and tetrabromo bisphenol A (68, 37, 25 μM). Our results validate the hepatocyte-like cells as a suitable experimental model allowing toxicity evaluation of low doses after long-term exposure.

517 CYCLOSPORINE A AND PALMITATE TREATMENT SYNERGISTICALLY INDUCE CYTOTOXICITY IN HEPG2 CELLS.

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The immunosuppressive compound Cyclosporin A (CsA) has been used for treating autoimmune diseases and in organ transplantation. However, severe side effects, such as nephrotoxicity, cardiotoxicity and hepatotoxicity have been attributed to CsA treatment. Cardiac and renal transplantation is often associated with the development of hyperlipidemia and obesity, probably due to immunosuppressive therapy. Elevated levels of free fatty acids have been linked to the etiology of metabolic syndrome, nonalcoholic fatty liver and steatohepatitis. The contribution of free fatty acids to CsA-induced toxicity is not clear. In this study we explored the effect of palmitate on CsA-induced toxicity in HepG2 cells. CsA induced little cytotoxicity in HepG2 cells as measured by ATP depletion and LDH release. Co-treatment with palmitate and CsA result in marked increase in cytotoxicity, and the responses are CsA and palmitate dose-dependent. Palmitate and CsA treatment also lead to synergistic induction of caspase-3 activity, suggesting that induction of apoptosis may contribute to the cytotoxicity. At sub-lethal dose, CsA reduced mitochondrial respiration, which is further exacerbated by the presence of palmitate. Inhibition of c-Jun N-terminal kinases (JNK) activity attenuated palmitate and CsA induced cell death, suggesting that JNK activation plays an important role in mediating palmitate/CsA toxicity. This novel finding of the synergized cytotoxicity induced by palmitate and CsA suggest that patients with underlying diseases that elevate free fatty acids may be predisposed to CsA-induced toxicity. Furthermore, hyperlipidemia/obesity resulting from immunosuppressive therapy may aggravate CsA-induced toxicity and worsen the outcome in transplant patients.
518 COMPARISON OF RAT AND DOG HEPATOCYTE-KUPFFER CELL COCULTURES AS A MODEL OF DRUG-INDUCED HEPATIC MICROGRANULOMA.

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During the conduct of routine safety studies, kupffer cell hypertrophy and subsequent microgranulomona formation occurred in dog liver after single and repeat oral administration within a series of drug candidates. The finding was not associated with parenchymal hepatocellular injury, was not monitorable by routine clinical pathology, and was not observed in repeat dose rat studies. The histopathology suggested a foreign body-type response to indigestible PAs+ material of which a component was likely test article. A primary hepatocyte/nonparenchymal cell coculture system of rat and dog was established to provide mechanistic insight and investigate the species-specificity of this finding, as well as to develop a model screening tool for potential back-up molecules. There was no difference between species in cellular toxicity in hepatocyte single culture or coculture systems. Uptake, however, of radio-labelled drug into either hepatocytes or nonparenchymal cells from rat and dog demonstrated pronounced hepatocellular uptake in rats and conversely, preferential uptake by nonparenchymal cells in dogs. This observation may explain the observed species-specific in vivo finding. Additionally, the system was used to evaluate the response to compounds in the series that did not have this finding in dog or rat, which further supported the hypothesis that increased uptake by nonparenchymal cells is a risk factor for microgranuloma formation. The presented approach demonstrates the utility of in vitro systems to understand and potentially de-risk, in vivo safety findings, as well as to provide a screening paradigm that will reduce animal use.

519 COMPARISON OF TWO CELL MODEL SYSTEMS, PRIMARY HUMAN HEPATOCYTES AND HIpsc-DERIVED HEPATOCYTES TO DETERMINE THE HEPATOTOXICITY OF THREE CANDIDATE DRUGS DEVELOPED FOR RHEUMATOID ARTHRITIS.

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In vitro hepatocyte culture serves as a very useful model system to detect potential hepatotoxicity without sacrificing animals. However, current in vitro model systems have limitations. Primary human hepatocytes (PHH) are known to exhibit donor-specific phenotypic variability and availability is too limited for high throughput assay. Immortalized hepatoma cell lines, such as HepG2 and HepaRG, offer more reproducible and homogenous culture systems, but interpreting the toxicant sensitivity in cells of tumor origin poses a considerable problem. Using human induced pluripotent stem cell (hiPSC)-derived hepatocytes is an important new tool which offers unlimited supply of euploid cells from single donors. Here, we tested the hepatotoxicity of three internal candidate drugs developed for the treatment of rheumatoid arthritis. In vivo, two of these compounds induced canine liver toxicity, while one compound showed no toxicity. This toxicological profile of three compounds is recapitulated in vitro, both in cultures of PHH and the hiPSC-derived hepatocytes. IC50 values of the three compounds were determined by 24 hour ATP assay (>50, 20, 14μM by PHH; >50, 24, 13μM by iPSc-derived HC) to be nearly identical. This pilot study shows that despite some difference in metabolism (cytochrome P450 and Phase II enzyme levels) between PHH and hiPSC-derived hepatocytes, stem-cell-derived hepatocytes may provide an invaluable model system for preclinical drug safety study and disease modeling.

520 URINARY BIOMARKERS DETECT RENAL DAMAGE IN HYPERTENSIVE AND OBSESE RATS.

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Hypertension and obesity are major risk factors for the development of kidney disease. Sensitive biomarkers are needed to detect renal damage at the earliest stages. The objective of this study was to determine if a suite of acute kidney injury biomarkers can be used to track the progression of nephropathy in an animal model of hypertension and obesity. Male spontaneously hypertensive obese (SHROB) and lean SHR (SHR-lean) rats were used to track the progression of kidney damage from 18 to 39 weeks of age. Body weight, blood pressure, and urinary biomarkers (albumin, NAG, Kim-1, NGAL, osteopontin, GST-π, fibrinogen and clusterin) were assessed at 3-week intervals. Weight gain was much greater in SHROB rats than in SHR-lean rats. Systolic and diastolic blood pressures were elevated throughout the study in both SHROB and SHR-lean rats with no significant difference observed between the two groups. Blood creatinine, BUN and plasma cholesterol and triglyceride levels were significantly increased in SHROB rats compared to SHR-lean rats at the end of study. The severity of renal lesions involving tubules, interstitium and glomeruli was graded on a scale of 0 to 5 (0=normal histology, 2=1-25%, 3=26-50%, 4=51-75%, and ≥76-100% of tissue affected). SHROB rats showed a higher overall chronic nephropathy score (4.9) than SHR-lean rats (3.9). Urinary albumin and fibrinogen were elevated at week 30 and remained elevated through week 36 in SHROB rats; however, an increase in albumin was not observed until week 36 in SHR-lean rats. Urinary NAG, clusterin and osteopontin levels were elevated at week 36 in SHROB rats only. There were no increases observed throughout the study in urinary levels of Kim-1, NGAL, RPA-1, GST-π and GST-Yb1 in both strains. In conclusion, urinary biomarkers such as albumin, fibrinogen, NAG, clusterin, and osteopontin, may be useful as tools to track the progression of chronic kidney injury associated with hypertension and obesity. (Funded in part by a FDA Office of Chief Scientist Challenge Grant).

521 AGE-DEPENDENT VARIATIONS IN BASELINE SERUM CARDIAC TROPONIN I LEVELS: A CROSS-SPECIES COMPARISON.

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Changes in the serum levels of cardiac troponin I (cTnI) are used as a specific indication of myocardial injury. Recently, factors such as strain, sex and arterial pressure have been found to affect baseline levels of cTnI in rats. The current study utilized the ultrasensitive Erenna immunoassay to determine whether animal age might also influence cTnI concentrations. Blood samples were collected from Sprague Dawley rats at 10 (n=5), 25 (n=7), 40 (n=11) and 80 (n=10) days of age. Blood was also obtained from Patas monkeys at birth (n=2), at two months (n=1) and one year of age (n=1). Samples of fetal and adult equine, porcine and bovine serum were also analyzed. The mean (±SD) concentrations of cTnI were 37.7±10.14±4.9, 7.3±4.8 and 5.8±3.1 pg/ml for 10, 25, 40 and 80-day-old rats, respectively. Examination of 10 day old rat hearts showed features such as hypercellularity and irregularly-sized nuclei with a moderate number of myocytes undergoing mitosis and apoptosis that were not apparent in the myocytes of 80-day rat hearts. The cTnI concentration in 2 monkeys at birth were 177 and 725 pg/ml compared to 8.29 pg/ml in the one year-old monkey. The levels of cTnI detected in fetal equine and porcine serum were considerably higher than those found in adult equine and porcine serum samples. Likewise, the levels of cTnI in fetal bovine serum were extremely high (>2,000 pg/ml) compared to adult caprine and laprine serum (2.9-2.7 pg/ml). These results indicate that levels of cTnI vary with higher cTnI with higher cTnI with higher C on and lower C on with lower C on, which could affect use and interpretation of cTnI in certain types of species. The age-related differences appear to be consistent across animal species.

522 TRANSCRIPTOME AND METABOLOME ANALYSIS REVEALS MECHANISM-LINKED BIOMARKER CANDIDATES IN CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY.

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Transcriptome and metabolome analyses were adopted to investigate the mechanisms underlying hepatotoxicity induced by carbon tetrachloride (CCH4), a highly toxic chemical agent used to elicit experimental liver damage. CCH4 (50 or 2000 mg/kg) or vehicle (corn oil) was administered to 8 to 9-week-old male rats (15 animals per dose group) via oral gavage. Some animals received a single dose and were sacrificed 6 and 24h after that single dose. Remaining animals received a total of 3 once daily doses and were sacrificed 24h after the 3rd dose. Five animals per group were euthanized via carbon dioxide hypoxia and immediately necropsied. Serum and urine samples were collected at each time point. NMR, LC/MS, LC/MS/MS, and GC/MS were utilized for metabolome analysis of the serum and
urine, and DNA microarrays were used for transcriptome analysis of liver tissue. Pathway analysis data revealed increased hepatic oxidative stress, increased bile acid secretion, a shift of energy metabolism from fatty acid beta-oxidation to glycolysis, and the microarray results showed increased progression of hepatic synthesis of triglycerides and cholesterol ester following the high dose. These results were consistent with histopathological indices in the liver of high dose animals such as necrosis and lipid accumulation, which were observed as early as 6 h. In addition, a decrease in serum arginine and urine 2-oxoarginine had a statistically significant correlation with serum alanine aminotransferase activity at 24h. Thus, an integrated analysis of transcriptome and metabolome profiles may provide a better understanding of the mechanisms and provide mechanistic-linked biomarker candidates of liver injury.

**523 ACUTE KIDNEY INJURY AND AGING INFLUENCE DIAGNOSTIC VALUE OF URINARY BIOMARKER-TO-CREATININE RATIO IN RATS.**

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 Recent research has revealed several useful urinary biomarkers of acute kidney injury (AKI). For adequate evaluation of altered urinary biomarkers, it is necessary to consider the influence of varied urine flow rate (UFR). Calculation of the excretion rate of a urinary biomarker (UFR-correction) is a gold standard for the correction. An alternative method is to calculate the urinary biomarker-to-creatinine ratio (Ucr-correction). To date, the correlation between correction values has been examined only in steady state such as chronic kidney disease, and the urinary biomarkers examined have been limited to proteinuria and albuminuria. The purposes of this study were to investigate the influence of AKI on Ucr-correction values of various urinary biomarkers, the influence of aging on Ucr-correction, and the backgrounds underlying the relationship between the two correction values. AKI was induced by cisplatin, gentamicin, carboplatin, bomoethymol, cyclosporin A, doxorubicin or puromycin aminonucleoside in rats. Comprehensive measurement of ten urinary biomarkers revealed larger amplitude increases in Ucr-correction values compared to UFR-correction values in AKI. Moreover, Ucr-correction showed higher diagnostic power than UFR-correction in receiver operating characteristic curve. We further observed a decrease in Ucr excretion in AKI accompanied by reductions in creatinine clearance (Ccrt) and mRNA expression of the renal organic cation transporter-2 known as a transporter for creatinine. Regarding aging, UFR-correction values were constant while Ucr-correction values showed age-dependent decreases, which were accompanied by decreases in Ucr excretion and Ccrt. In conclusion, Ucr-correction could over- or under-estimate the renal condition in AKI and aging. Thus, we should take into consideration of these backgrounds when using Ucr-correction.

**524 PROTEIN BIOMARKER PROFILES DISCRIMINATE BETWEEN SENSITIZING AND IRRITATING CHEMICALS IN CELL-BASED ASSAYS.**

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Introduction: Common environmental and industrial small molecular weight chemicals can cause allergic contact dermatitis. Current safety testing relies upon animal testing which will shortly be banned by the European Union and so alternative in vitro tests are urgently needed. In this study, we investigated whether protein expression signatures in MUTZ3 as a surrogate dendritic cell model can be used as biomarker read-outs to discriminate between chemical sensitizers and irritants.

Materials and Methods: MUTZ3 cells were exposed for 24 h to a set of training chemicals comprising 2 respiratory, 5 contact sensitizers, 3 non-sensitizers and vehicle controls. Cell extracts and cell culture medium were collected for proteomics analysis. Protein expression profiling was performed with isobaric tandem mass tag (TMT®) labelling for relative and absolute mass spectrometric quantitation.

Results: Using univariate statistics and linear prediction models (partial least square regression, PLS) we have developed a protein signature for testing contact and respiratory sensitizers comprising 130 proteins that are involved in inflammatory and stress response pathways. A subset of these proteins can be measured in cell culture medium by standard immunoassays (ELISA), and could be combined to a multi-marker panel with established protein markers. To verify the protein signature we currently developed multiplexed SRM (selected reaction monitoring) mass spectrometric assays which allow analyzing the 10 best performing biomarkers in one measurement.

Conclusions: Since a multi-marker panel allows measuring a combination of biomarkers it could provide additional information on pathways triggered by unknown chemicals.

**525 QUANTITATIVE ASSESSMENT OF ACETYL-CARNITINE EFFECTS ON ANESTHETIC-INDUCED NEURONAL DEATH BY MICROPET/CT IMAGING.**

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Recent reports indicate that combined administration of ISO and N2O triggers neuronal apoptosis in postnatal day (PND) 7 rats. Because high-resolution positron emission tomography (microPET) can provide in vivo molecular imaging at sufficient resolution to resolve neuronal activities in the rat brain, it has been proposed as a minimally invasive method for detecting apoptosis in the brain using the tracer [18F]-labeled DFNSH. In this study, the effect of ISO and N2O on the uptake and retention of [18F]-DFNSH in the brains of different aged rats and the potential protective effect of acetyl-l-carnitine (ALC) on anesthetic-induced neuronal cell death were investigated using microPET/CT imaging. On PND 7, rats pups in the experimental group were exposed to a mixture of 70% N2O/30% oxygen and 1% ISO for 8 hours with or without ALC and control rat pups were exposed to room air only. On PNDs 14, 21 and 28, [18F]-DFNSH (18.5 MBq) was injected i.p. and thirty minutes later microPET/CT images were obtained over 90 minutes. Radiolabeled tracer accumulation in a region of interest in the frontal cortex was correlated with standard uptake values (SUVs). In PND 14 rats the uptake of [18F]-DFNSH was significantly increased in gaseous anesthetically-treated rats. Additionally, the duration of tracer wash-out was prolonged in the gaseous anesthetic-treated animals. The uptake of the tracer was attenuated in brains of rats when they have been treated with ALC (100 mg/kg). No significant difference was found in radiotracer uptake in the frontal cortex of the brains of PND 21 and 28 rats compared with same aged controls. This result, which is consistent with our previous TUNEL studies, demonstrates that enhanced apoptotic effects are apparent during early developmental stages (PND 7-14) and that no significant neuronal apoptosis occurs on or after PND 21. Acetyl-l-carnitine effectively blocks the neuronal apoptosis caused by inhalation anesthetics in the developing rat brain.

**526 METABOLIC ANALYSIS OF ARGinine METABOLISM IN RAT PLASMA FOR AN INDICATOR OF HEPATIC INJURY.**


To clarify the relationship between arginine metabolism and hepatic injury, metabolic profile involved in arginine metabolism were investigated by metabolomic analysis in the rat hepatic injury models by monocrotaline (MCT), concanavalin A (ConA), and t-naptophyl isothiocyanate (ANIT), or the non-hepatic injury model by tetramethyl-p-phenylenediamine (TMPD). Male F344 rats were treated with a single dose of MCT (0, 30, 100 or 300 mg/kg, p.o.), ConA (0, 3, 20 or 30 mg/kg, i.v.), ANIT (0, 25, 50 or 100 mg/kg, p.o.) or TMPD (0, 3 or 9 mg/kg, p.o.), and plasma, serum or liver samples were obtained at several time points for metabolomic analysis by liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry, blood chemistry or histopathology, respectively. MCT, ConA and ANIT induced hepatocellular necrosis accompanied by increased plasma arginine and decreased plasma arginine levels in dose- and time-dependent manner, but TMPD treatment did not induce hepatic injury or changes in arginine levels even though ALT elevation was noted. A close correlation (r = -0.74 to -0.80, N = 60) between increased ALT and decreased plasma arginine was observed in line with decreased arginine levels in the arginine metabolic pathway. A subset of these proteins can be measured in cell culture medium by standard immunoassays (ELISA), and could be combined to a multi-marker panel with established protein markers. To verify the protein signature we currently developed multiplexed SRM (selected reaction monitoring) mass spectrometric assays which allow analyzing the 10 best performing biomarkers in one measurement.

Conclusions: Since a multi-marker panel allows measuring a combination of biomarkers it could provide additional information on pathways triggered by unknown chemicals.
plasma showed a high activity of arginase. These results indicate that arginine metabolism, particularly arginine to ornithine pathway is mainly involved in the cyclo- pound-induced hepatic injury, and plasma arginine and ornithine would be specific indicators for hepatic injury.

Acetaminophen (APAP) overdose is the foremost cause of acute liver failure in the West. The drug is metabolized to the electrophile N-acetyl-p-benzoquinone imine (NAPQI), which binds and depletes glutathione (GSH). The initiating event in the mechanism of injury is binding of NAPQI to proteins. Early studies found that loss of 70% of baseline GSH was necessary for protein binding and this has become a paradigm in electrophile-protein adduct toxicity. Based on this, it has been suggested that APAP-protein adducts could be measured clinically as a novel serum biomarker to confirm or exclude APAP as cause of liver injury when etiology is unknown. However, more recent data from therapeutic doses in humans have challenged that idea (Heard et al. BMC Gastroenterol. 2011). To study this in more detail, we treated mice with different doses of APAP and measured plasma enzymes, histology, liver GSH, and liver and plasma adducts between 0.5 and 24h. At toxic doses (150-600 mg/kg), liver GSH was rapidly depleted (0.3 ± 0.1, umol/g liver 0.5h after 300 mg/kg vs. 4.1 ± 0.2). As expected, increased liver adducts paralleled GSH loss. Interestingly, plasma adducts were easily detected in these mice within 0.5h of treatment and peaked at 3h (9,568 ± 1,399 ng/mL after 300 mg/kg), before the onset of necrosis. At a subtoxic dose (75 mg/kg), GSH levels were depleted (0.6 ± 0.1 umol/g at 0.5h vs. 4.5 ± 0.2 in controls) but quickly recovered. Importantly, plasma adducts were detected (95 ± 28 ng/mL at 0.5h) without enzyme release at any time point. Finally, while a therapeutic dose (15 mg/kg) caused only a minor decrease in GSH, liver adducts were readily detectable. Conclusion: Contrary to early reports, GSH depletion is not required for APAP-protein binding. Moreover, protein adducts were detectable in mouse plasma at non-toxic doses of APAP. Caution should be used in interpretation of plasma adducts as a biomarker of APAP overdose.

MicroRNAs (miRs) are short 20-22 nucleotide RNA molecules that are involved in the fine tuning of gene expression at the translational level. Numerous miRs are expressed in a tissue/organ specific manner and thus there has been considerable interest in miRs as specific biomarkers for organ toxicity. Liver specific/enriched miR-122 has been shown to increase in the serum of rats by thousands of fold when treated with acetaminophen. In an attempt to verify and potentially discover novel miRs associated with liver toxicity we conducted a serum miR profiling experiment using rats treated with thioacetamide (results in hepatocellular necrosis), alyl alcohol (results in hepato-cellular necrosis and biliary effects including bile duct hyperplasia (BDH)) or alpha naphthylisothiocyanate (ANIT) which results in BDH). Bile duct hyperplasia can be difficult to detect in 4 day rat studies and identification of a biomarker for BDH could help identify compounds associated with this liability. Serum miR profiling was conducted using TLA cards and two independent statistical analyses of the data were conducted. miRs increased in the serum were compared against specific pathology findings (BDH vs necrosis) and confirmed by single Taqman assays. Data analysis indicated that several miRs were increased in the serum of ANIT treated rats, but of these, only miR-34c was detected in the liver tissue (as well as brain and testes). Serum of rats treated with other BDH inducing compounds were examined for increases in miR-34c but, no consistent correlation between BDH and increases of miR-34c could be established. Analysis of our data indicates that liver specific/enriched miRs-122 and -193 were increased in the serum of rats in a dose dependent manner where hepatocellular necrosis was noted. Candidate miRs for other pathologies including cardiac (miR-499), testicular/brain (384-5p) and intestinal damage (miR-194) were identified, but require additional validation.

Urinary renal biomarkers (URB) are important new tools with which to monitor diagnosis, progression and recovery of drug-induced acute kidney injury (AKI). Drug-induced crystalluria is a problematic toxicity for which URMs could provide valuable translational tools to monitor compound safety in clinical development. This study investigated the URB profile associated with onset and development of crystalluria-induced AKI. Ethylene glycol (EG) is a well characterized nephrotoxicant resulting from formation and accumulation of calcium oxalate crystals in proximal tubules leading to AKI, and co-treatment with aluminum citrate (AC) can mitigate EG nephrotoxicity. Male Wistar rats (n=6/group) received (po) water (10 ml/kg; controls), EG (6 g/kg), or EG + AC (0.2 mmol/kg) and were placed in metabolic cages. Urine was collected at 12 h intervals for 72 h and analyzed for 8 renal biomarkers: cGST, GSTYb1, renal papillary antigen (RPA), clusterin, alubimin, lipocalin, osteopontin and KIM-1. Both GST isoforms were transiently increased at 12 h in EG and EG + AC-liver citrate treated rats indicative of an early non-segment specific injury. Clusterin, lipocalin and KIM-1, all markers for proximal tubule injury, were increasing in EG-treated rats compared to controls between 36 and 48 h and these increases were attenuated by treatment with AC. RPA was increased in both EG and EG + AC-treated animals compared to controls starting at 48 h and is likely due to the sheer stress of crystals passing through the distal portion of the nephron at the later timepoints. This study shows that calcium oxalate crystalluria resulting from EG treatment produces a unique toxicodynamic renal biomarker profile with dimensions of both treatment and time, and offers potential as a surrogate for repeated histologic monitoring to detect transient changes in progression of acute kidney injury.
Neutrophil gelatinase-associated lipocalin (NGAL) is a 178 amino acid protein secreted from specific granules of activated neutrophils. Its synthesis is also induced in epithelial cells during inflammation. Serum NGAL has been reported to be a useful biomarker for detection of inflammation and tissue damage, including kidney injury. Tissue inhibitor of metalloproteinase 1 (TIMP-1) is another marker of inflammation as an inhibitor of the metalloproteinases (MMPs). TIMP-1 is a 194 amino acid secreted glycoprotein that is widely expressed in many cells such as fibroblasts, endothelial cells, vascular smooth muscle cells and monocytes. The release of TIMP-1 is considered to be a modulator of an inflammatory response (anti-inflammatory). TIMP-1 has been proposed as a biomarker for many tissue injuries, including kidney and vascular toxicity. In rodent studies, NGAL and TIMP-1 have been employed as safety biomarkers to assess toxicological risk in the early stages of drug development. Their use has, however, been limited by the volume requirements for current single-plex assays combined with a loss of analyte sensitivity in multi-plex formats. We have identified an immunoassay platform that can provide sensitivity with microliter quantities of sample for quantitation of serum NGAL and TIMP-1 concentrations. The Gyros® immunoassay platform facilitated rapid development of assays with broad dynamic ranges, good sensitivity at low mini-plex and TIMP-1 serum samples may become valuable tools for monitoring development of assays with broad dynamic ranges, good sensitivity at low mini-plex and TIMP-1 concentrations. The Gyros® immunoassay platform facilitated rapid development of assays with high resolution accuracy and sensitivity to low minimum required dilutions, and tight precision. The Gyros assays developed for rat NGAL and TIMP-1 serum samples may become valuable tools for monitoring drug toxicity and safety assessments in the preclinical setting.

BMP particularly the 44:12 (22:6/22:8) lipid moiety is suggested to be a urinary biomarker of phospholipidosis (PLD). The purpose of this work was to characterize lysosomal phospholipid composition, with emphasis on phosphatidyglycerol (PG) and BMPs, in fluoxetine-induced PLD. Alveolar macrophages were isolated from male rats dosed with fluoxetine (30 mg/kg; 3 days), and lipids from isolated lysosomes were analyzed by high resolution accurate mass LCMS. PGs were more abundant than BMPs in control rat lysosomes, with PG (32:0) the predominant species (normalized value of 911 units/mg protein). The most abundant BMP (40:8) had a normalized value of 13, whereas BMP 44:12 was 1.6. In fluoxetine treated rats, all PGs and BMPs increased 1.7 to 5-fold in lysosomes. PG (32:0) remained most abundant, increasing 1.7-fold. BMP (40:8) increased 3.3-fold, whereas BMP (44:12) increased 1.9-fold. Lysosomal phosphatidylincholines (PCs) also increased 2-5 fold after fluoxetine treatment. In urine, BMP (44:12) was most abundant, increasing 3-fold with fluoxetine treatment, whereas BMP (40:8) increased approximately 10-fold. Excretion of other BMPs (38:6, 36:4, 42:8 and 40:6) increased 20 to 50-fold with early PLD. In contrast, urinary excretion of PCs was unchanged at 3 days. These results demonstrate that numerous BMPs increase in both urine and the subcellular target site for PLD (lysosomes), and suggest that BMPs other than or in addition to 44:12 may be candidate biomarkers of PLD.

Drug-induced kidney injury (DIKI) accounts for 25% of incidences of acute renal failure, and approximately 5% of failures of new drugs. The predictive safety testing consortium (PSTC) has been developing a set of urinary biomarkers and examining their utility as preclinical biomarkers of DIKI. These protein biomarkers demonstrate improved sensitivity over clinical standard-of-care biomarkers serum creatinine (SCr) and blood urea nitrogen (BUN) in the detection and monitoring of DIKI progression. An important feature of any biomarker of DIKI, however, is the ability to distinguish drugs that cause injury from those that impact kidney function without inducing injury. To determine whether this new generation of DIKI biomarkers is sensitive to altered renal function, we treated Wistar rats (8/sex/group) with enalapril (ACE inhibitor, 20 mg/kg/d), acetazolamide (carbonic anhydrase inhibitor, 10 mg/kg/d), probenecid (organic anion transporter inhibitor, 300 mg/kg/d) and cimetidine (organic cation transporter, 100 mg/kg/d) for 14 days with a 7 day recovery period. Renal histology and urinary protein biomarkers of kidney injury were monitored over the dosing and recovery periods, and revealed a trend towards increases in the distal tubule injury marker mu-GST in response to enalapril, acetazolamide, probenecid, and cimetidine, all in the absence of histological injury. In contrast, levels of KIM-1, clusterin, alpha-GST, albumin, lipocalin-2, and osteopontin are all relatively stable in response to these renally-active but non-nephrotoxic drugs. This work suggests that KIM-1, clusterin, alpha-GST, albumin, lipocalin-2, and osteopontin, but perhaps not mu-GST, distinguish between drug-induced changes in renal nephron function and injury in rats, enhancing their utility as DIKI biomarkers.

Biomarkers of cardiac toxicity are widely used in clinical settings to assess patient cardiovascular health, and a newer generation of cardiac biomarkers have recently been developed for use in rodents; however, there are no validated biomarkers suitable for use in NHP. We evaluated a variety of available rodent and human biomarkers in two NHP species: African green monkeys and Rhesus macaques. Endpoints included electrocardiographic (ECG) and Doppler echocardiographic (Doppler Echoscale Discovery® (MSD) rat and human muscle injury panels, and histopathology. Two animals/sex were administered a single subcutaneous (sc) injection of isoproterenol (IPIT: 4 mg/kg). Blood samples were collected pre-study and at 1, 4, 24, 48 and 72 hr postdose. Blood was evaluated with a Triage® card kit and MSD muscle injury kits (rat: cTnl, cTrt, FABP3, My3, cTnl; human: Tnl, CKMB). ITP production increases significantly in cardiac biomarkers: cTnl, cTrt, FABP3 and My3 were increased 150-, 1285-, 68- and 619-fold, respectively, over predose levels 24 hr after dosing in African green males, with similar results seen in females. Results in Rhesus macaques were similar, but less pronounced, with males at 24 hr showing increases of 13-, 10-, 3- and 25-fold over predose, respectively, and females had similar results. The peak response time appears to be either 4 or 24 hr for each parameter, though most parameters remained elevated 72 hr postdose. Human Tnl and CKMB were far less responsive with increases of only 0.9- to 4.4-fold for all parameters in both species at any time point. The traditional human Triage® panel showed modest activity for Troponin I and sporadic responses for myoglobin, but the sensitivity and reproducibility were far less than that seen with the MSD biomarkers. These results indicate that the MSD rat muscle injury panel provides excellent sensitivity for assessing cardiac effects in two different NHP species. Work supported by NIAID Contract N01-AI-70043.

During early preclinical development, a new drug candidate induced nephrotoxicity in rats following daily p.o. administration for 7 days. Renal lesions consisted of scattered nuclear enlargement in tubular cells at low-dose and tubulopathy (mixture of dilatation, degenerative and regenerative changes) at higher doses. Traditional serum kidney parameters remained silent, thus precluding their use as surrogates for further non-clinical or clinical studies. To identify early biomarkers, a mechanistic study in male rats was performed at low and high doses with sequential examination at 6h, 24h, 3 and 7 days combining kidney histology and transcriptomic profiling in the cortex and medulla, urinary and serum metabolomics as well as urinary kidney biomarkers. No histological kidney lesions were observed at 24h, while tubular changes were seen after 3 or 7 days of dosing with time-related severity scores. The transcriptomic fingerprints of treated and control rats were clearly different at 6h with a deregulation of genes not specifically associated with kidney injury, in contrast, a number of gene expression changes commonly associated with kidney toxicants (e.g. Calb1, Haverl/km1, clusterin, TIMP1, Sppl/osteopontin, Atf3) as well as overt changes in urinary microalbumin, KIM-1 and clusterin were
observed at 24h or after 3 days when histological lesions were either absent or minimal. In parallel, urinary metabolomics evidenced obvious and time-related changes in endogenous metabolites which could act as additional biomarkers for the observed toxicity e.g. citric acid cycle members in urine and serum phospholipids. A cross-parameter analysis identified a discriminating pattern of omics and urinary biomarkers considered as more sensitive than early histology alone for prediction of the observed toxicity. This combined analysis will add greater confidence in the selection and prioritization of back-up candidates during early preclinical development.

**HIGH CORRELATION OF NOVEL SKELETAL MUSCLE INJURY BIOMARKERS TO CK ACTIVITY IN HUMAN VOLUNTEERS AFTER PROLONGED EXERCISE.**

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Creatine kinase (CK) is a widely used blood based biomarker of skeletal muscle injury in humans and preclinical species. However, due to the cardiac and skeletal muscle expression of CK isoforms, the ability of current CK enzyme activity based assays to specifically diagnose skeletal muscle injury is limited. The Meso Scale Discovery ( MSD, Gaithersburg) Muscle Injury Panel 1 (MIP1) is an immunological electrochemiluminescent assay that has been developed to detect multiple rat proteins with restricted expression in heart and/or skeletal muscle. The assay detects fatty acid binding protein 3 (FABP3, heart/skeletal muscle), myosin light chain 3 (MyL3, heart/skeletal muscle) and cardiac troponin T and I (cTnT and cTnI, heart). We have performed the evaluation of the novel biomarkers in humans with exercise induced muscle injury. Plasma was collected from 37 human volunteers before and after prolonged exercise in sports competitions ranging from 48-150 hours in duration. CK enzyme activity was increased 29.5 fold after exercise (mean +/- standard deviation was 131 ± 62 U/mL before exercise and 3866 ± 3327 U/mL after exercise, P< 0.001). FABP3, MyL3 and sTNI were all significantly increased after exercise (9.0 fold, 41.7 fold and 11.0 fold respectively, all P<0.001), whereas cTnT and cTnI were undetectable, suggesting that the injury was restricted to skeletal muscle. FABP3, MyL3 and sTNI also showed a higher correlation to CK activity (Spearman r value of 0.93, 0.88, and 0.93 respectively). In summary we have shown that novel skeletal muscle injury markers are induced in humans in response to prolonged exercise, correlate highly with CK activity, and have potential utility for assigning skeletal muscle damage with more specificity than conventional assays.

**THE INTERPRETATION OF STRIATED MUSCLE TOXICITY BIOMARKERS IN NONCLINICAL SPECIES: A CASE SERIES.**


There are a number of serum biomarkers indicative of striated muscle toxicity. Traditional biomarkers including creatine kinase (CK), lactate dehydrogenase (LDH) and aspartate transaminase (AST) are commonly used. However, they lack the ability to differentiate between tissue-specific damage. Serum myoglobin, fatty acid binding protein 3 (Fabp3) and troponins (TrnT and TrnI isotypes, TnC) have been reported to provide improved specificity for the detection of cardiac and skeletal muscle toxicity. This case series describes a number of non-clinical studies examining the relationship between serum biomarkers and histopathological changes. Rats and dogs were exposed to sulfonil isoxazoline chemistries for 4 to 28 days via daily diet or gavage. Histopathology evaluation revealed a dose-dependent relationship in the muscle damage in both cardiac tissue and fast- and slow-twitch skeletal muscle (diaphragm, soleus and biceps femoris). Interestingly the temporal relationship and the magnitude of change in serum biomarkers did not correlate well with the severity of the observed muscle insult. There were also differences between species – in rats early change in tissue-specific tropinin isotypes (eg. cTnI), CK, LDH and AST was not detected, despite the presence of muscle pathology. In dogs, the converse relationship was often observed, significant early increases in tissue-specific biomarkers reflected only minimal histopathological findings. These data illustrate the temporal and species-specific complexity of serum biomarkers for predicting striated muscle toxicity. Further ongoing work includes transcriptome and protein profiling of tissues taken from these studies to explore mechanistically relevant pathways.

**INDIVIDUAL BILE ACID CHANGES ASSOCIATED WITH BILE DUCT HYPERPLASIA IN PRECLINICAL MODELS.**


Bile duct hyperplasia (BDH) findings are concern during drug development since they may be accompanied by various abnormalities, such as portal inflammation, cholestasis and biliary injury. The relevance and predictive value of BDH in animals for drug induced liver injury (DILI) in humans are under investigation. The purpose of this study was to evaluate potential biomarkers of BDH in mice and rats using tool compounds and company developed compounds. A validated LC/MS/MS model to quantitate three individual bile acids (IBAs), taurocholic acid, glycocholic acid and cholic acid, was deployed to analyze serum samples from these studies. Results were compared against histopathology findings and standard clinical chemistry parameters. The concentrations of taurocholic acid and glycocholic acid increased significantly in the animals with BDH findings. The three bile acids evaluated remained unchanged in animals dosed with compounds that did not induce BDH. The data were consistent for both species. No changes in clinical chemistry parameters, such as ALT, AST, ALP, GGT, GLDH and TBIL, were observed. Laboratory data has demonstrated that cholic acid in serum significantly increases in rats dosed with compounds causing necrosis and cholestasis. Cholic acid was more specific for necrosis than glycocholic acid and taurocholic acid, yet these two bile acids corresponded better with BDH in preclinical models. These results demonstrate the potential utility of individual bile acids as biomarkers of BDH in preclinical species, and the potential for relationships to be established between individual bile acids and specific liver toxicity findings.

**EVALUATION OF CALCINEURIN ACTIVITY AS A BIOMARKER OF IMMUNOSUPPRESSION IN LUNG TRANSPLANT PATIENTS.**

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Introduction: Optimal immunosuppression is difficult to achieve. Inadequate immunosuppression can provoke transplant rejection whereas excessive immunosuppression predisposes to infection or malignancy. To circumvent possible adverse events a biomarker of immunosuppression is needed. Since anti-calcineurin drugs are the standard for rejection prophylaxis after lung transplantation (LT), we have investigated whether calcineurin activity (CN-a) could be a biomarker of immunosuppression in LT patients.

Methods: 107 patients entered the study from March 2004 to August 2008. CN-a was measured once a month during the first 6 months after LT in mononuclear cells isolated from whole blood by quantifying by HPLC the dephosphorylation of a phospho-RiI peptide. Episodes of acute rejection were diagnosed on the basis of lung function and biopsy results. Pulmonary function was estimated according to the spirometric data FEV1 and FEF25-75. Chronic rejection was diagnosed according to the ISHLT criteria. Adverse events such as infections and malignancies were recorded.

Results: CN-a tended to be increased in patients who were treated for acute rejection as compared to patients who were not (66 vs 52 pmol/mg/min, p<0.09). An inverse relationship between CN-a levels and acute rejection was observed (r=-0.576, p<0.001). Moreover, we found that patients who displayed extremely low CN-a levels (<10 pmol/mg/min) continued to have altered evolution of their spirometric values as well as a lower freedom from chronic rejection (19% vs 67%, p=0.0326). Chronic rejection was diagnosed in 5% of the cases. CN-a levels outside the range of 10-100 pmol/mg/min were those who displayed an altered evolution of their spirometric values as well as a lower freedom from chronic rejection (19% vs 67%, p=0.0326). Moreover, we found that patients who displayed extremely low CN-a levels (<10 pmol/mg/min) possibly remained to suffer from immunosuppression developed more adverse events such as infection and malignancy was recorded.

Conclusion: These results suggest that CN-a could be a biomarker of immunosuppression and could be useful not only the occurrence of rejection related to an inadequate immunosuppression but also the development of severe complications related to exceedingly immunosuppression.

**BIOMARKERS FOR STEATOHEPATITIS (ASH, NASH, TASH).**

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Steatohepatitis may be caused by industrial chemical toxicants (TASH), obesity (NASH), or alcohol (ASH). The purpose of this study was to compare and contrast serologic biomarkers in human subjects with NASH (n=55), ASH (n=50), and...
TASH (due to occupational vinyl chloride exposures, n=16) vs. healthy controls (n=11). Serum CK18 was measured by ELISA (Diaplan) and adipokines were measured by multi-analyte chemiluminescent detection (Milliplex); and antioxidant activity was measured using a kit (Cayman). ASH and NASH were both associated with increased aminotransferase activities (ASH: ALT>AST; NASH: ALT>AST), while aminotransferases were normal in TASH. All steatohepatitis groups had increased CK18 M65 (necrosis biomarker) compared to controls; while CK18 M30 (apoptosis biomarker) was increased only in ASH and NASH. Hyperinsulinemia was present in all steatohepatitis groups; although the mechanisms for this appeared to be different. Adiponectin was low (vs. healthy controls) in NASH and TASH, while was lowest in the NASH group. The hepatocellular death biomarker, CK18, appears useful for the diagnosis and sub-classification of steatohepatitis. ASH and NASH were associated with significant degrees of hepatocellular apoptosis, while TASH was primarily necrotic. Insulin resistance was present in all etiologies of steatohepatitis, although adipokine profiles were divergent. ASH and TASH (to a lesser degree) were associated with increased inflammatory biomarkers. NASH was associated the greatest antioxidant depletion. Funding sources include NIH and State of Kentucky.

**543 URINARY MITOCHONDRIAL DNA AS A NOVEL BIOMARKER OF ACUTE KIDNEY INJURY.**

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Acute kidney injury (AKI) is defined by the abrupt reduction in kidney function and can be caused by numerous acute insults including drug toxicity, surgery, trauma, and ischemia/reperfusion (I/R). Animal models of AKI have demonstrated an important role for mitochondrial dysfunction, and mitochondrial components are released from cells and tissues after diverse insults to act as damage-associated molecular patterns (DAMPs) in signaling injury responses. Serum creatinine (SCr) is the standard clinical marker for AKI, but the limited sensitivity of SCr has led to recent efforts by multiple groups to identify novel biomarkers of AKI. The purpose of this study was to determine if urinary mitochondrial DNA (mtDNA) is a biomarker of AKI and of mitochondrial dysfunction in AKI. We established a rat model of renal I/R in which Scr increased from 0.5 to 2.2 mg/dl at 24 hr after surgery and returned to normal levels after 144 hr. We collected urine from sham and I/R rats at 24 hr and concentrated 1 ml of urine using centrifugal filters. DNA was isolated from concentrated urine, qPCR was performed using primers for the mitochondrial gene ND1, and ND1 was quantified using a standard curve of kidney DNA. There was a 184-fold increase in the levels of ND1 in urine from I/R rats compared to sham animals. We also examined mtDNA quantity in the urine of humans with AKI after cardiac surgery, control humans with normal renal function, and humans with no AKI after cardiac surgery. We found a 100-fold increase in urinary ND1 from humans with AKI versus healthy controls and cardiac surgery patients with no AKI. Our results indicate that urinary mtDNA may be a novel, non-invasive biomarker of AKI and could be indicative of mitochondrial dysfunction in renal toxicity.

**544 EARLY DETECTION OF KAINIC ACID NEUROTOXICITY IN RATS USING NONINVASIVE IN VIVO MAGNETIC RESONANCE IMAGING.**

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The current approach to neuropathology analysis in support of new drug submissions to the FDA (the use of 3 or 7 arbitrary histology slices) is prone to false-negative findings, meaning that small localized lesions can easily be missed. Modern magnetic resonance imaging (MRI) techniques can provide unique information about the whole brain structure and function in vivo with high resolution and contrast in three-dimensional space, non-invasively. The aim of this study was to develop sensitive MRI methods for detecting early neurotoxic changes in the brain of living rats using established models of neurotoxicity. Male Sprague-Dawley rats (64 days old, 389 ± 34 g) were injected with kainic acid (KA, N=2, 10 mg/kg, ip) or saline (N = 2, 2 ml/kg, ip) after baseline MRI scans. Animals were continually scanned for 2 hours in the MRI. Two days later animals were scanned again and euthanized for follow-up histopathological examination. MRI was performed using a Bruker 7tesa MRI with volume transmit and surface receive coils. Animals were anesthetized with isoflurane and the body temperature was maintained at 37 ± 0.6°C. T2 relaxation mapping of the whole brain was performed using pixel-by-pixel fitting of the signal decay in a multi-echo spin echo experiment. There were for the assessment of cardiac troponin I (cTnI) using an ultrasensitive assay and N-terminal propeptide (NT-proANP) concentrations. Histomorphologic evaluation was performed on Days 8 and 15.

Results/Discussion: Administration of M for 7 and 14 days resulted in CV changes to the left ventricle (LV) including an increase in chamber dimension, evident in diastole, with preserved indices of function. As a result, LV stroke volume increased with a concomitant decrease in heart rate. Coincident with these CV changes, NT-proANP increased on Day 3 and peaked on Day 7 and returned toward baseline while serum cTnI progressively increased starting on Day 3. All biomarker changes occurred in the absence of histomorphologic findings. These data suggest that sub-chronic M dosing induced a systemic vasodilatory response. As a result of the decrease in heart rate, LV filling was likely increased, resulting in chamber enlargement and the associated increase in serum biomarkers.

Conclusion: Herein, echocardiography, NT-proANP (stretch biomarker) and cTnI were more sensitive than histopathology to identify the CV response to M, while providing the advantage of being non-terminal. This multidisciplinary approach has translational implications for the early identification of CV liabilities of compounds in development.

**541 MASS SPECTROMETRY ASSAYS FOR STEROIDS IN TOXICITY ASSESSMENTS.**

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The accurate measurement of steroid levels in pre-clinical species has become increasingly important during drug development because changes in steroid levels have been linked to a variety of toxicities. Antibody-based methods such as radioimmunoassays (RIA) and enzyme-linked immunosorbent assays (ELISA) are commonly used for steroid analysis due to their ease of use and sensitivity. A major disadvantage of RIA and ELISA kit-based assays is the potential of the antibodies to cross-react with other analytes of similar structure resulting in false-positives. Additionally, many kits are non-transferable to different species or matrices, requiring the scientist to locate, purchase and evaluate multiple kits for the same analyte. To assess the feasibility of transferring steroid assays from kits to a mass spectrometer-based platform (MS), a direct comparison between platforms was conducted with aldosterone, corticosterone and 25-hydroxy (OH)2 vitamin D3. Three major benefits were observed when comparing MS to antibody-based assays: lower costs, shorter data delivery times, and increased specificity. For example, the MS assay for aldosterone costs ten times less to run and data is reported two days sooner; the corticosterone assay uses five times less sample volume; the 25(OH)2D3 assay can differentiate between D2 and D3 forms. Detection limits and assay precision for the MS assays are comparable to those observed in the kits. The quantitation range for aldosterone is 0.01 to 100 ng/ml, for corticosterone is 10 to 1000 ng/ml, and for 25(OH)2D3 is 5 to 150 ng/ml. Precision is within 25% for all three assays. These assays have been applied to multiple pre-clinical species, including dog, monkey, as well as to multiple biological matrices, including serum, plasma, and urine. As MS is becoming established in the clinical setting, this platform is broadening its impact in clinical pathology applications, enhancing drug development. These changes not only provide substantial cost and time savings, but they provide higher quality data for drug development.

**542 NT-PROANP, cTnI, AND ECHOCARDIOGRAPHY ARE EARLY MARKERS OF MINOXIDIL-INDUCED STRUCTURAL AND FUNCTIONAL CHANGES OF THE LEFT VENTRICLE IN RATS.**

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Introduction: Advances in biofluid assay technology combined with enhanced in vivo imaging now provide the sensitivity, accuracy, and reproducibility necessary for their validation and subsequent incorporation into routine toxicity studies. The usage of these biomarkers will beneficially impact the cardiovascular (CV) liability assessment of development compounds.

Methods: Male rats were orally dosed with vehicle or Minoxidil (M) (100 mg/kg/day) for 7 or 14 days. Echocardiography was performed the day prior to dosing (-1) and on Days 7 and 14. Serum was collected on Days -1, 3, 7, 11 and 14 for the assessment of cardiac troponin I (cTnI) using an ultrasensitive assay and N-terminal propeptide (NT-proANP) concentrations. Histomorphologic evaluation was performed on Days 8 and 15.

Results/Discussion: Administration of M for 7 and 14 days resulted in CV changes to the left ventricle (LV) including an increase in chamber dimension, evident in diastole, with preserved indices of function. As a result, LV stroke volume increased with a concomitant decrease in heart rate. Coincident with these CV changes, NT-proANP increased on Day 3 and peaked on Day 7 and returned toward baseline while serum cTnI progressively increased starting on Day 3. All biomarker changes occurred in the absence of histomorphologic findings. These data suggest that sub-chronic M dosing induced a systemic vasodilatory response. As a result of the decrease in heart rate, LV filling was likely increased, resulting in chamber enlargement and the associated increase in serum biomarkers.

Conclusion: Herein, echocardiography, NT-proANP (stretch biomarker) and cTnI were more sensitive than histopathology to identify the CV response to M, while providing the advantage of being non-terminal. This multidisciplinary approach has translational implications for the early identification of CV liabilities of compounds in development.
Circadian rhythms in many physiological processes can impact drug response and toxicity. The sensitivity of the central nervous system to neurotoxicants may also be dependent on the phase of the circadian cycle and this may be due to variations in the activity of neurotransmitters and other neural systems. The goal of this study was to evaluate diurnal changes in neurometabolite profiles in naïve rats measured using non-invasive magnetic resonance spectroscopy (MRS). Male Sprague-Dawley rats (69.0 ± 1.5 days old, 367 ± 31 g) were subjected to MRS one hour after light onset (7 am, N = 5, AM group) or offset (7 pm, N = 5, PM group) during the regular light cycle. MRS was performed using a Bruker BioSpec 7T/30 system with a 72 mm bird-cage transmit and a 4-channel phased array receive coil. Animals were anesthetized with isoflurane and the body temperature was maintained at 37.0 ± 0.8°C. Spectroscopic voxels were positioned at the left anterior hippocampus. Dynamic proton spectra were acquired for up to 2 hours at -11 min/spectrum.

LCModel software was used to extract metabolite concentrations from individual spectra and the statistical analysis was performed using ANOVA. Concentrations of glutamate, N-acetylaspartate, taurine, and choline were all significantly lower in AM group comparing to PM group (2 hrs after light onset or offset). However, at the end of scanning (3 hrs after light offset or onset) all these differences disappeared. Concentrations of other significant neurometabolites (GABA, creatine, glutamine, and myo-inositol) were not significantly different between groups at any time points. The data demonstrate that brain neurochemical composition is influenced by diurnal variation which may be one of the mechanisms underlying circadian rhythms in many physiological processes. The primary objective of this study was to discover surrogate biomarkers which are correlated with hepatotoxicity induced by acetaminophen (APAP) using urinary proton nuclear magnetic resonance (1H NMR) spectral data. A procedure of 1H NMR urinalysis using pattern recognition was proposed for early screening of the hepatotoxicity of APAP in humans. The hepatotoxicity occurrence was tested through clinical chemistry. APAP (3 g/day, two 500-mg tablets every 8 h) was administered to 20 healthy males (20-29 yr) for 7 days and urine was collected daily before and during dosing and 6 days after final dosing. All the subjects didn’t show significant changes in alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GTP), and lactate dehydrogenase (LDH) by APAP compared to before treatment. However, urinary metabolite profiles were obtained from subjects showing mild liver injury, as indicated by increase in ALT or AST to a level more than 1.7-fold the before treatment. 1H NMR spectroscopy revealed evidently different clustering between pre-dosing and APAP treatment in global metabolomic profiling through principal component analysis (PCA) and partial least square (PLS)-discrimination analysis (DA). In targeted profiling, urinary endogenous metabolites of trimethylamine, citrate, 3-chlorotyrosine, phenylalanine, malonate, glycine, creatine, taurine, betaine, glutamine, glutamate, and taurine were selected as diagnostic metabolites for APAP-hepatotoxicity. These results might be applied to predict or screen other potentially hepatotoxicity caused by drugs using urinary 1H NMR analysis.

The prediction of phospholipidosis (PLD) was investigated by in silico/in vivo methods using 50 drugs with known PLD inducing potential in vivo. For in silico prediction of PLD 3 models were used. They are based on physico-chemical descriptors for a simple rule model (modified Ploemen model: logP and pKa) or more sophisticated for decision tree models (C5 RuleQuest and KNIME model). For in vitro analysis, phospholipid (PL) accumulation was measured in HepG2 cells 24h/48h after incubation using the HCS LipidTOX kit, including a cytotoxicity evaluation by ATP content and neutral red uptake. Finally, for in vivo analysis the urinary PL biomarkers BMP (Bio(Bis(monosaccharide)glycerophosphate) and PAG (phospholactylglycine) were analyzed by LC-MS and NMR in different species (rat, dog, hamster, human) for some examples. Additionally, accumulation of PLs was analyzed in different organs using a photometric enzyme-assay and PLs was confirmed by histopathology.

A combined approach using the 3 in silico models together with in vitro result showing PL increase below 2μM was able to identify most of the in vivo PL-inducing compounds. Sensitivity up to 96% could be achieved with acceptable specificity (70-95%) depending on the combinations. These results were confirmed by the urinary PL biomarker BMP and the PL analysis and histopathology mainly in lung and liver, providing also more information about time course and NOEL. Urinary PAG was increased by PLS in rats, but only low or no effects were seen in other species. These validation results showed that an integrated testing strategy from in silico models to in vitro assays to in vivo biomarkers is able to identify and to characterize the PLS inducing potential of drugs very early by combination of different tools depending on available data and development status.

The primary objective of this study was to develop non-invasive biomarkers for the early preclinical detection of neurotoxicity would benefit public health by extending the number of tools available for safety evaluation of new drugs. (Supported by NCTR/CDER, FDA, #E07418801).
549 URINARY BIOMARKERS IDENTIFICATION USING METABOLOMICS FOR KIDNEY TOXICITY IN SPRAGUE DAWLEY RATS.

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Identification of new biomarkers for detection of nephrotoxicity is important for evaluation for new drug development and screening. Recently, several biomarkers have been identified for detection of nephrotoxicity. In this study, we compared the sensitivity of biomarkers with conventionally used biomarkers for kidney toxicity. The animals were allocated to several groups. A single dose of cisplatin (10 mg/kg, i.p.) and D-galactosamine (20 mg/kg, i.p.) was administered to Sprague Dawley rats. Urine, plasma and kidney tissue samples were collected at 1, 3, 7 days after drug injection. To discover biochemical biomarkers useful for sensitive identification of nephrotoxicity, measurement of urinary indexes and blood biochemical parameters were performed. Urinary metabolites were also measured using NMR-based spectroscopy. In the cisplatin-treated rats, urinary blood urea nitrogen (BUN) and creatinine levels were dramatically decreased and protein, glucose and LDH levels were significantly increased, which were thought to be caused by the dysfunction of proximal tubule injury. However, no significant differences in urinary biomarkers were observed in D-galactosamine-treated rats. A number of metabolites were changed in urine of rats treated with cisplatin. Among them, trigonelline, 3-indoxylsulfate, hippurate, and citrate levels were dramatically reduced in rats, but lactate, acetate, and glucose levels were markedly increased. In cisplatin treated group, LDH level was significantly increased in the urine, but LDH activity was decreased in kidney tissues. Based on these results, detection of combined urinary metabolites may be a powerful tool for detection of nephrotoxicity, and LDH was considered useful for broad detection of nephrotoxicity.

550 LOW-DOSE 3-IODOTHYRONAMINE INCREASES ACUTE LIPOLYSIS FOLLOWED BY PROTEIN CATABOLISM DETECTABLE BY BREATH AND SERUM BIOMARKERS.

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3-iodothyronamine (T1AM) is a recently discovered fast-acting thyroid hormone derivative. Treatment with T1AM produces profound metabolic effects. This study monitored the effect of worklong, daily, low dose T1AM administration on weight and metabolism in spontaneously overweight mice. Real-time analysis of exhaled 13CO2 by cavity ringdown spectroscopy detected increased lipolysis shortly after and metabolism in spontaneously overweight mice. Real-time analysis of exhaled 13CO2 by cavity ringdown spectroscopy detected increased lipolysis shortly after treatment with T1AM produces profound metabolic effects. This study monitored the effect of worklong, daily, low dose T1AM administration on weight and metabolism in spontaneously overweight mice. Total RNA including small RNA species was isolated from urine samples at day 3, 5, 8, 10 and 14 after single dose treatment with Cp. Using TaqMan®miRNA Cards (Array Gene Signature Rodent A and B Cards) miRNA profiles were generated for each time point for both treatment and control groups. MiRNA levels were normalized to a non-endogenous spiked-in control miRNA of plant origin (Arabidopsis thaliana miR-159a); in addition urine miRNA were normalized to urinary creatinine. In plasma from Cp-treated rats as well as in urine from Cp-treated rats, microRNAs were found in urine from Cp-treated rats, microRNAs were found in significantly higher amounts compared to vehicle treated rats, including the liver-specific miR-122 in plasma (1) and the kidney-cortex enriched miR-192 in urine (2), respectively. The miRNAs found at altered levels bear the potential of being used as new and sensitive BMs for liver injury. References:

551 HEPATIC AND PLASMA MiRNAs—SENSITIVE BIOMARKERS OF LIVER INJURY INDUCED BY A METHYL-DEFICIENT DIET IN MICE.


MicroRNAs (miRNAs) are a class of small, conserved, tissue-specific regulatory non-coding RNAs that modulate a variety of biological processes, including metabolism, cell differentiation and proliferation, and apoptosis. Aberrant expression of certain microRNAs may play a fundamental role in disease pathogenesis, including liver; however, the association between the susceptibility to any given pathological state and altered expression of miRNAs is largely unknown. In order to determine whether or not individual susceptibility to liver injury is associated with an altered miRNA expression, we fed inbred male A/J, C57BL/6J, C3H/HeJ, 129S/SvImJ, CAST/EiJ, PWK/PhJ, and WSB/EiJ mice a methyl-deficient diet for 12 weeks, a relevant model of nonalcoholic fatty liver disease (NAFLD) in humans. miRNA levels in livers and plasma of mice were determined using TaqMan RT-PCR. Feeding the methyl-deficient diet induced liver injury in all mouse strains; however, the severity of liver pathological changes was strain-specific, with A/J mice being the least susceptible and WSB/EiJ mice being the most sensitive to liver injury induced by methyl-deficiency. There was a significant up-regulation of miR-34a, miR-200b, miR-181a, and miR-221. Importantly, expression of these miRNAs was highly correlated with the extent of NAFLD-associated pathomorphological changes in the livers. Microarray gene expression data and target-prediction miRNA-mRNA analysis showed that the predicted targets for these miRNAs affect cell proliferation, apoptosis, inflammation, oxidative stress, and metabolism. More importantly, we found that the levels of miR-34a, miR-122 and miR-192 were upregulated in the blood plasma and this was significantly correlated with the extent of liver pathomorphological changes. In conclusion, the severity of NAFLD is strongly associated with the level of hepatic and plasma miRNAs. This suggest that circulating miRNAs may be used as biomarkers of liver toxicity, and more importantly, as a potential indicator of susceptibility to liver injury.

552 MiRCONAS IN PLASMA AND URINE AS NEW SAFETY BIOMARKERS FOR DRUG-INDUCED TISSUE INJURY IN RATS.

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MicroRNAs (miRNAs) are highly conserved, noncoding, approx. 22 nucleotides long, small RNAs that play an important role in post-transcriptional regulation of gene expression. Because of their tissue specificity and stability in plasma and urine, they are suggested as new biomarkers (BM) of tissue injury in easily accessible bioluids in preclinical studies. For evaluation of miRNA preclinical BMs plasma and urine were collected from male Wistar rats treated either with the hepatotoxicant methapyrilene (MPy) or with the nephrotoxicant cisplatin (Cp). Tissue injury was confirmed by histopathology and chemical pathology parameters, i.e. we measured increased ALT (Alanine transaminase) activity in serum and liver injury and increased protein levels in urine after kidney injury, respectively. In addition new urinary protein BMs for nephrotoxicity, (e.g., KIM-1), showed a time-dependent increase after Cp treatment. Total RNA including small RNA species was isolated from plasma at day 4, 8 and 10 after daily treatment with MPy and after a 10 day recovery period. Total RNA was also isolated from urine samples at day 3, 5, 8, 15 and 26 after single dose treatment with Cp. Using TaqMan®miRNA Cards (Array Gene Signature Rodent A and B Cards) miRNA profiles were generated for each time point for both treatment and control groups. MiRNA levels were normalized to a non-endogenous spiked-in control miRNA of plant origin (Arabidopsis thaliana miR-159a); in addition urine miRNA were normalized to urinary creatinine. In plasma from MPy-treated rats as well as in urine from Cp-treated rats, microRNAs were found in significantly higher amounts compared to vehicle treated rats, including the liver-specific miR-122 in plasma (1) and the kidney-cortex enriched miR-192 in urine (2), respectively. The miRNAs found at altered levels bear the potential of being used as new and sensitive BMs for liver or kidney injury. References:

553 MiRNA BIOMARKERS OF OCULAR TOXICITY.

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Ocular toxicity has been associated with numerous systemic medications and is a serious concern during drug development. Drugs can disturb blood-aqueous and blood retinal barriers ultimately allowing drug to enter the eye resulting in toxicity. Otherwise, small molecules such as miRNAs can transfer into blood due to ocular toxicity. Therefore, the fluctuation of ocular miRNAs in body fluids such as plasma reflects ocular cell and tissue injury just like miR-122 in liver injury since ocular miRNAs are differently enriched in retina, lenses and cornea determined by microarray, ISH and RT-PCR. More evidence indicates the deregulation of microRNA expression correlates with a number of diseases such as cancers, liver and heart injury. In this study, we evaluated ocular enriched miRNAs in the organotypic culture of the rat eye – posterior segment by real time RT-PCR with SYBR green based detection.
The posterior globe of Sprague-Dawley rat eye was dissected into 4 pieces, washed and incubated in a 12-well plate with -1.5 mL culture media. After 24 hours of initial culture, oncology compounds known to induce ocular toxicity were tested. Supernatants of culture media (~200 µL) were extracted for miRNA with Qiagen miRNAasy kit and the miRNAs were assayed via qRT-PCR. The relative fold change was calculated and normalized with control. The PCR product was confirmed to be single product in each of the miRNA amplifications by melting curve analysis; whereas no amplification in negative control was detected above baseline. Of the 5 ocular miRNAs tested, 2 miRNAs had Ct values of ~20 to ~22; comparatively less abundant miRNAs had Ct values of ~24 to ~30 in 48 hour culture media. At least two ocular miRNAs had a 3-6 fold elevation in a dose and time-dependent response; one ocular miRNA had 5-10 fold reduction in one of the compounds tested. In conclusion, the ocular miRNAs was validated in an ex vivo culture model as potential ocular injury biomarkers. These ocular miRNAs will be further evaluated as potential biomarkers in animal model for ocular toxicity.

**554 EVALUATION OF BLOOD AND URINARY miRNAs IN GENTAMICIN-INDUCED NEPHROTOXICITY.**


The focus of the present work was to evaluate microRNAs (miRNAs) in whole blood and urine as potential markers for drug-induced kidney injury. Male Sprague-Dawley rats were given gentamicin at doses of 0, 50, 100, 150, or 200 mg/kg/day for 3 consecutive days by subcutaneous injection. Urine and blood samples for miRNA analysis were collected 24 hours after the last dose. Kidney samples were collected for histopathological evaluation and miRNA expression analysis. Blood and urinary miRNAs were detected using quantitative real-time PCR (qRT-PCR)-based TaqMan® low-density arrays. Histopathology confirmed proximal tubular injury with gentamicin treatment at 200 mg/kg, and in whole blood, miR-125a-3p and miR-30b were detected in gentamicin treated rats but not in controls. These two miRNAs were detected in the control rat kidney cortex. Analysis of urine samples revealed significant differences in the composition and levels of miRNAs in drug-treated rats compared to controls, including 108 miRNAs that were detected only in injured rats and an additional 187 miRNAs that were at least 2-fold higher in gentamicin-treated rats relative to controls. miR-4676, miR-706, miR-4606, miR-667 and miR-193b increased (114, 113, 108, 67 and 49-fold, respectively) to controls. Abundant miRNAs detected only in the urine of gentamicin-treated rats included miR-466b, miR-297a, miR-30d, miR-452 and miR-339-3p. These miRNAs were detected in control kidney cortex samples, suggesting that their increase in urine may be related to nephrotoxicity. These data indicate that gentamicin markedly affects urinary excretion of miRNAs and suggests that urinary miRNAs may be sensitive markers of kidney injury.

**555 THE SERUM MICRORNA PROFILE IS NOT ALTERED IN RATS AFTER 14 OR 28 DAYS OF DOING WITH ROSIGLITAZONE OR 3, 3', 5-TRIOIDO-L-THYRONINE.**

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Background: Increased heart weight is a common cardiac finding in preclinical safety studies. In this study, we investigated the relationship between serum cardiac troponin I (cTnI) concentrations, heart weight, and histopathology with serum microRNA profile following the administration of known cardiac hypertrophic agents in the rat. Methods: male Wistar rats were administered rosiglitazone at 80 mg/kg/day or T3 at 20 µg/kg/day for 14 or 28 days followed by 14 or 27 days of recovery, respectively. Heart weight, serum cardiac troponin I (cTnI), routine clinical pathology; and histomorphology were evaluated at necropsy. These data were compared to serum microRNAs measured on the day of necropsy with the Affymetrix microRNA chip. Results and Discussion: Compared to age-matched vehicle-treated controls, rosiglitazone caused an increase in heart weight to brain weight ratio of 10% and 17% after 14 and 28 days of dosing, respectively. This increase in rats administered T3 was 21% and 30% after 14 and 28 days of dosing, respectively. This increase in heart weight reversed after the recovery periods. There were no serum cTnI concentration increases or histomorphologic findings in rats administered rosiglitazone. In contrast, rats administered T3 had serum cTnI concentration increases which occurred concurrently with histiocyctic infiltration of the myocardium after 14 and 28 days of dosing, and with necrosis after 28 days of dosing. In addition, there were no treatment-related changes in the serum microRNA profile with any of the test compounds. Conclusion: These results indicate that a serum-based microarray strategy was not adequate to identify candidate microRNA biomarkers of increased cardiac weight, histiocyctic infiltration of the heart, and myocardial necrosis.

**556 NOVEL DIAGNOSTICS OF TOXICANT INDUCED METABOLIC DYSFUNCTION DETECTED IN BREATH AND Plasma BY SELECTIVE ISOTOPE-ASSISTED LABELING (SIAL).**


OBJECTIVE: Metabolomics is the study of a unique fingerprint of small molecules present in biological systems under healthy and disease conditions. One of major challenges in metabolomics is verification of fingerprint molecules to identify specifically perturbed pathways in metabolic aberrations. This step is crucial to the understanding of budding metabolic pathologies and the ability to identify early indicators of common diseases such as obesity, diabetes mellitus type II, metabolic syndrome, polycystic ovary syndrome, and cancer. We present a novel approach to diagnosing aberrations in glucose utilization including metabolic pathway switching in a disease state.

METHODS: We used a well-defined prenatally exposed glucocorticoid mouse model that results in adult females with metabolic dysfunction. We applied the complementary technologies of nuclear magnetic resonance spectroscopy, and cavity ringdown spectroscopy to analyze serial plasma samples and real-time breath measurements following selective 13C-isotope assisted labeling (SIAL). These platforms allowed us to trace metabolic markers in whole animals and identify key metabolic pathway switching in prenatally cortisol-treated animals.

RESULTS: Specifically, increased glucose flux and a shift in the major oxidative pathway in glucose metabolism from glycolysis to the pentose phosphate pathway were apparent in the prenatally cortisol-treated animals.

CONCLUSION: This novel approach is fast, non-invasive and sensitive for determining specific pathway utilization, and provides a direct translational application in the healthcare field.

**557 CYP2B10 INDUCTION AS A BIOMARKER FOR A CAR-MEDIATED, RODENT-SPECIFIC, MECHANISM OF LIVER FOI FORMATION AND CARCINOGENESIS DEVOID OF HUMAN RELEVANCE: EVIDENCE FROM 4-WEEK TO 2-YEAR GLP STUDIES.**


Pharmaceutical compounds under development undergo non-clinical safety testing from 4-week to 104-week durations in rodents; the latter being for lifetime carcinogenesis risk assessment. In a 104-week study with an advanced compound, male mice demonstrated statistically increased rates of hepatocellular adenoma/carcinoma accompanied by liver weight increase. Because the compound was earlier proven free of genotoxic potential, a non-genotoxic mechanism of carcinogenicity was hypothesized. Activation of the nuclear receptor constitutive androstane receptor (CAR) is a widely established promoter of non-genotoxic rodent-specific hepatic tumors, and was therefore explored in priority. Hepatic Cyp2b10 induction typifies CAR activation. We therefore monitored hepatic Cyp2b10 mRNA and its enzymatic activity in a large subset of male mice from the 104-week study. Quantitative real time PCR (qRT-PCR) and enzymatic activity monitoring evidenced strong induction of Cyp2b10 at all dose levels. Despite expected large inter-individual variations, these results reached high statistical significance. These data strongly suggested CAR activation, a mechanism of tumor formation known to be irrelevant to the human situation. With another test compound in a different project, foci of altered hepatocytes appeared in male rats of a chronic 26-week toxicity study. Microarray profiling of the corresponding 4-week rat and mouse GLP toxicity studies had previously shown strong induction of miRNAs encoding Cyp2b2 and Cyp2b10 (mouse) in both genders, as was further confirmed by qRT-PCR (up to 100-fold), in the absence of other possible proliferative pathways. These results show feasibility of Cyp2b screening in rodent liver from 4-week to 104-week studies, for prediction of a CAR-mediated, rodent-specific, mechanism of hepatocarcinogenesis known to be devoid of human relevance.
dyne of the arachidonic cascade. Using TCC-containing soaps deposits TCC on human skin. A low but significant portion of absorbed TCC may be deposited on the skin. Absorbed TCC is metabolized by cytochrome-P450 monoxygenases to monohydroxylated derivatives, 2′OH-TCC, 3′OH-TCC and 6-OH-TCC. These metabolites are quickly conjugated in the liver, and their glucuronides account for the major metabolites in mammalian bile. In human plasma, 2′SO2-O-TCC is the dominant metabolite and TCC is excreted as N-glucuronides via the urine. We recently demonstrated that further oxidation of the ortho-hydroxylated metabolites 2′OH-TCC and 6-OH-TCC leads to quinone imines that covalently bind to glutathione and model proteins in vitro. We investigated if this metabolic activation of TCC by two subsequent oxidations might occur in skin. Spontaneously immortalized human epidermal keratinocytes (Sik) were incubated with 2 μM TCC. After 10 min, the intracellular TCC concentration reached 1.21 ± 0.27 nmol/mg protein and increased at 20 h to 2.04 ± 0.27 nmol/mg protein. LC-MS/MS analysis revealed that a small but significant portion of the absorbed TCC was metabolized by Sik with 2′OH-TCC as a main metabolite. Pre-incubation of TCC with 10 nM TCCD increased metabolism of TCC in Sik and the relative conversion (cellular metabolite concentration versus cellular TCC concentration) increased from 0.5% to 15%. Up to 165 ± 28 pmol/mg protein of free 2′OH-TCC formed after 20 h incubations. To investigate whether this metabolite is further activated to reactive metabolites that bind to protein, covalent protein binding is currently under investigation utilizing LC–MS/MS in vitro. Thereafter, to accelerate TCC production, we recently supported by NIH56499 and NCRR13461.

560 DOES THE ANTIBACTERIAL TRICLORECARBAN (TCC) FORM REACTIVE METABOLITES IN THE SKIN?  
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TCC is a common antimicrobial ingredient in bar soaps. Human exposure to TCC is a concern because it accumulates in the aquatic environment and has biological effects on mammals. TCC is a potent inhibitor of soluble epoxide hydrolase, an enzyme involved in the metabolism of endogenous and exogenous epoxyeicosatrienoic acids. TCC is also known to inhibit inflammatory responses, including pro-inflammatory cytokine release and leukocyte recruitment. TCC is commonly applied to the skin to prevent UVB induced skin diseases. Recently, we have shown that UVB irradiation of human keratinocytes results in activation of the AhR and associated EGFR signaling leading to an enhanced expression of CYP1A1 and proinflammatory COX-2, respectively. The initial step is the UVB induced intracellular formation of the tryptophan photoprotein 6′-formylindolo[3,2-b]carbazole (FICZ), a high affinity AhR ligand. Thus, the FICZ activated AhR is an important mediator of the DNA damage independent part of the UVB response. Our current study aims to identify further aspects of AhR mediated UVB responses. Therefore, we analysed changes in protein expression, proliferation and apoptosis in AhR+/+ and AhR−/− keratinocytes (NCTC 2544) by western blot, flow cytometry and BrdU incorporation. UVB exposure of NCTC cells led to a dose-dependent increase in apoptosis. Compared to AhR+/+ cells, AhR−/− cultures exhibited an increased amount of apoptotic cells. This finding was confirmed in irradiated AhR+/+ cells, pretreated with the AhR antagonist 3′-methoxy-4′-nitroflavone. Moreover, the proliferation of sham as well as UVB irradiated AhR−/− cells was significantly decreased. In AhR−/− cells we found a reduced expression of checkpoint kinase 1 (Chk1), an important cell cycle regulator that arrests the cell in G2/M upon DNA damage. Interestingly, UVB exposure led to a higher phosphorylation of Chk1 in AhR−/− cells in comparison to AhR+/+ cells. This pathway is responsible for the observed AhR-dependent differences in proliferation and apoptosis. Further expression analyses of Chk1 client proteins emphasize our hypothesis. In conclusion our study identifies the AhR as an anti-apoptotic player in UVB-induced skin diseases. We propose that the AhR is a suitable target to prevent UVB-induced skin diseases.

561 THE ARYL HYDROCARBON RECEPTOR (AHR) IS A MODULATOR OF ULTRAVIOLET B (UVB)-INDUCED PROLIFERATION AND APOPTOSIS IN HUMAN KERATINOCYTES  
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The AhR is a ligand-activated transcription factor that mediates the toxicity of dioxins and related compounds. Upon ligand binding the AhR translocates into the nucleus and dimerizes with ARNT to modulate gene expression, e.g. of CYP1A1. Recently, we have shown that UVB irradiation of human keratinocytes results in activation of the AhR and associated EGFR signaling leading to an enhanced expression of CYP1A1 and proinflammatory COX-2, respectively. The initial step is the UVB induced intracellular formation of the tryptophan photoprotein 6′-formylindolo[3,2-b]carbazole (FICZ), a high affinity AhR ligand. Thus, the FICZ activated AhR is an important mediator of the DNA damage independent part of the UVB response. Our current study aims to identify further aspects of AhR mediated UVB responses. Therefore, we analysed changes in protein expression, proliferation and apoptosis in AhR+/+ and AhR−/− keratinocytes (NCTC2544) by western blot, flow cytometry and BrdU incorporation. UVB exposure of NCTC cells led to a dose-dependent increase in apoptosis. Compared to AhR+/+ cells, AhR−/−cultures exhibited an increased amount of apoptotic cells. This finding was confirmed in irradiated AhR+/+ cells, pretreated with the AhR antagonist 3′-methoxy-4′-nitroflavone. Moreover, the proliferation of sham as well as UVB irradiated AhR−/− cells was significantly decreased. In AhR−/− cells we found a reduced expression of checkpoint kinase 1 (Chk1), an important cell cycle regulator that arrests the cell in G2/M upon DNA damage. Interestingly, UVB exposure led to a higher phosphorylation of Chk1 in AhR−/− cells in comparison to AhR+/+ cells. This pathway is responsible for the observed AhR-dependent differences in proliferation and apoptosis. Further expression analyses of Chk1 client proteins emphasize our hypothesis. In conclusion our study identifies the AhR as an anti-apoptotic player in UVB irradiated human NCTC cells. Therefore, we propose that the AhR is a suitable target to prevent UVB-induced skin diseases.
of these cytokines from unirradiated keratinocytes. RNase inhibited this activity, thus the trigger of cytokine release by UVB-damaged keratinocytes was likely an RNA specie. Whole-transcriptome sequencing through RNA-Seq analysis of UVB-treated keratinocytes identified several unique non-coding RNAs, specifically U RNAs, that undergo alterations in stem-loop domains. A specific UVB-modified U RNA (U1 RNA) was identified that was able to stimulate TLR-α and IL-6 from keratinocytes. Synthetic oligoucnucleotides based on the stem-loop sequences modified by UVB were found to readily stimulate keratinocyte cytokine release. In isolated keratinocytes, this response was toll-like receptor 3 (TLR3)-dependent and inhibitable by chloroquine, an inhibitor of endosomal TLR activation. TLR3/- keratinocytes were also unresponsive to UVB. Intradermal injection of synthetic U1 RNA into the ears of wild-type C57BL/6 mice, but not TLR3/- mice, resulted in induced skin inflammation. These knock-out mice also produced little or no cutaneo us TNF-α and IL-6 after irradiation with 500 mJ/cm² UVB when compared to wild-type controls. These findings establish that TLR3 detects UV damage to U1 RNA to induce an inflammatory cytokine response.

**563 DIFFERENTIAL GENE EXPRESSION RESPONSES DISTINGUISH BETWEEN DERMAL AND RESPIRATORY SENSITIZERS AND NONSENSITIZING IRRITANTS IN THE LLNA.**


Genomic approaches have the potential to enhance the specificity and predictive accuracy of existing toxicology endpoints, including those for chemical sensitization. The present study was conducted to determine whether gene expression responses might distinguish among dermal sensitizers (dinitrochlorobenzene (DNCB) and hexyl cinnamic aldehyde (HCA)), respiratory sensitizers (ortho-phthalaldehyde (OPA) and trimellitic anhydride (TMA)) and non-sensitizing irritants (methyl salicylate (MS) and nonanoic acid (NA)) in the LLNA. Female Balb/C mice received doses of each chemical per the standard LLNA dosing regimen on days 1, 2 and 3. Auricular lymph nodes were analyzed for 3HTR incorporation on day 6 and gene expression on days 6 and 10. All chemicals induced dose-dependent increases in stimulation index values with EC3 values of 0.084% (DNCB), 8.6% (HCA), 0.015% (OPA), 0.17% (TMA), 48% (MS), and 18.8% (NA). The number of differentially expressed genes was dose-dependent for each chemical and correlated strongly with the corresponding stimulation index. A majority of the genes modulated by the irritants were similarly altered by the sensitizers, consistent with the irritating effects of the sensitizers. However, a select number of responses involved with dendritic cell activation were unique to the sensitizers and may offer the ability to distinguish sensitizers from irritants. Genes for the mast cell proteases related strongly with the corresponding stimulation index. A majority of the genes associated with ESI. Results indicate that the majority of the Ac-EEMQR-amide is unabsorbed and removed from skin after washing. Decreasing amounts of peptide was detected in the deeper layers of stratum corneum (0.76%) to the viable epidermal layer (0.016%). Peptide was not detected in the dermis of the skin or receptor fluid. This absorption profile is useful for understanding the safety of cosmetic products containing short-chain peptides.

**565 SCREENING OF TOXIC INDUSTRIAL COMPOUNDS FOR CUTANEOUS INJURY.**

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A weanling swine model has been used to characterize superficial dermal (SD) and deep dermal (DD) cutaneous lesions following exposure to vesicants and toxic chemicals. This study utilized this weanling swine model for screening toxic industrial chemicals with potential dermal toxicity including acrolein, acrylonitrile and hydrofluoric acid (HF). Three exposure methods were evaluated for acrolein and acrylonitrile including vapor cap, vapor cap with simulated sweat and liquid. HF was evaluated using only vapor caps. Assessments conducted on Day 2 included photographs, lesion size and modified Draize scoring. Tissues were excised on Day 2 for histopathology. Acrolein exposure by all methods resulted in some degree of dermal injury as indicated by basal cell necrosis and der mal coagulation. Increasing exposure time resulted in significantly increased injury in both lesion area and modified Draize score for animals exposed using the vapor with sweat and the liquid models. Acrolein vapor exposures with sweat resulted in the most consistent injury with a mean range of 0%-65% basal cell necrosis and a mean range of 38%-100% dermal coagulation. Acrylonitrile vapor exposure for up to 45 minutes did not result in dermal injury as evidenced by no basal cell necrosis or der mal coagulation. The lesions created by the Acrylonitrile vapor with sweat or liquid method lacked uniformity and were SD to mid-dermal in depth. HF vapor exposures consistently resulted in both SD and DD dermal injuries as demonstrated by basal cell necrosis mean range 35%-100% and dermal coagulation mean range 11%-100%. Many endpoints for the HF exposures were found to be highly signific ant including modified Draize score, basal cell necrosis, dermal coagulation, PMN epidermal infiltrate, and total inflammation severity. Clinically, the HF lesions appeared uniform and increased in severity with the increased exposure time as evidenced by the increased severity in modified Draize scores and dermal coagulation.

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**566 SYSTEMIC AND TOPICAL ANTI-INFLAMMATORY DRUG EFFICACY IN TREATING CUTANEOUS SULFUR MUSTARD LESIONS.**

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Sulfur mustard (HD)-induced dermal lesions in the weanling swine model were used to evaluate the efficacy of topical anti-inflammatory compounds and systemic anti-cytokine treatments. This study evaluated the efficacy of diclofenac sodium (DICLO) and clotobetasol propionate (CLO) with and without systemically-administered drugs, including a tumor necrosis factor (TNF)-α inhibitor (Enbrel®), an interleukin-6 (IL-6) receptor inhibitor (Actemra®) and an IL-1β receptor antagonist (Kineret®). Superficial dermal (SD) or deep dermal (DD) lesions were generated by exposure to 400 mL of HD for 8 or 30 min, respectively. Post-exposure assessments on Days 2, 7 and 14 included lesion area, modified Draize scoring, photographs, reflectance colorimetry, transepidermal water loss (TEWL) and infrared imagery. Tissues were excised on Day 14 for histopathology. Generally, SD lesions were healed (75-100% re-epithelialization) and were similar to untreated control skin by Day 14. Epidermal and dermal necrosis, collagenolysis and inflammation were minimal in untreated SD lesions and had slightly increased scores following systemic and/or topical treatments. Fibroplasia was significantly decreased in SD lesions by Actemra® and Enbrel® without topical treatment. Generally, DD lesions were less well healed at Day 14 with severe dermal necrosis and less than 25% re-ep ithelialization. Untreated DD lesions were characterized by almost complete reten tion of the necrotic epidermis and significant dermal coagulation. Topical treatment of DD lesions with or without systemic treatment significantly decreased mean Draize scores and TEWL on Day 7. For DD lesions, CLO and DICLO+CLO significantly decreased inflammation and fibroplasia across all systemic dose groups.

In summary, the data indicate that systemic treatment with DICLO+CLO trended toward improved healing relative to all other therapeutic regimens when the primary and secondary parameters are considered.
Dicyclohexylamine (DCHA) is commonly used in the metalworking industry to prevent corrosion of fabricated materials. There is no published information describing the dermal absorption of DCHA in metalworking fluid (MWF) formulations. Machine workers are most likely to be exposed to DCHA by the dermal route. The objective of this research was to quantify the dermal absorption of DCHA in vitro using porcine skin because of its similarity to human skin both anatomically and biochemically. DCHA was applied to pig skin in water and in 3 generic MWF formulations commonly used in industry: Soluble (SO), synthetic (SYN), and semi-synthetic (SS) oil. Dermatomed pig skin (n=4) was loaded onto a flow-through diffusion cell system with individual dose areas of 0.64 cm² and perfused with media containing 4.5% bovine serum albumin for 8h to mimic occupational exposure conditions. Pig skin was dosed with 5-10% DCHA in 7 vehicles: Water, water + 5% SO, SYN, or SS oil; or neat SO, SYN, or SS oil. Dermal absorption of DCHA was similar between SO, SYN, and SS oil mixed with water, as well as between neat SO, SYN, and SS oil. Dermal absorption of DCHA from water + SS (0.08%) > water + SO (0.02%) > water + SYN (0.005%). Dermal absorption of DCHA from neat SS (0.11%) > neat SYN (0.099%) > neat SO (0.03%). The highest overall dermal absorption was at 1.4% from the water vehicle. These results suggest that water facilitates the dermal absorption of DCHA across skin whereas DCHA in MWFs may partition more with the vehicle due to its partitioning behavior. In conclusion, the varied dermal absorption of DCHA across MWF formulations must be taken into account within the machining industry and MWF manufacturing as repeated occupational exposure to this metalworking fluid additive may prove to be detrimental to human health. (Supported by NIOSH grant R01-01-03669)

Vehicle effects on the absorption of finite and infinite saturated doses of caffeine, mannitol, and testosterone in porcine skin.

The flow through diffusion cell system is an in vitro technique often used for the toxicological assessment of chemical absorption across skin. This approach routinely employs porcine skin as a surrogate membrane for human skin. Review of the literature highlights variations in experimental variables such as dosing solutions (finite/infinite or saturated/unsaturated and delivery vehicle), and receptor fluid. With such vast variations, data comparison can be difficult. This study aimed to standardize dosing solutions for three model 14C-labeled compounds (caffeine CF, mannitol MN, and testosterone TS) to evaluate the effect of delivery vehicle on absorption through porcine skin. A finite (20 μL) and infinite (1000 μL) saturated dose was applied in one of three vehicles: propylene glycol (PG), water (W), and ethanol (EtOH). Flux of each compound (n=3) into the receptor phase was monitored over 24 hours. Levels of radioactivity were also determined in the stratum corneum (by tape stripping) and the remaining skin. Apparent permeability coefficients and absorbed dose (μg) were then calculated and compared. Each compound showed unique absorption (abs.) profiles, such that from the infinite Ws doses, absorption was greater from MN (solubility 81442 μg/mL; abs. 50.4 μg) > CF (solubility 29092 μg/mL; abs. 24.9 μg) > TS (solubility 15.8 μg/mL; abs. 0.65 μg). The same profile was observed in the amount remaining in skin after 24 hours: MN (264 μg) > CF (193 μg) > TS (0.49 μg). The absorption and skin deposition profile from the infinite PG and EtOH doses followed the parent: TS > CF > MN. The skin absorption and skin deposition profile was maintained for the finite EtOH doses for all three compounds, but altered for the finite PG and Ws doses. This data highlights the variable effects a vehicle may exert on dermal absorption, suggesting that the contribution from the vehicle should be incorporated in dermal absorption models. (Supported by Novartis Animal Health, Inc.)

Xenobiotic metabolism capacities of human skin in comparison to 3D epidermis models and keratinocyte-based cell culture as in vitro alternatives for chemical testing: Phase I and II.

The metabolic competence of skin has so far not been fully characterized, although human skin fulfills important tasks in uptake, distribution and metabolism of chemicals, such as voluntarily applied substances and anthropogenic pollutants. Since the 7th Amendment to the EU Cosmetics Directive will prohibit the use of...
animals in cosmetic testing in the coming years, there is an urgent need to und-erstand the toxicological potential and test these en-zyme activities to in vitro models that may be used to replace animals in skin chem-ical testing. In this work, the enzymatic competences of the most prominent Phase I cytochrome P450 (CYP) and Phase II enzymes were investigated in ex vivo human skin, in the 3D epidermal model EpiDerm 200 (EPI-200), immortalized keratinocyte-based cell lines and primary normal human epidermal keratinocytes. Furthermore, cyclooxygenase (COX) activity was assessed, since also capable of phase I oxidation of chemicals through peroxidation. For phase II enzymes, we as-sessed activities of glutathione S-transferases (GST), N-acetyltransferases (NAT) and UDP-glucuronosyltransferases (UGT) in human skin and the alternative in vitro systems. Our data showed that basal Phase I xenobiotic metabolism enzyme activities of reconstructed epidermis are very similar to human skin microsomes. Monolayer cells, however, differ from EPI-200 and from each other in basal and in-ducible Phase I enzyme activities while they have similar phase II detoxication ca-pacities than human skin. Due to distinct phase I enzyme, especially COX, activi-ties in monolayer compared to 3D organotypic cultures, enzymatic activities of EPI-200 models are closer to human skin than monolayer cultures.

572 MAP KINASES REGULATE NITROGEN MUSTARD-INDUCED ACTIVATION OF DAMAGE-ASSOCIATED MOLECULAR PATTERNS IN MURINE KERATINOCYTES.

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Exposure to chemical vesicants results in skin inflammation and blistering. To in-vestigate mechanisms underlying these responses, we evaluated the effects of the ni-trogen mustard vesicant 2-chloro-N-(2-chloroethyl)-N-methylthethylamine (HN2) on cellular signaling pathways initiated by endogenous molecules released by dying cells, damage-associated molecular patterns (DAMP). Proteins active in DAMP sig-naling include members of the toll-like receptor (TLR), TLR adaptor protein, and NOD-like receptor (NLR) families. Using RT-PCR, we found concentration and time related increases in mRNA for TLR2, 3, 6, 7 and 8 following treatment of PAM 212 murine keratinocytes with 1-30 μM HN2. Increases in TLR mRNAs ranged from 4-15 fold, reached maximal levels by 24 hr and persisted for 72 hr. HN2 also increased TLR adaptor protein mRNA for TRAF6 (10-fold), MyD88 (4-fold), and NOD2 (13-fold) maximal responses were observed after 12 hr. HN2 also increased mRNAs downstream of DAMP including COX2, IL-1beta, NF-KB and PPAR gamma; maximal effects were evident 24-72 hrs after HN2 treatment. Taken together these data indicate that DAMP signaling is altered in response to HN2 in a process mediated by TLR and NOD. In further studies, SB203580, a p38 MAP kinase inhibitor, was found to suppress HN2-induced expression of MyD88, TRAF6 and NOD2 indicating that MAP kinases regulate DAMP responses to HN2. We speculate that DAMP signaling is important in vesicant-induced skin toxicity and that regulation of DAMP by MAP kinases may modulate HN2-in-duced injury in the skin. Supported by AR055073.

573 ASSESSMENT OF HUMAN SKIN PENETRATION OF TWO IRITANT HERBICIDES.

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Two common herbicides; isoproturon and bentazon, are strong skin irritants and cross the skin barrier easily. Assessment of percutaneous absorption of these sub-stances is a very important step in the evaluation of any dermal or transdermal dose, especially among agricultural workers who frequently have dermal exposures during crop treatment. The aims of the study were to determine the permeation rate of human skin for both herbicides in vitro, and historically evaluate skin damage due to irritation at different concentrations. Skin penetration was assessed using a dynamic flow-through in vitro penetration system and analysis were performed with ion chromatography (LC-IC50) and water activities (αw). Two concentra-tions of bentazon (75 and 150 μg/mL) and isoproturon (125 and 250 μg/mL) in saline solution were applied on excised human skin from several donors. Saline water was used as receptor fluid. Collection times were 4, 8, and 24 hours. After the experiments, the skin was removed and examined by histopathology for apop-tosis, acanthosis, acantholysis and epidermolysis. The skin permeation rate, J, was calculated from the slope of the cumulative amount permeated as a function of time. The lag time, tL, was assigned from the time-axis intercept of the extrapola-tion of this linearity. Our results showed that tL for bentazon and isoproturon for both concentrations tested were similar, 2, 1.5 hours, respectively. Bentazon had a lower J compared to isoproturon; 350, 600 ng/cm²/h, respectively. Some acan-thosis was observed after 8 hours of exposure to either of the two substances. In conclusion, our in vitro experiments demonstrate that bentazon and isoproturon cross the skin barrier within 2 hours even at very low concentrations, and showed some signs of skin damage. Future tests involve concentrations found in commer-cial products.

574 THE USE OF EXCISED HUMAN SKIN IN THE IN VITRO PREDICTION OF DERMAL CHEMICAL IRRITATION.

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Since the 1940s preliminary skin irritation evaluation has been conducted using the Draize test. Ethical concerns over the use of animals and recognition that the re-sponses in rabbits are not always predictive of human responses have initiated the development of in vitro which include RHE models, animal skin organ cultures and monolayer cell assays. However all of these differ from human skin by varying degrees therefore making the use of human skin the ideal model if its availability can be secured. The aim of this study was to investigate the response of testicular skin to a range of known chemical irritants and corrosives to determine and com-pare those that obtained in the in vivo Draize test. Human testicular skin (permission was obtained from South East London Research ethics committee 4 REC reference 10/H0807/51) was collected, prepared and mounted onto Millicell inserts and placed in RPMI 1640 (10% FCS, 2mM L-Glutamine, 50 IU/ml Penicillin, 50 μg/ml Streptomycin, 50 mg/ml Cholera toxin, 5 μg/ml Hydrocortisone) for 24h. The viability of the skin in culture was determined by the MTT assay; this demonstrated that viability could be maintained over the culture period (60% initial viability at 96h). To look at the response to irritants and corrosives chemicals were selected with varying Draize scores (Di-propylene glycol, IPA, Eugenol, limonene, tea tree oil, methacrolen, isostearic acid, KOH, SDS and Methyl butyric acid). Tissues were treated with 100μl of each chemical for 1h then washed with PBS to remove. The tissues where then placed in fresh media and the viability of the tissues was assessed at 48h by MTT assay and conditioned media was analysed for both IL-1α and IL-8 by ELISA. By combining the results from the cytokine response and viability assay it was possible to correctly assign 80% of the chemicals to their Draize classification i.e. non-irritants, irritants or corrosives. In conclusion the data generated supports the use of the human testicular skin in the in vitro predication of dermal chemical irritation.

575 SKIN METABOLISM OF CINNAMIC ALCOHOL AND CINNAMALDEHYDE: ITS ROLE IN CONTACT DERMATITIS.


Skin is an extrahepatic metabolizing organ. It can be involved in the metabolism of chemicals which could have penetrated it. Contact dermatitis results from the inter-action between human beings such as adhesives and endogenous proteins to form anti-genic complexes. Cinnamic alcohol (CIN-OH) and Cinnamaldehyde (CIN-CHO) are very well known pro-hapten and hapten, respectively. In this work, we studied the cutaneous metabolic fate of these two compounds. We used normal human skin (NHS) and three reconstructed human skin models (skin models) from SkinEthicTM laboratories to assure the relevance of their use. Results showed that CIN-OH is oxidized into CIN-CHO by alcohol deshydrogenases (ADH) and CIN-CHO is quickly oxidized into cinnamic acid by aldehyde deshydrogenases (ALDH). No other metabolite was detected. Apparent enzymatic parameters such as affinity (Km), maximal velocity (Vmax) and metabolic clearance, estimated by the Vmax/Km ratio, were calculated from metabolites assay in dose-effect studies. We observed that Vmax/Km ratio for ALDH (~ 0.8 ± 0.1 and 1.7 ± 0.6 μL/min×mg prot-1 for models and NHS, respectively) was more important than ADH ac-tivity (~ 0.2 ± 0.1 μL/min×mg prot-1 for NHS and models). ALDH activity was higher in NHS than in skin models but with a high individual variability. Regarding these results skin stratum corneum 300 μm will be triggered more likely when the aldehyde local concentration exceeds the capacity of ALDH isozymes to metab-olize it. Moreover, people who would be deficient in the gene expression of a crucial ALDH isoform could represent a sensitive target to CIN-CHO sensitization. In conclusion, findings show that, for these metabolic pathways, skin reveals a more detoxifying behavior than a bioactivating one. On the other hand, skin models can replace NHS to study the cutaneous metabolism of primary alcohols and aldehydes and determine the saturating concentration of substrates which could become toxic.
COMPARISON OF XENOBIOTIC METABOLIZING ENZYME ACTIVITIES IN NORMAL HUMAN SKIN AND RECONSTRUCTED HUMAN SKIN MODELS FROM SKINETHIC™ LABORATORIES.


Skin stands for the major protective barrier of the body to its environment. Also, skin is an organ involved in the metabolism of xenobiotics and its ability to metabolize them can become consequent when considering its total surface area (2 square meters). Consequently, research on skin metabolism would need a real scientific effort to characterize skin metabolizing enzymes and their activities. In addition, the European amendment to the cosmetic directive forbids the use of animal testing to assess the safety of new cosmetic ingredients. This policy has forced the cosmetic industry to develop in vitro tools such as reconstructed human skin models (skin models) as alternative methods to animal experiments. For these reasons, these skin models require to be characterized and compared with normal human skin (NHS) samples in terms of metabolic capabilities. mRNA expression of several enzymes (CYP450, Esterase, ADH, ALDH, NAT, GST, UGT, SULT...) were previously demonstrated. This work presents their apparent catalytic parameters determination (apparent Km, Vmax and the ratio Vmax/Km) in skin models compared with NHS. Results show that all these enzymes involved in the metabolism of xenobiotics are expressed and functional in the NHS and skin models. Also, the Vmax/Km ratios (estimating the intrinsic metabolic clearances) show that the metabolic abilities are the most often comparable between the skin models and NHS. These results indicate that the skin models can substitute themselves for NHS to select cosmetic ingredients on the basis of their metabolism.

SKIN SUBSTITUTE TISSUE BIOENGINEERED TO INHIBIT EXCESSIVE PROTEASE ACTIVITY IN CHRONIC CUTANEOUS WOUNDS.

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Successful wound closure requires balanced matrix metalloproteinase (MMP) and tissue inhibitor of metalloproteinase (TIMP) activity for proper granulation tissue formation and keratinocyte growth and migration. Chronic cutaneous wounds exhibit a highly proteolytic environment due to an increase in MMP activity and a decrease in TIMP activity inhibiting the healing process. Our hypothesis is that a human skin substitute genetically modified with a non-viral vector to express ele-

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In the U.S. and Europe, exposure and risk associated with dermal contact with media containing environmental chemicals are now primarily assessed using steady-state permeability data obtained from in vitro diffusion-cell experiments, or model predictions that (upwardly) adjust physico-chemical regression (PCR) fits to such data to address non-steady-state, short-term exposure conditions. This study tested the reliability of the latter approach to predict short-term dermal uptake of dilute aqueous lipophilic organic chemicals (LOCs). Effective permeability (Kup) predictions by two skin models were compared to values observed or derived from in vivo experiments that used the “difference method” to measure net chemical loss from solution over ~1 hour, with vs. without skin contact. In vivo Kup measures for over-lapping sets of 14, 17, 21 and 23 dilute LOCs were all well predicted (to within ~2-fold, r 0.89, p < 10⁻⁷) by set-specific PCR models. In contrast, the in-vitro-based Kup-prediction approach developed 20 years ago by the U.S. EPA, and still widely used, underestimates every experimentally based in vivo Kup value considered, by a median factor of ~10. Another similar model examined also tends to underestimate in vivo values substantially. Until wide discrepancies between in vivo measures and predictions based on in vitro data are better understood for aqueous organics, the in vivo database should be expanded, and it offers the more conservative basis for assessing dermal exposures by this route.
burden and is currently being used to calculate specific dose-associated decreases in tumor burden for both agents. The results support use of this model for the determination of ant-cancer therapy efficacy and surrounding normal tissue toxicity.

581 TCDD ENHANCES LIPID METABOLISM, CORNEOCYTE EXPRESSION, AND REACTIVE OXYGEN SPECIES, ACCELERATING KERATINOCYTE DIFFERENTIATION: AN INTEGRATED GENOMIC/METABOLOMIC APPROACH.

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Chloracne is commonly observed in people exposed to dioxins, yet the mechanism of toxicity is not well understood. The pathology of chloracne is characterized by hyperkeratinization of hair follicle cells, as well as a metabolic response of the ductular, sebum secreting, sebaceous glands. In vitro studies using normal human epidermal keratinocytes (NHEKs) to model interfollicular human epidermis demonstrate a 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-mediated acceleration of differentiation. By harnessing the combined strengths of microarray analysis and LC-MS, we integrated the effects of TCDD on NHEKs at the genomic, as well as functional level. HPTLC was used to measure changes in lipid quantities of the epidermal barrier - cholesterol, free fatty acids (FFA), and ceramides. Eight of nine ceramide classes in the lipid matrix were increased significantly by TCDD, with almost no change noted in cholesterol and FFA. Expression of numerous genes associated with cornocyte formation was increased by TCDD. Along with these increases in expression and cornocyte genes, TCDD increased mitochondrial ROS formation. Mechanisms by which TCDD increased ROS include (1) decreased levels of mitochondrial reduced glutathione with corresponding increases in oxidized glutathione, as a result of reduced mitochondrial glutathione reductase activity, and (2) reduction of enzymes and metabolites of the glycolytic pathway. Increases in ROS have been associated with mitochondrial apoptotic-like events that occur during keratinocyte terminal differentiation, indicating that increases in ROS, as well as increases in expression of lipid matrix and cornocyte genes, are mediating the acceleration of differentiation by TCDD.

582 PERCUTANEOUS ABSORPTION OF 14C-AMINOETHYLETHANOLAMINE, IN VITRO, USING THE FRANZ FINITE DOSE MODEL WITH HUMAN AND RAT SKIN.

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Aminoethylethanolamine (AEEA) is an organic base used in the manufacture of fuel/oil additives, chelating agents, surfactants and fabric softeners. This in vitro study was aimed at evaluating the effect of AEEA exposure on skin barrier integrity and to characterize its absorption in human and Wistar rat skin. Studies were conducted in 1cm² Franz diffusion cells using cryopreserved human skin trunk and rat skin. The skin barrier integrity test was based on the amount of 3H2O absorbed 30 min after a 5 min pulse exposure. The effect of AEEA on barrier integrity was assessed before and after (1 & 16-hr) on 8-hr exposure (5 µL/cm²) to 0, 0.25, 2.5, 10.0 and 25% AEEA (in water). The absorption of AEEA (5 µL/cm²) was measured in 3 skin donors using 0.25, 2.5 and 25% 14C-AEEA. 14C-AEEA flux was monitored over 24-hr; with removal of AEEA at 8-hr. Mass balance was assessed at 24-hr. An 8-hr exposure to 25% AEEA increased 3H2O permeability 1.5-fold (human) and 4.4-fold (rat) at 16-hr post-exposure. At lower AEEA concentrations (n=3), no changes in 3H2O permeability of human and rat skin were noted, except a 5.8-fold increase at 10% AEEA (16-hr) in rat skin. The water permeability of vehicle-only exposed rat skin, but not human skin, increased 4-fold at 16-hr post-exposure. Total AEEA absorption ranged from 14-31% (rat) and 3.2-3.6% (human). Absorption of 14C-AEEA showed a rapid early peak at ~30 min in rat, and a slow rise to a peak at ~7 hrs in human. In both, AEEA flux progressively declined after its removal at 8 hr. Thus, an 8-hr exposure to 25% AEEA concentration can moderately alter rat and human skin barrier integrity. However, the increased 3H2O permeability observed was within the normal range known for untreated human skin and is unlikely to represent a biologically relevant change in barrier integrity. AEEA can penetrate human and rat skin, but human skin is 4-10-fold less permeable than rat skin.

583 STRATAGRAFT® SKIN TISSUE AS AN ALTERNATIVE TO AUTOGRAFTING IN PROMOTING HEALING OF DEEP PARTIAL-THICKNESS BURNS.

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Deep partial-thickness burns are clinically managed in the same manner as full-thickness burns; by surgical excision with subsequent autologous skin grafting. Alternatives to autografting to reduce the pain and morbidity associated with donor site wounds are needed. Stratagraft, a novel living human skin substitute, has been developed to treat severe burns and other complex skin defects. This skin tissue consists of a dermal layer containing normal human dermal fibroblasts and a fully-stratified, biologically-active epidermis derived from NIKS® keratinocytes. In a phase I/IIa clinical trial, Stratagraft skin tissue was well tolerated and comparable to cadaver allograft in the ability to prepare full-thickness wounds for autografting. No product-related adverse events were observed and exposure to Stratagraft tissue did not elicit an acute immune response. A phase Ib clinical trial is being conducted to examine the safety and efficacy of Stratagraft skin tissue as an alternative to autografting for deep partial-thickness burns. Up to 20 patients will have excised deep partial-thickness burns covered with Stratagraft tissue to determine if it promotes healing and eliminates the need for autografting. The primary efficacy outcomes are wound closure after 3 months and the percentage of the initial wound area that requires autografting by 28 days after Stratagraft tissue application. Additional assessments include wound cosmesis, immunological responses, and persistence of allogenic DNA. It is anticipated that Stratagraft tissue may promote healing, improve survivability and reduce pain by serving as a barrier to water loss and infection, 2) promoting healing without autografting, 3) reducing pain by covering exposed nerve endings, 4) enhancing functional and cosmetic outcomes by quickly closing the wound, and 5) avoiding painful donor site wounds by eliminating the need for autografting. Results of this study will guide development of late stage clinical trials of Stratagraft skin tissue for use in severe burns and other complex skin defects.

584 AN INTEGRATIVE APPROACH FOR THE PREDICTION OF ACUTE SYSTEMIC TOXICITY, VALIDATION OF THE MODEL, COMBINING CELL TOXICITY, AND PHARMACOLOGICAL AND PHYSICO-CHEMICAL PROPERTIES.

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Developing alternatives in the area of acute systemic toxicity remains a challenge because of the complexity of the biological processes involved. However, we have demonstrated that applying a realistic approach, based upon the combination of multiple parameters could provide promising results. We showed that considerations of cell-death data, pharmacological profiles and physico-chemical properties resulted in a significant improvement of the LD50 model originally developed by CeTox (standard model). 75% of the chemicals pertaining to a revised GHS categorization were correctly classified versus 50% with the standard model. In addition, at an LD50 threshold of 500 mg/kg, the sensitivity and specificity were 85% and 89% against 71% and 83% with the standard model. The study presented herein describes results obtained with a new selection of chemicals. A validation set that consisted of 17 compounds was evaluated in the improved method and compared to in vivo data. Cytotoxicity and pharmacological parameters were processed in conjunction with specific physico-chemical properties in order to generate LD50 predictions. The results confirmed that the integrative approach was robust and that improvements made improved the predictions of highly toxic chemicals. The results showed that 16 out of the 17 chemicals were correctly categorized. In addition, by pooling the training and validation sets, the overall predictive performance remained high, with sensitivity and specificity of 87% and 89% at an LD50 threshold of 500 mg/kg. This study is a clear demonstration that one way to address acute systemic toxicity is the combination of multiple, mechanism-based parameters and key chemical properties. However, efforts are still required to expand the applicability domain of the model and anticipate the complexity and diversity of consumer products in terms of chemical reactivity and classes of solubility profiles.

585 METHODS FOR COMPARING COMPOUND SETS BASED ON THEIR PRECLINICAL IN VIVO STUDY OUTCOMES.

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In recent years there has been increasing interest in developing in vitro assays that are predictive of in vivo outcomes. In order to validate in vitro assays it is necessary to have a large set of compounds where the in vivo outcomes are known and can be
compared to the responses in the assay. Most efforts have focused on comparing assay responses to specific lesions like proximal tubule damage or broader grouped phenotypic responses like hepatotoxicity to see if trends and patterns exist.

However, recent publications have described other approaches using a more holistic comparison of the in vivo responses of compounds. One method used the systemic exposure at which the first sign of injury was observed irrespective of the nature of that injury to compare compound responses [1]. Other methods used the survival of a compound to a first in human clinical trial to look at properties of compounds that are most likely to succeed [2].

Here we describe an approach to provide a quantitative measure of the level of severity of in vivo toxicity thus combining findings across dose groups to yield a single metric of in vivo toxicity at the compound-study level to enable comparisons across compounds. This method was then applied to a set of Pfizer compounds to see how this measure relates to chemical properties and in vitro assay profiles when compared to a previously used system for classifying compounds. In general, the trends previously observed with physicochemical parameters such as lipophilicity and polarity remain unchanged yet there are improvements in the correlation to cytotoxicity measures [3] suggesting that this approach to comparing compounds improves upon previous classification systems.


587 ZEBRAFISH AS A MODEL FOR QUANTITATIVE ASSESSMENT OF HUMAN DEVELOPMENTAL TOXICITY.

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The zebrafish model has been shown to be a useful screening tool for qualitatively predicting mammalian developmental toxicity, both in terms of whether or not a chemical is teratogenic and the types of effects produced. However, application in risk assessment requires quantitative dose-response information. This study addresses the quantitative relationship between effect levels for developmental endpoints in zebrafish and rodent assays. To allow for comparison of effect levels, zebrafish studies using aqueous exposure with no estimate of dose to the egg (e.g. mg/kg) were excluded. For each chemical, rodent studies were identified which provided dose response data for similar endpoints as those measured in the zebrafish assays. The LOAEL (based on maternal dose) in the rodent studies was then compared to the estimated dose (e.g. mg/kg) to the zebrafish with suitable zebrafish studies were identified: 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), ethanol, caffeine, domoic acid (DA), and all-trans retinoic acid (tRA).

The ratios of rodent LOAELs to zebrafish LOAELs for specific developmental endpoints in these studies were as follows: TCDD, 2.9-3.8; ethanol, 1.0-2.3; caffeine, 2.1; DA, 1.5-2.7; tRA, 6.3-6.7. Comparing study LOAELs across all endpoints rather than for specific effects, the rodent:zebrafish ratios were 1.2-2.7. In all cases, developmental LOAELs from rodent studies were within an order of magnitude of developmental LOAELs in zebrafish. For most endpoints, the zebrafish was slightly more sensitive than rodents. This suggests that in cases where rodent developmental data are not available, the zebrafish may serve as a viable alternative for rapid and inexpensive quantitative assessment of developmental toxicity. Further study with a wider array of chemicals is necessary to validate this approach. The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the US EPA.

588 ACTIVITY OF US EPA's TOXCAST COMPOUNDS USING A C. ELEGANS GROWTH SCREEN.

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Tox21, an intergovernmental toxicity community, is exploring the use of high-throughput in vitro tests and alternative model organisms for prioritizing the large numbers of chemicals with limited toxicological data for more comprehensive testing and to develop prediction models for human disease. As part of this effort, the US EPA is evaluating collections of chemicals in a program known as ToxCast™. The ToxCastTM Phase I library contains 309 unique compounds, mainly pesticide active ingredients with well-characterized mammalian toxicities. The ToxCast™ Phase II library contains 67 novel chemicals including food additives, and industrial products. Toxicity assays in 96-well formats have been developed for C. elegans including reproduction, growth, and feeding using the COPAS Biosort. The C. elegans growth assay, which measures changes in size after a 48-h exposure, was used to screen both ToxCast™ libraries. Chemical activity was evaluated when possible by half-maximal effective concentrations (EC50s) and lowest effective concentrations (LECs). Previously, we reported that 64% of the Phase I chemicals were classified as active using an activity score based on decreased size at each concentration as well as steepness of negative concentration-response trends. Because the Phase II compound set is more chemically diverse, we expected a lower percentage of active compounds than for Phase I. However, 57% of chemicals were found to decrease C. elegans growth and development at the highest concentration tested (ranging from 48 μM to 100 mM). Excellent reproducibility was observed for the eight chemicals replicated in the Phase II library: seven were active and one was inactive in all replicates. Information and experience gained from the C. elegans screening of the ToxCast™ libraries will be used to inform the design of future toxicity screens of the Tox21 library, which contains approximately 10,000 compounds.

589 PROLIFERATION AS A KEY EVENT IN DEVELOPMENTAL TOXICITY: CHEMICAL SCREENING IN HUMAN NEURAL STEM CELLS USING HIGH-CONTENT IMAGING.


New toxicity testing approaches will rely on in vitro assays to assess chemical effects at the cellular and molecular level. Cell proliferation is imperative to normal development, and chemical disruption of this process can be detrimental to the organism. As part of an effort to elucidate toxicity pathways, we screened 38 known developmental toxicants for effects on proliferation of neural stem cells. Human neuroprogenitor (ReN CX) cells were grown in 96-well plates and exposed to chemicals (0.001 to 100 μM) for 24 h. BrdU, which incorporates into replicating DNA, was added to the cells 20 h post exposure. Proliferating (BrdU positive) cells were identified using immunocytochemistry and automated image analysis; cell viability was assessed in duplicate plates. The concentration which caused a 30% decrease from control (EC30) and the lowest statistically effective concentration (LEC) were compared across endpoints and chemicals. Of the 38 developmental toxicants tested, 16 induced at least a 30% decrease in proliferation, and in 14 cases this effect was also statistically significant. Thirteen out of the 16 chemicals affecting proliferation were known developmental neurotoxicants. For the chemicals that decreased proliferation, 6 did so at concentrations that did not affect cell viability. Seven chemicals with no reports of developmental toxicity (negative controls) were
also tested, and did not affect proliferation or cell viability. These results demonstrate the ability to screen rapidly chemicals for effects on cell cycle, a key event in development. Effects of this chemical set will be examined in other model systems (embryonic stem cells, zebrafish) to identify common pathways leading to developmental toxicity. This abstract does not necessarily reflect US EPA policy.

590 DEVELOPMENTAL EXPOSURE TO PFOS OR PFPA INDUCES SIMILAR BEHAVIORAL ALTERATIONS IN ZEBRAFISH AND IN MICE.

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The Zebrafish (ZF) has emerged as a relevant whole-organism model attractive also for behavioral neurotoxicologists, since the fish shows most of the behavior seen in terrestrial species. We have implemented ZF as a model for developmental neurotoxicity (DNT) studies and the initial aim is to evaluate our ZF behavioral data after ethological validation against the background of knowledge from rodent models. In this study we have tested the visual locomotor response in ZF larvae following developmental exposure to perfluorooctanoic acid (PFOA) or perfluorooctane sulfonate (PFOS) and compare the results with our behavioral data from PFOS- and PFOA-exposed mice. Fertilized ZF eggs were continuously exposed to 0.1 or 1 mg/L PFOS or PFOA in the water starting at 2 hpf (1-2 cell stage). The visual locomotor response was tested at 96 hpf by exposing the fish to a brief alternating period of light and darkness in a controlled environment that allowed live video tracking. Abrupt changes in light intensity induce a burst of swimming activity that typically subsides within 10 min after switching the light off in the observation chamber. Mice were exposed to 0.3 or 3 mg/kg PFOS or PFOA via maternal food throughout pregnancy. Locomotor activity was recorded at 5-6 weeks of age within 2 h after moving to a novel environment. After deriving similar parameters for the analysis of locomotor activity in both species, we found that both chemicals had similar effects in mice and ZF with similar dose-response curves. We found that exposure to either PFOS or PFOA resulted in hypoactivity and an accelerated decay in locomotor activity during the observation period. In light of additional behavioral observations, these findings are suggestive for a decrease in neuromuscular endurance in the animals exposed during early developmental stages. Taken together, our data point to ZF as a promising model for DNT studies designed for screening purposes and/or identification of mechanisms of neurotoxicity.

591 INVESTIGATION OF ZEBRAFISH YOLK SAC INJECTION.

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Zebrafish (ZF) is an upcoming animal model for toxicity assessment. However, unlike mammalian models, routes of drug administration for ZF are limited to exposure to either PFOS or PFOA via maternal food injection (p < 0.05, N = 5-8). D25 may block OCT3 or OCT3-like uptake 2 sites in mouse and zebrafish brains. Using a polyclonal rabbit anti-rat OCT3 antibody, we found that OCT3 mRNA type-labeling was 30% higher in zebrafish whole brain than in the mouse hippocampus, where OCT3 is richly expressed (N=5). Saturation binding of [3H] D22 in zebrafish whole brain and mouse hippocampal homogenates revealed a common KD of P = ± 1.5 mM, and a Bmax of 3532 ± 1187 fmol/mg protein in the zebrafish brain, and a Bmax of 1186 ± 231 fmol/mg protein in mouse hippocampus (N=3). Binding of [3H] D22 D25 uptake was blocked by the neurotoxic MPTP metabolite MPP+, a known substrate of OCT3. In OCT expressing HEK cells, [3H] MPP+ uptake was blocked with D25 with IC50 values (µM) of 0.9 ± 0.4 for OCT1, 0.3 ± 0.1 for OCT2 and 0.8 ± 0.2 for OCT3 (N=5). OCTs have affinity for and/or transport many endogenous and xenobiotic compounds, and it appears that their properties in zebrafish and mice are preserved. Further study of uptake 2 in zebrafish is necessary to determine if D22 is blocking OCT3-like transporter(s) in brain. Support by: NIMH064489, NIMH086708 & T42CCT610417.

592 BEHAVIORAL AND BINDING PROPERTIES OF DECENCYNNUM-22 IN ZEBRAFISH AND MICE.

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Uptake 2 mechanisms such as organic cation transporters (OCTs) modulate monoamine neurotransmission in brain. Their blockade produces effects similar to selective serotonin reuptake inhibitors. For example, decencynnum-22 (D-22) blocks 5-HT uptake and produces antidepressant-like effects in mice (Baganz et al., 2008, PNAS 105:18976-18991). We explored the behavioral and neurochemical properties of D-22 in mice and zebrafish. Administration of D-22 (0.01 mg/kg) or dizapam (1 mg/kg) significantly increased social approach in BTBR mice 50 min after injection (p < 0.05, N = 6). However, unlike dizapam, D-22 did not reduce marble burying in BTBR mice. In adult zebrafish, 5 min bath exposure to either 12.5 ± 0.2 or 125 ± 24 h D-22 did not affect swimming behavior, but without a reference dye. In the drive-tank or light/dark plus maze anxiety tests. In contrast, 25 mg/L buspirone increased top-dwelling in the drive-tank (p < 0.05, N = 5-8). D25 may block OCT3 or OCT3-like uptake 2 sites in mouse and zebrafish brains. Using a polyclonal rabbit anti-rat OCT3 antibody, we found that OCT3 mRNA type-labeling was 30% higher in zebrafish whole brain than in the mouse hippocampus, where OCT3 is richly expressed (N=5). Saturation binding of [3H] D-22 in zebrafish whole brain and mouse hippocampal homogenates revealed a common KD of P = ± 1.5 mM, and a Bmax of 3532 ± 1187 fmol/mg protein in the zebrafish brain, and a Bmax of 1186 ± 231 fmol/mg protein in mouse hippocampus (N=3). Binding of [3H] D-22 D22 uptake was blocked by the neurotoxic MPTP metabolite MPP+, a known substrate of OCT3. In OCT expressing HEK cells, [3H] MPP+ uptake was blocked with D25 with IC50 values (µM) of 0.9 ± 0.4 for OCT1, 0.3 ± 0.1 for OCT2 and 0.8 ± 0.2 for OCT3 (N=5). OCTs have affinity for and/or transport many endogenous and xenobiotic compounds, and it appears that their properties in zebrafish and mice are preserved. Further study of uptake 2 in zebrafish is necessary to determine if D-22 is blocking OCT3-like transporter(s) in brain. Support by: NIMH064489, NIMH086708 & T42CCT610417.

593 COMPARATIVE EVALUATION OF THE CONVENTIONAL RADIOACTIVE LOCAL LYMPH NODE ASSAY AND NONRADIOACTIVE ALTERNATIVES.

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The Local Lymph Node Assay is able to detect not only the sensitizing properties of chemicals but also the potency of a potential allergen, by evaluating different test concentration. OECD 429 includes the radioactive determination of incorporated 3H-Thymidine in the proliferating lymph node cells, whereas OECD 442B describes the nonradioactive determination by the ELISA-technique. The presentation will describe a comparative evaluation of both methods and the use of additional parameters included in the LLNA for discriminating sensitization from irritation. For this DNBC, HCA, Eugenol, Croton Oil and Benzoic acid were investigated in the LLNA following OECD 429 and 442B. As additional parameters for discriminating sensitization from irritation, evaluation of the total cell count and of the ear and lymph node weights were used. The results indicate that the conventional radioactive method, the ELISA method and the determination of the total number of cells allowing a clear differentiation between moderate, strong, extreme and nonsensitizers. It was shown that the determination of the ear thickness as an additional parameter has only limited value because of the inaccuracy of the method. The data lead to the conclusion that the LLNA ELISA method 442B is a useful substitute to the conventional radioactive LLNA (OECD 429), which will lead to minimisation of occupational radioactive exposure and to the elimination of radioactive waste and as well as to the performance of the LLNA in laboratories which are not licensed to use radioactive reagents.

594 ANIMAL STRAINS IMPACT THE LLNA-BRDU RESULT.

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OECD 442B describes the Local Lymph Node Assay (LLNA): BrdU_ELISA method for evaluation of skin sensitisation potential of chemicals without the use of radiotopes. The method utilizes non-radiolabelled 5-bromo-2-deoxyuridine
in composition due to source of the extracts. The toxicity class was established by a literature review on KE indicated 10 principal constituents with small variations. The compound was recovered. There is no strong scientific evidence to support such claims. It is considered beneficial to the skin, helping to maintain skin hydration, barrier function, and pH. In addition, geniposide is known to have antioxidant activity, anti-inflammatory effects, and anti-apoptotic properties.

Intestinal microflora is able to produce toxic or carcinogenic metabolites and induce more potent cytotoxicity against cells than non-metabolites. This study was performed to investigate the cytotoxic responses of geniposide and its metabolite to determine the metabolism of cytotoxic activities. Genipin, geniposide metabolite, increases cytotoxic effect in cells, but not with geniposide. Incubation of geniposide with intestinal microflora, its aglycone genipin could be produced by β-glucosidase, and cytotoxicity was detected which was highly increased when compared to geniposide. Western blot analysis revealed the phosphorylated e-Jun NF2 terminal kinase (JNK), caspase-3 and bax protein significantly increased in a dose-dependent manner after treatment of activated geniposide. In our thinking, the activation of JNK maybe result in the increase of the bax protein, which further induced cell apoptosis death. However, geniposide alone did not induce cytotoxicity as well as caspase-3. In addition, activated geniposide-induced apoptosis was confirmed by apoptosis assay, and generated reactive oxygen species (ROS), and N-acetyl-L-cysteine (NAC) suppressed activation of ROS and apoptotic cell death. Taken together, these findings suggest that the human intestinal microflora is capable to metabolize geniposide to genipin and its related biological activities and induced apoptosis. This research was supported by a grant (09172KFDAA916) from Korea Food & Drug Administration in 2011.

A WEIGHT-OF-EVIDENCE APPROACH FOR THE SAFETY EVALUATION OF KOMBUCHA EXTRACT IN COSMETIC PRODUCTS.

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Kombucha Extract (KE) is derived from the fermentation of yeasts and bacteria with black tea, sugar, etc. It is used in a variety of cosmetic products. As Kombucha Tea, it is consumed as a beverage in many parts of the world and thought to exert a number of therapeutic benefits in metabolic diseases, arthritis, psoriasis, constipation, indigestion, hypertension, etc., although there is no strong scientific evidence to support such claims. It is considered beneficial to the skin, helping to maintain moisture and elasticity so it appears more even in tone and texture. The KE components vary with the species of bacteria and yeasts, fermentation time, quantity of substrates in culture and exposure conditions. Being a complex mixture, safety data is not readily available, so we utilized a weight-of-evidence (WoE) approach to support its safe use in personal care products. This evaluation entails an initial in silico screening, read-across review, in vitro skin irritation estimation, establishing allowable exposure values based on TTC, and a clinical study to confirm skin tolerance. A literature review on KE indicated 10 principal constituents with small variations in composition due to source of the extracts. The toxicity class was established by the Cramer rules using ToxTree, DEREK (for structural alerts) and OECD Toolbox (for “read-across”) evaluations were performed. Octanol-water partition coefficient and water solubility (from EPISUITE) with the molecular weight were used to estimate dermal penetration. An exposure calculation for each KE constituent was done for a face cream at use level of 3%. The exposure was then compared to allowable levels of systemic exposure for each constituent. Results from in silico analysis verified each KE constituent was at a safe use level. An in vitro EPISKIN assay with the 3% KE face cream demonstrated it would not be a skin irritant. A clinical study revealed the face cream was neither a skin irritant nor sensitizer. Therefore, Wolf approach is very useful in confirming the safety of a cosmetic ingredient in a product prior to market launch.

A WEIGHT-OF-EVIDENCE-BASED APPROACH FOR THE SAFETY ASSESSMENT OF JUNIPERUS COMMUNIS BERRY EXTRACTS IN PERSONAL CARE PRODUCTS.


Natural, plant-derived ingredients are increasingly being used in personal care products but the safety evaluation of these poorly characterized complex mixtures is a challenge. We previously demonstrated the safe use of Juniper Berry Oil (JBO) utilizing a combination of the Threshold of Toxicological Concern (TTC) with a weight-of-evidence approach. JBO is typically steam distilled and has low molecular weight components with a significant potential for percutaneous absorption. Juniper berries can also be extracted using organic solvents or CO2 yielding different concentrations and components. As with JBO, we classified extract constituents according to generic groups using the JECPA approach. For each chemical constituent, its maximum known concentration in the oil, its molecular weight, and the estimated skin penetration potential were used to estimate a maximal daily systemic exposure. These nominal exposure values were compared to the respective TTC and/or the food intake values of “no safety concern.” In cases where systemic exposure exceeded TTC levels, additional evaluations were done. For each generic group, constituents with adequate safety data were identified and extrapolated to other members of the congenic group in a “read-across” approach. In the extracts, a new component, 1,2-epoxy-1,5,8,9-tetramethyl-undeca-5,9-diene, exceeded its TTC value at the initially set use level. Potential structural alerts and a metabolic profile for this component were generated using Derek Nexus and Time computer programs. These did not allow a presumption of safety; therefore, the use concentration of this extract needs to be reduced to exposure values below its TTC value. Using juniper berry extracts as an example, we are able to demonstrate the practical utility of a weight-of-evidence approach using TTC, read across and other in silico tools to evaluate and limit the safe use of complex plant-derived materials in cosmetic and personal care products.

IN VITRO KINETICS OF CHLORPROMAZINE IN CYTOTOXICITY ASSAYS WITH CACO-2, BALB/c 3T3 AND HEPG2 CELLS.


The toxicity of compounds can be tested in in vitro assays using different types of cells. The cells are usually exposed to the test compound for 24-72 hours after which the viability of the cells is determined. However, different cell types need different culture conditions, including the presence of serum in the medium. The aim of this project, part of the European project Predict-IV, was to study the in vitro kinetics of the pharmaceutical chlorpromazine (CPZ) in three different cell assays. Three cell types were used for the cytotoxicity assays: the human intestinal cell line Caco-2 cells, mouse fibroblast Balb/c 3T3 cells and the human liver cell line HepaRG cells. The cells were cultured for 24 hours and the Caco-2 cells for 1 week. The HepaRG cells were cultured for 4 weeks, with 2%DSMO in the medium in the last 2 weeks. Next, the cells were exposed to different concentrations of CPZ for 48 hours. Medium samples were taken at the beginning and at the end of the exposure. Viability of the cells was measured by the Alamar Blue method. At the end of the exposure, CPZ was extracted from the cells and well plastic. Nd-SPME was used to determine the free concentration of CPZ in the medium. All samples were analyzed by HPLC-UV.

The amount of CPZ in the medium decreased over time; the most pronounced decrease was seen in the metabolically competent HepaRG cells. Plastic binding of CPZ is negligible in all three systems. At the end of the exposure, all CPZ was recovered from the Caco-2 and Balb/c 3T3 cells; in the HepaRG cells less parent compound was recovered. The Balb/c 3T3 cells appear to be the most sensitive to chlorpromazine based on the nominal concentrations. Protein binding influences the cytotoxicity results; therefore the free concentration is an important factor. In conclusion, chlorpromazine differ in vitro kinetics in different cell systems. Thus, for a better interpretation of cytotoxicity results, it is important to take these in vitro kinetics into account.
Drug-induced phototoxicity can be caused after the exposure of skin to photoreactive drugs, triggered by sunlight exposure to the body surface. The establishment of effective methodology to predict the phototoxicity has been attempted over the past few years. Recent studies demonstrated that generation of reactive oxygen was responsible for the induction of early phototoxic events. Reactive oxygen species (ROS) assay to determine ROS generated from photoirradiated chemicals was proposed for recognizing their phototoxic potential. The present study was undertaken to evaluate the intra- and inter-laboratory accuracy and precision of the ROS assay using 44 coded chemicals, and the validation study was supervised by the Japanese Center for the Validation of Alternative Methods (JaCVAM). Most phototoxic chemicals tended to generate singlet oxygen and superoxide under light exposure, but non-phototoxic chemicals did not. The intra- and inter-day precisions (coefficient of variation; CV) for the determination of ROS from irradiated quinine (200 μM), a typical phototoxic drug, were found to be 3.3 and 4.5%, respectively. The inter-laboratory CV for quinine averaged 10.2% for singlet oxygen and 26.0% for superoxide. Z’-factors for the determination of singlet oxygen and superoxide were calculated to be 0.92 and 0.87, respectively, demonstrating a large separation band between samples and blank signals. These data would be indicative of suitability of ROS assay for phototoxicity prediction.

Human hepatocytes represent the “gold standard” for drug metabolism, drug-drug interactions, and in vitro hepatotoxicity studies. One drawback of the use of human hepatocytes in culture is that drug metabolizing enzyme activities such as CYP3A4 would decrease rapidly in culture (approximately 90% decrease per day) to 10% of the initial activity after several days. This drawback limits the use of the hepatocytes, especially for prolonged metabolism (e.g., for slowly metabolizing compounds), or to mimic low-dose, chronic exposure to hepatotoxic compounds. We report here the results of culturing human hepatocytes in a novel medium, Li’s Differentiation Maintenance Medium (LDMM). Cryopreserved hepatocytes from three donors were cultured in Cryopreserved Hepatocyte Plating Medium (CHPM) or LDMM for 14 days. CYP3A4 activities were quantified using Lucifer-IPA as substrate from days 1 (day of plating) to 14. While both media were able to maintain viability of the hepatocyte cultures, CYP3A4 activity decreased as expected in CHPM to nearly nondetectable level at day 14, while that in LDMM decreased from day 1 to approximately 50% at day 4, then increased to near day 1 level from days 7 to 14. This phenomenon was observed in all of the three lots of cryopreserved human hepatocytes. Treatment of hepatocytes cultured in LDMM with CYP3A4 inducer led to activities higher than that on the day of plating, thereby modeling in vivo situation. Evaluation of gene expression showed that human hepatocytes cultured in LDMM expressed near normal level of differentiation markers, uptake and efflux transporters. The results suggest that LDMM represent an improved culture medium which would extend the application of human hepatocytes in drug metabolism, drug-drug interactions, and hepatotoxicity studies.

The human liver contains a variety of metabolic enzymes, and understanding the role of these enzymes in drug metabolism is an important area of research that impacts human toxicology testing, and ultimately drug safety. Approximately 40% of drugs metabolized in the liver due to liver metabolism, and it is not identified until the late stage in the drug development process. Efforts have been made to position predictive in vitro screens earlier in the drug discovery process. To address this need, Solidus Biosciences has developed the DataChip/MetaChip platform that couples a 3-D cell culture microarray with a complementary microarray consisting of recombinant enzymes to identify metabolism-induced toxicity in a high-throughput manner. The DataChip platform has contained 532 elevated micropillars, each supporting 60-nL alginne 3-D matrices consisting of Hep3B cells, that become submersed in microwells of the complementary MetaChip. Various mixtures of recombinant enzymes are used to metabolize drugs, and the results are compared to the in vivo data to identify metabolic liabilities.

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binant metabolic enzymes that are designed to mimic the metabolic reactions in the human liver were encapsulated within 120 µL spots of Matrigel in the microwells to enable the in situ generation of metabolites. To validate the DataChip/MetaChip platform for metabolism-induced toxicity, 10 literature compounds which carry warnings for hepatotoxicity or have been withdrawn from the market due to idiosyncratic hepatotoxicity were tested. The toxicity of 7 compounds out of 10 was correctly identified using this approach. In addition, 22 proprietary Pfizer compounds were selected for validation as well. Of the 22 Pfizer compounds, 10 showed deactivation on metabolic enzyme spots, whereas 3 showed activation of the toxic response. Troglitazone was included as a control compound on every chip for standardization. The DataChip/MetaChip platform shows minimal day-to-day and chip-to-chip variability, and high-throughput capability and predictability to identify metabolism-induced toxicity at early stages in compound safety assessment.

**604 TEAMCHIP FOR HIGH-THROUGHPUT GENE TRANSFECTION AND METABOLISM-INDUCED TOXICITY SCREENING.**

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Variation in metabolic enzyme expression among segments of the human population may cause deviations from the expected pharmacokinetic profile of a drug, resulting in idiosyncratic toxicity or a lack of efficacy. Cell lines that stably express individual or combinations of metabolic enzymes are emerging tools for prediction of these rare clinical events; however, it is difficult to create and maintain a library of stable cell lines that mimic the diversity of metabolic profiles that could arise to unanticipated outcomes. In addition, it is difficult to control the expression levels of each of the enzymes in stably-transfected cell lines. To address this need, we have developed a Transfected Enzyme and Metabolism Chip (or TeamChip) for high-throughput analysis of systematic drug metabolism and toxicology. The TeamChip is prepared by simultaneously infecting an array of miniaturized 3-D cell cultures with varying concentrations of recombinant adenosines carrying genes for different metabolic enzymes, which generates an array of cell cultures with differentiated metabolizing capabilities. We have demonstrated the controlled expression of individual and multiple reporter proteins (GFP and RFP) and drug-metabolizing enzymes (CYP3A4, CYP2C9, and UGT1A4) in three human liver cell lines (HepG3B, HepG2, and THLE-2 cells) on a microarray platform by altering the multiplicity of infection of the various recombinant adenosines. As a proof of concept, 10 model compounds that were shown to be hepatotoxic, in some cases idiosyncratically and withdrawn from the market, were tested to simulate enzyme-specific hepatotoxicity. These studies demonstrate that the TeamChip platform can provide critical information necessary for evaluating metabolism-induced toxicity in a high-throughput manner.

**605 SELECTING GENES FOR USE IN A HIGH-THROUGHPUT MECHANISM-BASED TOXICITY SCREEN USING C. ELEGANS.**

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The high level of homology between the stress response pathways and genes in mammals and nematodes can be leveraged to create a high-throughput toxicological screen in C. elegans to predict responses in higher organisms. Transcriptional response was measured in several strains of transgenic C. elegans containing fluorescent protein driven by the promoters of stress-inducible C. elegans genes. This system captured both changes in pathway activation, as well as tissue-specificity of gene expression. Genes of interest encompassed a broad variety of identified mechanisms of toxicity including: heat shock proteins, metallothioneins, UDP-glucuronosyl transferases, P450s, and glutathione-S-transferases. To select genes with a sensitive and specific stress response, we compared transcription of stress response genes using an array of well understood compounds: paraquat, ethyl methanesulfonate, cadmium, chlorpyrifos and tunicamycin. These chemicals were used to create a toxicant response profile, representing oxidative stress, DNA damage, metal toxicity, neurotoxicity and ER stress pathways, respectively. Nematodes were treated with low, medium and high concentrations (covering 1-2 orders of magnitude) of each toxicant. Florescence intensity was measured from images of transgenic nematodes via heat shock. For example, a transcription at the highest concentrations was confined by overt toxicity. These results illustrate that a model of toxicity based on gene regulation in C. elegans is a viable method for detecting subtoxic responses in a high-throughput assay.

**606 USE OF IN VITRO BIOASSAY AND CLINICAL DATA FOR MODELING DRUG-DRUG/Chemical Interactions (DDCIs) MEDIATED BY CYTOCHROME P450 3A4.**

Y. Tie and E. Demchuk. ATSDR/CDC, Atlanta, GA. Sponsor: B. Fowler.

Cytochrome P450 3A4 is responsible for metabolism of many xenobiotics. Co-administration of drugs and environmental chemicals may cause changes in 3A4 metabolism that can lead to different health outcomes. Such DDCIs are difficult to evaluate during the pre-clinical stage of drug development. In the present work we focus on the feasibility of using in vitro 3A4 inhibition data from high-throughput screening (HTS) assays to estimate the in vivo DDCIs that were observed. The in vitro HTS data were obtained from the PubChem database, in which 13,072 compounds previously have been categorized as active, inactive, or inconclusive based on the inhibition constant, IC50. Alternatively, 702 drugs categorized in the literature based on the drugs’ clinical effects were used: 241 of them inhibited 3A4 and 461 did not. For the 702 drugs only 260 were in common with the HTS database. The overlapping drugs were further categorized in PubChem as active, 142 active and 69 inactive. Using the clinical data as a reference and HTS data as a predictor, we evaluated the utility of HTS as a classifier of clinical DDCIs and obtained a 43.5% sensitivity, 84.4% specificity, and 69.6% overall accuracy. The potency of 241 drugs that inhibit 3A4 in vivo was examined. Based on clinical categorization given by the Merck Manual, 35 were attributed to potent and 88 to weak inhibitors of 3A4. 46 of the 121 compounds were in common with the HTS dataset, of which only 17 were labeled in PubChem as active. Their IC50 values were then extrapolated to in vivo pharmacokinetic area-under-curve ratios, and subsequently subjected to categorization according to their inhibition potencies following the FDA guide. Unfortunately, 10 of the 17 inhibitors could not be categorized because of insufficient statistical power of the data, although among 7 drugs that were successfully classified, no misclassification of metabolic reactions was observed. If the HTS study design is improved, in vitro data may become useful for classification of clinical DDCIs mediated by 3A4. However at present, HTS data are not yet fully representative of the in vivo potency of inhibition.

**607 USE OF PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING (PBPK) IN CONJUNCTION WITH CELLCHIP™ TO PREDICT COMPOUND SAFETY THRESHOLDS AND IMPROVE LEAD OPTIMIZATION.**

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The CellCiphr™, an in vitro High Content Screening (HCS) Toxicity platform has been used by us to delineate mechanisms of toxicity as well as to predict the maximum plasma concentration of a drug (Cmax) at which a compound is classified as presenting toxic side effects through retrospective analysis of compounds with in vivo exposure and toxicity data. However, at early stages of drug discovery, before safety information is generated in vivo in animals and humans, the practical use of this information in prioritizing compounds is limited by the absence of in vivo exposure data. Here we explored if in the absence of in vivo data, one can apply CellCiphr™ in conjunction with Cloe PK, an in silico Physiology Based Pharmacokinetic (PBPK) modeling program that predicts Cmax from oral dosing, to predict dosing regimens for both rodent and human testing. We demonstrate that Cloe PK, can be mathematically inverted in order to predict an oral dosing regimen from a given Cmax safety threshold. This mathematical development, based predictive toxicology platform. We show how technical issues, (e.g., varying metabolism of different compounds in vivo and varying dosing protocols), affect the outcome of the simulations, and how these considerations can provide guidance to proper preclinical in vivo experimental design. We demonstrate the effectiveness of this predictive model for members of the piperazine family of antidepressants, oxaprozin, disoproxil, and buspirone as well as members of the endothelin receptor antagonists family of pulmonary hypertension drugs; sakanexan, bosentan, and ambrisentan.
PS 608 DEVELOPMENT OF ORGANotypIC IN VITRO HUMAN MODELS WITH ENGINEERED GENE KNOCKDOWN OR TOXICOLOGICAL REPORTER FUNCTIONS.


In vitro human 3D epithelial models including skin (e.g., EpiDerm, EpiDerm–FT) and airway (e.g. EpiAirway, EpiAirway–FT) are important advances over traditional monolayer cell cultures. These models provide more realistic, in vivo-like structure, barrier properties, metabolic functions and dosing capabilities compared to monolayer cultures. Here we describe development of organotypic 3D epithelial models with added features of engineered gene knockdown or toxicologic reporter functions. To create NFKB and Nr2 reporter models, early passage normal human epidermal keratinocytes, dermal fibroblasts, tracheobronchial epithelial cells and pulmonary fibroblasts were transduced with lentiviral vectors containing the respective transcription factor (TF) response elements linked to either GFP or luciferase. To create gene knockdown models of NFKB, IL-1t, MMP-1 and MMP-7, lentiviral vectors containing tetracycline (Tet) inducible shRNA and RFP were utilized. Stably transduced cells were selected by puromycin resistance, expanded several passages and cryopreserved to produce large pools of cells for organotypic skin and airway model production. Organotypic structure and barrier properties of models produced from the transduced cells were similar to models produced from untransduced cells, as determined by histological assessment and barrier function measurements. NFKB and Nr2 reporter models readily responded to positive control treatments, TNFα and r-butyldihydroquinone, respectively. GFP was detected in fixed paraffin sections by fluorescence microscopy. Luciferase activity in tissue extracts was quantified by microplate luminometer. In gene knockdown models, exposure of models to Tet induced robust RFP expression and >70% knockdown of IL-1t gene and protein expression. These gene knockdown and TF reporter models, together with additional models that may be produced by the methods demonstrated in the current work, will provide important new tools for conducting mechanistic toxicological studies.

PS 609 INCORPORATING THE THREE R’S INTO CONSUMER AND HEALTH PRODUCTS SAFETY TESTING.


Assessing the potential toxicity of pesticides, metals, and environmental compounds using current animal-based methods are expensive, difficult to standardize and cannot scale to the throughput required for screening hundreds of thousands of potential active chemicals required by new regulations. Compliance costs are escalating, with estimates in the millions of animals and billions of dollars. Incorporating the “Three R’s” for alternative animal testing (Replacement, Reduction, Refinement) provides ethical principles to help minimize animal use for research. Thus, the need for increasing throughput but reducing in vivo models necessitates the use of in vitro technologies and automation for preliminary safety assessment.

We tested a subset of natural, industrial and pharmaceutical compounds and environmental contaminants (including but not limited to dicofol, estradiol, lead, and acetonitrile) with three separate biological assays. By employing the same battery and concentration of compounds for each assay using quantitative fluorescent imaging, general toxicity was assessed by monitoring specific biological features. Endocrine activity was evaluated with estrogen and androgen receptor cell lines. Neuroscreen-1 cells treated with these compounds were used to assess developmentnal neurotoxicity (i.e., morphology, loss of neurites and branching). Genotoxicity (via micronucleus frequency) was compared with the same compounds in CHO-K1 cells. Time to complete all three assays took less than two weeks from setup to overall evaluation of each compound, compared to months using animal models. A variety of biological profiles were observed with the tested compounds, showing how general toxicants can act by various mechanisms of action, while others are more localized and only warrant specific outcomes.

PS 610 IDENTIFICATION OF A CELL AMOUNT INDICATOR TO NORMALIZE IN VITRO METABOLOMICS DATA.


In vitro screening systems are particularly well suited to preclinical toxicology testing at an early stage of drug development as they have the advantage of being fast and requiring only a small amount of test substance. The demands for in vitro screening assays for systemic toxicity are multiple and include the need of organ specific cell systems, the use of optimal cell numbers, cell passages and incubation times. Even minimal changes in the conditions of the test system may lead to significant changes of the biological system. Therefore a reliable normalization compensating biological variability is crucial prior to any interpretation of results generated from a biological system. BASF SE has developed an in vitro metabolite profiling assay and a subsequently tuned normalization strategy allowing the prediction of specific organ toxicity. The in vitro assay consist of exposing cells lines to test substances and to determine the metabolite profile using chromatography coupled to mass spectrometry systems. Herein we compare five different normalization strategies referring to their suitability in the application to in vitro metabolite profiling data. The strategies comprise statistical approaches, approaches referring to reference values from each individual sample or samples generated in dependent batches. Best results were achieved by an individual strategy using a new reference value correlating well over a large range of cell counts previously used for generating corresponding cell extracts. Statistical analysis revealed the normalization based on the new reference value greatly improved the quality of the results compared to non-normalized samples as well as to all remaining strategies. Generation and application of this new reference value and the corresponding normalization strategy will be presented the first time. Validation will be featured on the basis of extracts of the human hepatocellular carcinoma cell line Hep G2.

PS 611 HARNESSING CHEMILUMINESCENCE TO DELIVER MECHANISTIC-INFORMATIVE IN VITRO ASSAYS FOR TOXICOLOGICAL SCREENING.

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We have developed a generic technology to support animal-free molecular toxicology assays and applied it to two areas of interest, steroidogenesis and genotoxicity. This technology utilizes a detection system based on acridinium ester (AE) chemiluminescence which allows sensitive and reproducible quantitation of gene expression without the use of a reporter system or the need to amplify mRNA targets. The light output is directly attributable to the number of transcripts present. This method has in the past been used successfully to measure genetic indicators for endpoints of the Rat Uterine assay and also for endpoints specific for endocrine disruption in several fish species. The use of molecular-endpoints to identify and classify compounds provides significant potential for high-throughput in vitro screening. A limited suite of molecular endpoints, when measured in concert, will allow prediction of the mode of action and provide mechanistic information of the test compound by identifying the pathways affected. We developed and optimised seven assays to established indicators. These include endpoints for measuring genotoxic activity (tumour suppressor p53, RAD51C, cystatin A) and effects on steroidogenesis enzymes (CYP21A2, CYP19A1, HSD2). An assay for beta-actin transcript has been developed and optimised to be used in conjunction with the other assays to allow standardisation. Inter- and intra-assay variation is generally low with an average coefficient of variance of less than 15%. Target concentrations as low as 0.5 – 1 fmol can be measured and discrimination between targets with a single base pair difference can be made. To assess genotoxic or endocrine disruption, the effect of chemicals on HEPG2 or H295R cell lines respectively will be determined by using the probes to measure specifically these RNA transcripts. Employing genetic endpoints in this way will provide a basis for a rapid screening technology allowing accurate assessment of the action of new drug/chemicals.

PS 612 A SYSTEMATIC APPROACH FOR STUDYING THE MITOCHONDRIAL TOXICITY OF ENVIRONMENTAL CHEMICALS.

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A goal of the Tox21 program is to transit toxicity testing from traditional in vivo models to novel in vitro assays that assess how chemicals affect cellular responses and toxicity pathways. A critical NCGC contribution to Tox21 is implementation of a quantitative high throughput screening (qHTS) effort, using cell-based and biochemical assays, to generate toxicological profiles for thousands of compounds. In the present study, changes in mitochondrial membrane potential produced by different chemicals were evaluated in HepG2 cells using a cationic fluorescence dye, an assay optimized for qHTS. Of the 1343 unique compounds in the NTP library...
screened over 14 concentrations (0.59 nM—92 μM), 221 (16.5%) and 189 (14%) compounds disrupted the mitochondrial potential in HepG2 cells after treatment for one and five hours, respectively. Eighty-five compounds positive at both time points were clustered by structural similarity, resulting in 17 clusters and 26 singletons. Thirty-nine compounds covering most of the chemical space represented by the 85 compounds were more extensively evaluated. All 39 compounds were confirmed to disrupt mitochondrial membrane potential using a fluorescence plate reader and a high content imaging approach. Among these 39 compounds, 4 and 8 induced some cytotoxicity (LDH release) at 1 and 5 hours, respectively, and 12 induced caspase 9 activity at 5 hrs of treatment. To further understand the mechanisms of toxicity for these compounds, we measured changes in oxygen consumption rates (OCR) during treatment. Fourteen compounds, including trichlorophenol and captan, two known uncouplers, increased OCR and four compounds decreased OCR. This approach allows us to evaluate thousands of environmental chemicals for their potential mitochondrial toxicity and define mechanisms of toxicity. Supported by NIEHS Interagency Agreement Y2-ES-7020-01.

614 CHARACTERIZING CHANGES IN POLYUNSATURATED FATTY ACID PROFILES IN C. ELEGANS IN RESPONSE TO DEVELOPMENTAL STAGE, FOOD, AND CADMIUM EXPOSURE.


Understanding the mechanisms by which exposure to the environmental toxicant, cadmium, causes multiple disease phenotypes is relevant to human health. Metals are known to affect insulin and retinoic acid signaling, and cadmium has been implicated as a factor of transcription of fatty acid synthesis enzymes. Deregelation of proper intake and metabolism of fatty acids is associated with human diseases such as hypertension, diabetes, inflammatory disorders, and cancer. Defecation cycles in the nematode C. elegans are caused by rhythmic muscle contractions and have been used as a model for human cardiac cycles. We have shown that defecation cycles in cadmium-exposed nematodes phenotypically copy nematodes with disrupted fatty acid synthesis. Based on this evidence, we suspected that cadmium exposure may disrupt fatty acid synthesis in C. elegans and may also contribute to cadmium-induced disease. To understand how cadmium affects fatty acid metabolism, we have used gas phase chromatography tandem mass spectrometry (GCMS) to characterize changes in fatty acid metabolites in nematodes exposed to cadmium. Initial results show that cadmium-exposed nematodes accumulate the saturated fatty acids palmitic acid (16:0) and steric acid (18:0), indicating that cadmium may decrease that activity of delta 9 desaturase in C. elegans. This is consistent with evidence that cadmium decreases activity of delta 9 desaturase in rat liver and decreases transcription of delta 9 desaturase in hepatocytes. Because cadmium is a neurotoxicant and affects both feeding and development in C. elegans, it is possible that the effects observed here may be due to changes in feeding and development. Therefore, we also defined the fatty acid metabolic profiles of nematodes in response to food availability and throughout development. Our results indicate that fatty acid metabolism may be affected by life stage and food availability, however, cadmium exposure affects fatty acid composition in C. elegans independent of developmental stage or food availability.


Cardiototoxicity is one of the most prevalent adverse effects of drugs that affects patients and, in the pharmaceutical industry, is a leading cause of drug candidate attrition and the withdrawal of FDA-approved drugs from the market. We have developed an in vitro assay that predicts a drug’s propensity to induce cardiotoxicity. To accomplish this goal, several types of cardiac cells, including primary cardiomyocytes, human embryonic stem cell-derived cardiac precursors, and human induced pluripotent stem (hiPS) cell-derived cardiomyocytes, were evaluated. As a result, the hiPS cell-derived cardiomyocytes were selected for further study based on their purity, availability, reproducibility, and ability to function similarly to their in vivo counterparts. Preliminary studies with these cardiomyocytes indicated that a cell density of 50K cells per well in a 96 well plate provides an optimal level of secreted factors to be evaluated using mass spectrometry. hiPS cell-derived cardiomyocytes were then exposed to a training set consisting of compounds from different chemical classes that are of known inducers (15 compounds) and noninducers (10 compounds) for cardiotoxicity. Doses for the compounds used for metabolic analysis were based on known human cardiac exposures and the effects on cell viability measured in culture. The cardiomyocytes were exposed to compounds for 72 hours and the spent culture media was analyzed following treatment using LC-ESI-QTOF mass spectrometry. Univariate and multivariate statistical analysis was used to identify a metabolic signature of features that altered in response to cardiotoxicants. These features were then used to test the potential of predictive models capable of classifying cardiotoxic compounds from noncardiotoxicants. Our current activities are aimed to understand the small molecules that gave rise to the observed LO/MS features and map them with in metabolic pathways.

615 IDENTIFICATION OF BIOMARKERS OF CARDIOTOXICITY USING METABOLOMICS OF HUMAN PLURIPOTENT STEM CELLS-DERIVED CARDIOMYOCYTES.

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Both topically applied and systemically administered medications have the potential to induce photosensitivity. According to current regulatory guidelines, photo-safety testing is required for a substantial number of drug development submissions (e.g., due to light absorption in the range of 290–700 nm or because the new compound partitions into the skin or eyes). However, there has been growing concern regarding the performance of the only approved, non-animal in vitro phototoxicity assay, 3T3 Neutral Red Uptake Phototoxicity Test (3T3 NRU-PT), as being overly restrictive in predicting the in vivo photosafety hazard to humans. EpiDerm™, a highly differentiated NRU-3D skin model, is highly reproducible, contains an in vitro-like barrier, possesses in vivo-like biotransformation capabilities and has been pre-validated for determining phototoxicity of topically applied materials. Here, we utilized EpiDerm to develop an in vitro assay for screening phototoxic potential of pharmaceuticals after systemic administration (Epi-SPH0). Test materials are added into the culture medium, allowed to partition into the epidermal skin model, and then exposed to solar radiation. Phototoxic effects are determined by comparing the tissue viability of UV irradiated vs. non-irradiated tissue models, as determined using the MTT assay. A prediction model was established: a material is
**INCREASED OXYGEN CONSUMPTION ASSOCIATED WITH ALTERATIONS IN CARDIAC FUNCTION IN ADULT ZEBRAFISH AFTER ACUTE AQUEOUS EXPOSURE TO BETA-NAPHTHOFLAVONE.**

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Polycyclic aromatic hydrocarbons (PAHs) cause developmental cardiac deformities in fish as well as functional cardiac abnormalities. In contrast, acute adult fish exposure to PAHs is generally thought to have little effect. However, increasing numbers of studies in adult mammals report acute PAH exposure causes altered cardiac function. In order to determine if similar acute PAH effects could be detected in adult zebrafish, the model PAH-like compound, beta-naphtoflavone (BNF), was used. Adult zebrafish were aqueously exposed to increasing concentrations of BNF (0, 0.1, 1 and 1000 μg/L) for 48 hr, then subjected to swimming tests with concurrent oxygen consumption measurements (n=10 fish/treatment) or echocardiography performed to determine cardiac function (n=5 fish/treatment). While swim endurance was not altered by BNF exposure, oxygen consumption (MO2) was significantly higher (p<0.0001 for BNF factor in 2-way ANOVA) compared to control; but swimming at higher water velocities magnified this increase. MO2 at zero water velocity was highly positively correlated with ventricular volume at diastole (r=0.957), and ejection fraction (r=0.859), but negatively correlated with acceleration of blood through the ventricle (r=−0.588) in resting fish. These parameters clustered together in the first component in principle components analysis, while heart rate and cardiac output clustered in component 2. Unexpectedly, heart rate was the only cardiac end-point that did not correlate well with MO2 (r=0.027). In conclusion, acute exposure to the PAH-like compound, BNF, increased oxygen consumption. This in turn appeared to drive an increase in cardiac output via increased ventricular filling during diastole, not increased heart rate. More toxic and environmentally relevant PAHs are therefore predicted to pose a greater cardiorespiratory stress and need to be examined in future studies.

**IDENTIFICATION OF THE ARYL HYDROCARBON RECEPTOR (AHR) AS A NEGATIVE REGULATOR OF NUCLEOTIDE EXCISION REPAIR (NER).**

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Ultraviolet B (UVB) radiation is a major environmental hazard that causes DNA damage which may initiate the development of skin cancer. Efficient removal of UVB-induced cyclobutane pyrimidine dimers (CPDs) critically depends on NER. Although some cytokines were shown to enhance NER activity, their complex regulation is still poorly understood. We now report that keratinocytes are equipped with a negative regulator of NER: the AHR, a cytosolic transcription factor that is rapidly activated upon ligand-binding to stimulate gene expression. We have previously shown that FICZ, an intracellularly formed tryptophan photoproduct, is a strong AHR ligand, which triggers the activation of the EGF and downstream MAPK signaling upon UVB exposure resulting in a pro-inflammatory response.

Now, we have investigated if AHR activation also influences the DNA damage-dependent UVB-response. By using southwestern doblon analyses, we have found that chemical AHR inhibition in human keratinocytes leads to a faster repair of UVB-induced CPDs. Accordingly, Ahr-/− keratinocytes exhibited significantly lower amounts of CPDs after UVB-irradiation as the control cells. In order to assess the in vivo relevance, we irradiated Ahr+/+ and Ahr-/− SKH-1 mice with 180m2 UVB. The CPD amount in skin samples was measured by HPLC-MS/MS immediately, 24h and 48h after UVB-exposure. Strikingly, 48h after irradiation, the skin of Ahr-/− mice contained only 50% of the CPDs measured in skin samples from Ahr+/+ mice. Mechanistic studies indicate that the AHR represses NER activity in two ways: i) via modulation of cell-cycle by altering the expression and phosphorylation of checkpoint kinase-1, and ii) via activation of the EGF/MAPK pathway. In conclusion, the AHR is a negative regulator of NER that may serve as a novel target for chemoprevention against UVB-induced skin damage.
levels of AP sites whereas variant allele of CYP1B1 Leu432Val were positively correlated with the background levels of AP sites in breast cancer patients. (This work was supported by the National Science Council, Taiwan, through Grants NSC98-2314-B-371-004-MY2 and NSC99-2314-B-005-001-MY3).

621 SPONTANEOUS POINT MUTATIONS AND DELETIONS ACCUMULATE IN A DIFFERENT MANNER WITH AGING OF GPT DELTA TRANSGENIC MICE.


Chronic exposure of endogenous mutagens and DNA replication errors induce spontaneous gene mutations. The mutations accumulate in the genome throughout lifetime and may cause cancer or genetic diseases. Point mutations such as base substitutions are thought to increase with age in an organ-specific manner. However, effects of aging on the other types of mutations such as deletions are not well studied. In this study, we investigated the accumulation of spontaneous point mutations and deletions in young to aged mice. Mutation assays using gpt delta transgenic mouse have a feature that can detect point mutations and deletions by gpt and Spi assays, respectively, in the same organ. To characterize spontaneous gene mutations accumulated in mice, male gpt-delta transgenic mice were fed normal diets for two years. The organs were collected at week 4, 26, 52, 78 and 104, and mutant frequencies were examined for point mutations and deletions. Spontaneous gpt mutant frequencies in liver significantly increased with age up to 78 weeks, and then slightly decreased at week 104. In tests, the gpt mutant frequencies didn’t increase up to 78 weeks but increased 3 times at week 104 although the difference was not significant because of large standard deviations. The gpt mutation spectra of young and aged mice had similar characteristics, so the unique mutation types induced by aging were not observed. In contrast, Spi deletion frequencies were similar at week 4 to 78 but increased 2-3 fold at week 104 in both liver and testis. These results raised the possibility that endogenous DNA lesions that induce point mutations accumulate with age in liver but not in testis and also that deletions are induced with age in a different manner from that of point mutations. Catastrophic events that may induce deletions may occur at week 104 in the liver and tests although inter-individual differences are substantial.

622 SITE-SPECIFIC IN VIVO ANALYSIS: POSSIBLE INVOLVEMENT OF GENOTOXIC MECHANISMS IN THE MODES OF ACTION FOR OCHRATOXIN A (OTA)-INDUCED RENAL CARCINOGENESIS.

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Ochratoxin A (OTA), a mycotoxin and known food contaminant, can induce renal tumors arising from the S3 segment of the proximal tubules in rodents. The results from conventional mutagenicity tests investigating the role of genotoxic mechanisms in OTA-induced carcinogenesis are controversial. Human exposure to OTA from conventional mutagenicity tests investigating the role of genotoxic mechanisms in OTA-induced carcinogenesis is necessary for accurate estimates of the human risks. In the present study, gpt delta rats were first exposed to a carcinogenic dose of OTA for 13 weeks, enabling us to detect in vivo mutagenicity in the target organs. OTA induced karyomegaly and apoptosis at the outer stripe of the outer medulla (OSOM) in the kidney, but failed to affect the reporter gene mutations in DNA extracted from whole kidneys. This site-specificity resulting from the kinetics of specific transporters might be responsible for the negative outcome of in vivo mutagenicity. Next, kidneys from gpt delta rats exposed to OTA for 4 weeks were macroscopically divided according to anatomical characteristics in the cortex, and the outer and inner medulla, each of which was confirmed by histopathology. Spi- mutant frequencies (MFs), but not gpt MFs, in the outer medulla and mainly the OSOM, were significantly higher in OTA treated rats than in controls despite the absence of cortical changes. These results strongly suggest the involvement of a specific transport system in OTA-induced renal carcinogenesis. In addition, the reporter gene mutation assay using DNA from target sites could be a more powerful tool to investigate in vivo genotoxicity.

623 MAPPING DNA DAMAGE RESPONSE IN PLURIPOTENT STEM CELLS THROUGH INTEGRATION OF RNA SCREEN WITH GLOBAL TRANSCRIPTOMICS AND PHOSPHOPROTEOMICS.

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Damaged DNA contributes to aging when (stem) cells accumulate cytotoxic lesions and to cancer through mutagenic lesions. It is also the mechanism of action of anti-cancer radio- and chemotherapy. The anticancer drug, cisplatin causes DNA cross-links, stalled replication forks, and as a consequence double strand breaks. We analyze the signaling response to such broad-range genotoxic stress in pluripotent stem cells where repair pathways and triggering cell death when damage is beyond repair must be particularly robust. In an RNA interference screen targeting all known kinases, phosphatases, and transcription factors we identify cispalitin response modifiers in embryonic stem (ES) cells. A number of such modifiers are found to play similar roles in p53 mutant breast cancer cells. Subsequently, the RNAi screens are combined with global transcriptomics and phospho-proteomics (SILAC) to build integrated networks. In addition to the expected pathways, these point to alterations in self-renewal signaling. In particular, our findings demonstrate that genotoxic stress in ES cells elicits Wnt signaling through a novel mechanism to constrain p53-mediated apoptosis.

624 PARP-1 ACTIVATION IN ANILINE-INDUCED DNA DAMAGE: PROTECTIVE EFFECT OF ANTIOXIDANTS.

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Mechanisms by which aniline exposure elicits splenocyte response, especially the tumorogenic response, are not well-understood. Earlier, we have shown that aniline exposure leads to oxidative DNA damage and up-regulation of base excision repair (BER) enzymes, i.e., OGG1 and NER1/2 DNA glycosylases in rat spleen. This study was focused on evaluating if poly (ADP-ribose) polymerase-1 (PARP-1), another BER enzyme, is also involved in protecting aniline-induced DNA damage, and whether supplementation of antioxidants N-acetyl cysteine (NAC) and aminoguanidine (AG) could provide protection against aniline-induced DNA damage. To achieve this, four groups of male SD rats were orally treated with aniline (1 mmol/kg/day), aniline plus NAC (300 mg/kg/day), and aniline plus AG (200 mg/kg/day) for 7 days, while controls received drinking water only. Aniline treatment led to a 2-fold increase in 8-hydroxy-2-deoxyguanosine (8-OHdG) levels compared to controls. The aniline-induced increases in 8-OHdG levels were associated with significant increases in PARP-1 levels which were 2.2 fold greater than the controls as analyzed by ELISA and Western blot analysis. NAC or AG supplementation in aniline-treated rats led to decreased DNA damage which was evident from significant decreases in 8-OHdG levels compared to aniline-treated rats. NAC and AG supplementation also led to reduced PARP-1 activation compared to aniline-treated rats. Thus, NAC and AG supplementation not only attenuated the aniline-induced DNA damage, but also PARP-1 activation in the spleen. These results suggest that aniline-induced oxidative stress is associated with increased oxidative DNA damage and PARP-1 activation, and NAC and AG supplementation can provide protection by preventing oxidative DNA damage. Supported by NIH ES06476.

625 ANTIOXIDANT DYSFUNCTION COMPROMISES UVB-INDUCED DNA DAMAGE REPAIR.

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Mutagenic disruption of the nucleotide excision repair (NER) pathway, a specific repair pathway to remove UVB-induced DNA damage, can cause xenodermic pigmentation in mouse and predisposing affected individuals to development of skin cancer. The xeroderma pigmentosum C (XPC) protein is essential for initiating the cancer-preventive global genome NER by recognizing the DNA lesion and recruiting downstream factors. Here we show that inhibition of the long isoform of the nuclear factor-erythroid-2-related factor-1 (NRF1), a cytoprotective transcription factor critical for the expression of multiple antioxidant response element-dependent genes, impairs gpt plus gpt- induced renal carcinogenesis. In addition, the reporter gene mutation assay using DNA from target sites could be a more powerful tool to investigate in vivo genotoxicity.

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XPC. Adding XPC or glutathione restores the DNA repair capacity in NRF1-inhibited cells. Meanwhile loss of NRF1 severely depresses keratinocytes to UVB-induced apoptosis through up-regulating the expression of the pro-apoptotic Bel-2 family member Bik. Knocking down Bik reduced UVB induces apoptosis in NRF1-inhibited cells. Finally, we demonstrate that NRF1 levels are significantly reduced in human skin tumors as compared with normal skin, and by UVB radiation in mouse skin. These results suggest that NRF1 acts as a tumor suppressor through its role in DNA repair.

DNA polymerases help maintain genomic stability and protect cells from DNA damage. Based on sequence homology, C. elegans contains homologues to eleven DNA polymerases; however, the role these polymerases play in maintaining genomic stability of mitotic and meiotic cells has not been well characterized. DNA polymerase θ (POLQ-1) is an α family polymerase that contains an N-terminal helicase-like domain and a C-terminal polymerase domain connected by a large central domain. Previous studies showed that POLQ-1 is important in the defense against interstrand DNA crossovers and γ-irradiation. POLQ-1 may also participate in base excision repair due to intrinsic 5-deoxyribose phosphate lyase activity. In this study, we examined the removal of base damage and embryonic lethality after exposure to DNA damaging agents, hydrogen peroxide (H2O2) and methyl methanesulfonate (MMS), in wild type nematodes and two polq-1 mutants, one containing a deletion mutation in the polymerase domain (tm2026) and the other containing an insertion-deletion mutation in the helicase domain (tm2752). A qPCR-based assay revealed that rates of DNA lesion repair were comparable between wild type and the polq-1 mutants, suggesting POLQ-1 does not affect mitotic cell repair. We also examined the number of offspring and embryonic lethality. After exposure to H2O2 or MMS, brood size was similar in all strains for the doses tested. However, when assessing embryonic lethality, tm2752 nematodes showed a 2-fold increase in embryonic lethality after exposure to 0.5 mM H2O2 and both mutant strains showed an increased rate of embryonic lethality after MMS exposure compared to wild type nematodes. These results suggest that POLQ-1 plays a role in meiotic cell repair. The difference in sensitivity between assays used to evaluate DNA repair in mitotic and meiotic cells indicates that POLQ-1 may perform a specific function during meiosis.

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Pyrollozidine alkaloids (PAs) are the most common plant constituents that poison livestock, wildlife, and humans. Riddelliine is a prototype genotoxic PA. Riddelliine induced high incidence of liver tumors that were observed in both mice and rats. In this study, we explored whether riddelliine treatment could alter microRNA (miRNA) expression in rat liver and whether the possible deregulation of miRNA were related to mutagenicity and carcinogenicity of riddelliine. Groups of 6 rats were administered riddelliine at a carcinogenic dose of 1 mg/kg body weight, with control vehicle 5 days a week for 12 weeks. The animals were sacrificed one day after the last treatment and the livers were isolated for miRNA expression analysis using miRNA microarrays. miRNA expression was significantly altered by riddelliine treatment. Principal component analysis and hierarchical clustering analysis showed that the miRNA expression profiles were clearly classified into two groups, riddelliine treatment vs. the control samples. Forty-seven miRNAs were significantly downregulated by riddelliine treatment, among which 38 were upregulated and 9 were downregulated. Functional analysis of these differentially expressed miRNAs by riddelliine revealed that these miRNAs were involved in liver carcinogenicity and toxicity, such as liver proliferation, liver necrosis/cell death, hepatocellular carcinoma, liver hepatomegaly, liver inflammation, and liver fibrosis. These results suggest that miRNAs actively respond to a carcinogenic dose of riddelliine and the pattern of miRNA expression has the potential to be used as a biomarker of carcinogenicity for riddelliine and possibly other PAs.

Early life exposure to mitochondria toxicants, including paraquat, rotenone, and manganese, has been hypothesized to promote early onset of genetic mitochondrial disorders as well as common degenerative diseases such as Parkinson’s disease and Alzheimer’s disease. The current study aims to investigate the biochemical and physiological effects of early life exposure to mitochondrial genotoxicants in juvenile and adult C. elegans. In the first experiment, a laboratory method was developed to selectively generate mitochondrial DNA (mtDNA) damage (but not nuclear DNA (nuDNA) damage). Twelve-hour old first-larval stage (L1) C. elegans was treated with three serial low-intensity (10 J/m²) doses of ultraviolet C (UV-C) radiation. This resulted in 1.3 ± 0.3 mtDNA and 0.2 ± 0.0 nuDNA lesion per 10,000 base pairs in 36-hr old third-larval stage (L3) C. elegans. In the second experiment, ATP level and mtDNA : nuDNA copy number ratios in C. elegans were measured after serial L1 UV-C doses (7.5 J/m²). Both ATP level and mtDNA : nuDNA ratios significantly decreased during the first 48 hr after the doses, but rebounded in the fourth larval stage (L4, 60 hr old; p < 0.05). The UVC doses significantly increased ATP levels in 108-hr old adults (p < 0.05) without any detectable effect in mtDNA : nuDNA ratios (p > 0.05). In the third experiment, paraquat, cumene hydroperoxide, rotenone, maneb, cadmium (II) chloride, and manganese (II) chloride were screened for mitochondrial genotoxicity in 108-hr old adults. Exposures to paraquat, maneb, cadmium (II) chloride, and manganese (II) chloride all significantly decreased mtDNA : nuDNA ratio (p < 0.05). Both paraquat and cadmium (II) chloride caused detectable DNA damage, but only paraquat caused significantly more mtDNA than nuDNA damage (p < 0.001). The results indicate that early life exposure to mitochondrial genotoxicants leads to late life alterations in ATP level and mtDNA copy number, thereby representing a potential human health hazard for further investigation.
miR-34a by transfection chemically synthesized miR-34a precursor into human lymphoblast TK6 cells. The mutant frequency (MF) in the thymidine kinase locus was consistently increased in miR-34a over-expressed TK6 cells. Further more, the over expression of miR-34a can abrogate the X-ray induced MF in transfected cells. These data indicate miR-34a plays a significant role in regulating the MF in TK6 cells. WT1 (mutant P53 gene) cell line derived from the same lymphoid progenitor as TK6 (wild type P53 gene) cells showed WT1 cells has a very high level of MF and very low level of miR-34a expression in comparison with TK6 cells. Interestingly, when miR-34a was transfected into WT1 cells, the high basal level of MF in WT1 cells didn’t show any change. miR-34a over-expression was also not able to diminish X-ray induced mutation in WT1 cells. These data suggest P53 is required for miR-34a modulation regulation. Consistent with the role of miR-34a involved in apoptosis, our data indicate the over-expression of miR-34a can induce the apoptosis of TK6 cells with the presence of wild type P53. In summary, these results suggest that miR-34a plays a role in suppression of mutation induction under pathological conditions. The role of miR-34a in mutagenicity regulation is P53 dependent. The mechanism of miR-34a suppresses TK mutation induction may be mediated through apoptosis.

631 CHANGE OF DNA LIGASE I PHOSPHORYLATION INCREASES DNA DAMAGE, DELAYS CELL CYCLE, AND LEADS TO SENESCENCE.

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Human DNA ligase I (hLigI) plays a key role in DNA replication and several different DNA repair pathways. Our previous studies have shown that physical and functional interactions between hLigI and the replicative clamp loader replication factor C (RFC) and the checkpoint clamp loader BRAD17-RFC are sensitive to the phosphorylation status of hLigI. The objective of this study is to elucidate the role of hLigI phosphorylation in regulating its cellular functions. We isolated derivatives of the hLigI-deficient cell line 46BR1.G1 that stably express mutant versions of hLigI, in which 4 serine residues phosphorylated in vivo, were replaced with either alanine or aspartic acid. The cell lines expressing the phosphorylation site mutants of hLigI exhibited a dramatic reduction in proliferation, high level of cellular senescence and were also hypersensitive to DNA damage. Our studies have identified Ser51 as a critical residue in the regulation of the cellular functions of hLigI by phosphorylation.

632 ATR DEPLETION IN ATM-DEFICIENT HUMAN MAMMARY EPITHELIAL CELLS CAUSES SIGNIFICANTLY ATTENUATED G2 CHECKPOINT AND SYNERGISTIC LEATHALITY TO DOUBLE-ST RAND BREAKS AND DNA-DAMAGING AGENTS.

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DNA double-strand breaks (DSBs) pose a great threat to genome stability and cell viability. Normally cells respond to DSBs by activating cell cycle checkpoint allowing DNA repair to occur. These response pathways are induced mainly by three members of the phosphatidylinositol 3-kinase–related kinase (PIKK) family: the ataxia-telangiectasia mutated kinase (ATM), the ATM and Rad3-related kinase (ATR), and the catalytic subunit of the DNA-dependent protein kinase (DNA-PKcs). ATM and ATR are key players in homologous recombination (HR), which predominates after DNA replication, while DNA-PKcs plays a critical role in non-homologous end-joining (NHEJ), which is the primary repair pathway when the sister chromatid is not available. Previous studies in our lab utilizing ATM-deficient human mammary epithelial cells (HME-CC) have shown that DNA-PKcs is playing a role in the activation of IR-induced G2 checkpoint when ATM is depleted, suggesting cross-talk between HR and NHEJ. In order to dissect the signals behind this cross-talk and the role of ATR in particular, we recently generated inducible ATR knockdown cell lines by lentiviral infection of both ATM proficient and deficient HME-CC cell lines. Preliminary data suggests that depletion of ATR in ATM deficient cells causes significant G2 checkpoint attenuation and synergistic lethality when cells are treated with ionizing radiation or DNA-damaging reagents such as bleomycin sulfate and etoposide. We are currently investigating the changes in the ATM/ATR double deficient HME-CC cells in their response to IR-induced DNA damage. While the ATR/ mouse is embryonic lethal, precluding many studies of ATR function, and ATR inhibitors generally lack specificity, these inducible knockdown cell lines allow us to study DNA damage responses in an ATR-deficient background. Furthermore, the synergetic lethality caused by ATM and ATR depletion may be potentially useful in cancer therapeutics.

633 DIFFERENTIAL P53 DNA DAMAGE DOSE-RESPONSE IN HUMAN FIBROSARCOMA HT1080 AND LYMPHoblAST AHH-1 CELLS.

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p53 protects cells from DNA damage-induced mutation by initiating DNA repair, cell cycle delay, and apoptosis. The pathway that the cell chooses to follow depends upon the type, amount, and duration of DNA damage, as well as the specific cell type. In order to better define the dose-dependent transition in p53 response after chemically-induced DNA damage, we examined markers of p53 activation, DNA damage and subsequent cellular responses in human HT1080 fibrosarcoma cells and AHH-1 lymphoblast cells following exposure to etoposide (ETP). HT1080 cells showed 10-fold greater sensitivity to DNA damage than AHH-1 cells based on micronuclei formation and H2AX levels. In both cell lines, cell cycle arrest (G2/M) was observed at lower doses than initiation of apoptosis. While total p53 expression was up-regulated in both cell types, the favored phosphorylation site for p53 and the downstream cellular response differed. In AHH-1 cells, phosphorylation of p53 at serine46 (apoptosis inducer p53) and induction of cleaved caspase 3 (apoptotic marker) were 35% and 50% respectively, much greater than HT1080 cells (8% and 8%), suggesting that p-p53 (ser46) and apoptosis were favored in AHH-1 cells. In contrast, in HT1080 cells maximum induction of phosphorylation of p53 at serine 15 was greater than AHH-1, indicating that p-p53 (ser15) is favored in HT1080 cells. The differential response of these cells could result from alterations of downstream apoptotic or cell arrest signaling in cancer cells. It may also be indicative of differences in cellular response in blood and epithelial cells. Understanding the relevance of both these factors will be important in achieving the vision of the Toxicity Testing in the 21st Century paradigm linking in vitro assay data to mechanistic pathway perturbation. Genome wide RNA expression is being evaluated in order to better characterize the differential pathway activation in these two cell lines and to identify context-dependent mechanisms that determine cell fate after DNA damage.

634 MODULATION OF O6MG REPAIR ACTIVITY BY MGMT SINGLE-NUCLEOTIDE POLYMORPHISMS.

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The therapeutic efficacy of popular antitumor alkylating chemotherapeutic agents such as Temozolomide and Caramustine depends largely on the generation of O6-methyl guanine (O6mG). This lesion, which is highly cytotoxic to tumors, is repaired by a specialized enzyme (O6 methyl guanine methyl transferase or MGMT), that removes the O6 methyl adduct by irreversible transfer to its own active site. The correlation between MGMT activity and sensitivity of tumors to alkylating therapy is well established. There are 10 known non-synonymous MGMT SNPs in humans that could affect the response of individual patients to alkylating agent chemotherapy, I have developed a complementation system that detects O6mG repair by human MGMT with great accuracy. This system is based on expressing human MGMT in an E. coli strain deficient in O6mG activity. This strain carries the R323W mutation in its DNA repair function (the functional homolog of MGMT); which abolishes O6mG repair without interfering with transactivation of other DNA repair genes. Using this system, I confirmed the results of 4 previously characterized polymorphisms and characterized the repair activity of 6 previously uncharacterized ones. I found that the L53F variant of MGMT (present in about 0.15% of the population) exhibits a 100-fold increase in activity towards O6mG lesions, whereas other variants exhibit slight to significant decreased activity. This wide range of O6mG demethylase activities that I observe should have an impact on the sensitivity of tumors to alkylating agent therapy, particularly if MGMT is dominant or haplosufficient.

635 INCREASED NUCLEOTIDE EXCISION REPAIR ACTIVITY OF AFLATOXIN-B1’-N’-GUANINE ADDUCTS IN LUNGS AND LIVERS OF MICE EXPOSED CHRONICALLY TO AFLATOXIN B1.

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Aflatoxin B1 (AFB1) is produced by species of Aspergillus, moulds that can grow on grains, oilseeds and spices. Following inhalation or ingestion, AFB1 is biotransformed into the highly reactive AFB2-exo-epoxide, which binds preferentially to the
DNA REPAIR CAPACITY IS MODULATED BY XPC HAPLOTYPES.

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The impact of single nucleotide polymorphisms (SNPs) of the DNA repair gene XPC on DNA repair capacity (DRC) has not been comprehensively determined. We constructed a haplotype map of XPC by aligning all XPC SNPs existing with a minor allele frequency ≥0.05. We identified 21 haplotypes that segregated into 6 phylogenetic groups (PGHs A-F). We evaluated the relationship between these PGHs and DNA damage associated with smoking, using chromosome aberrations (CA) as a biomarker. We observed a significant interaction between PGH-C and smoking for baseline CA (P = 0.0046). Using mutagen sensitivity as a biomarker of cancer risk, we observed significant interactions between smoking and PGH-D (P = 0.023) and -F (P = 0.007). To provide mechanistic explanations to our findings, we exposed human lymphoblastoid cells representing different XPC haplotypes to UV radiation to generate the NER substrates 6-4 photoproducts (6-4PP) and cyclobutane dimers (CPD). We then evaluated the effect of XPC haplotypes on DRC using ELISA. We hypothesized that if XPC haplotypes have functional effects, there would be a correlation between these haplotypes and repair of 6-4PP and CPD. We found significant differences in dimer repair as a function of XPC haplotypes. For example, cells with the PGH-EE repaired 6-4PP more efficiently than those with DD or FF. Our results highlight the potential effects of Corexit 9500 on essential biological processes in marine species in response to its use as a chemical dispersant in the Gulf of Mexico.
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In Guizhou province, China, arsenicosis caused by burning coal was found since 1976. The villagers were suffering severe symptoms of arsenicism. According to our investigation, the happening of the disease existed individual differences. In order to find it clearly, we selected Excision repair cross-complement group (ERCC6) and Xeroderma pigmentosum group A gene(XPA), analyzing the relationship between their gene polymorphisms and the risk of arsenicism, to provide the evidences for screening the susceptible persons and protecting high risk population in the endemic area. Methods According to Chinese National Arsenicism Diagnosis Criteria (WS/T211-2001), 205 diagnosed arsenicism patients were chosen as patients group. 187 villagers who lived about 13km away were chosen as control group, sharing a similar lifestyle but not using high arsenic coal. PCR-RFLP was used to analyze the gene polymorphisms of ERCC6 and XPA. Results 1. Unconditional Logistic regression analysis showed after the adjustment of gender and age, the individual carrying G allele(AG+GG) in ERCC6 A3368G has significantly lower risk of arsenicism than that carrying AA gene(with ORadj=0.282,95%CI:0.126-0.628,P=0.0002). 2. The risk of arsenicism decreased for the individuals carrying the two gene types as follow: ERCC6 3368(AG+GG) and ERCC6 -6530 CC; ERCC6 3368(GG)and ERCC6 -6530(CG+GG) and XPA 23 AA;ERCC6 3368(AG+GG) and XPA 23(AG+GG);ERCC6 -6530(CG+GG) and XPA23 AA. Conclusions 1. ERCC6 A3368G gene polymorphism is related to the risk of arsenicism caused by burning coal. More protection should be done for population carrying AA gene type in the area. 2. Combined effects of different gene loci exist in the process of arsenicism development.

A vital challenge that all cells and organisms constantly have to face is the generation of diverse harmful DNA lesions in the genome caused by oxidative and environmental agents. The DNA lesions within highly transcribed genomic regions must be dealt with in a timely and efficient manner to prevent stalling of the transcription machinery and subsequent cell death. A significant body of evidence indicates that RNA polymerase II (Pol II) is a highly selective DNA damage sensor, since it constantly scans the transcribed genome during transcription. Pol II can bypass small DNA lesions. Certain types of DNA damage frequently cause nucleotide misincorporation into an RNA transcript (error-prone transcription bypass) in a manner similar to translesion synthesis by error-prone DNA polymerases. However, other types of DNA lesions have little effect on transcription fidelity (error-free transcription bypass). Error-prone transcription bypass, termed transcriptional mutagenesis, may be an important pathway for the generation of mutant proteins and therefore play a role in tumor development. However, the molecular mechanisms that dictate error-prone versus error-free transcription are not fully understood. Here, we use a combined approach including pre-steady state transient kinetics, X-ray crystallography, and molecular dynamics simulations to molecular basis of factors in controlling transcription fidelity. We further elucidated the roles of electrostatic effects and of steric effects in controlling Pol II error-prone versus error-free transcription bypass.

Arsenic is a recognized carcinogen. Environmental arsenic pollution will cause arsenic and even cancers. Up to now, no evidence has been discovered about the mechanism of arsenic intoxication and carcinogenesis. This study analyzes the relationship between methylation in detoxification gene-GSTP1 gene promoter region, mRNA transcriptional expression and protein expression and the occurrence of arsenicism. Methods The blood samples were collected from 123 arsenicism patients and 47 controls. DNA methylation regulation of GSTP1 gene in blood of arsenicism patients was detected by methylation-specific PCR. The mRNA expression of GSTP1 was detected by real-time quantitative reverse transcription polymerase chain reaction, the tiss e samples of skin were collected from 53 patients with endemic arsenicism and from 15 controls. GSTP1 proteins were detected by immunohistochemical method. Results It showed that the positive rate of DNA methylation of GSTP1 gene increased with disease development and dermatic lesion of arsenicism. The average levels of GSTP1 mRNA in promoter methylation group of GSTP1 was lower than that in non-methylation group. Compared with the control group, the GSTP1 protein expression was higher in arsenicism group, which was associated with the degree of skin damage, the GSTP1 protein expression in promoter methylation group of GSTP1 was higher than that in non-methylation group. Conclusions Arsenicism may lead to hypermethylation of the GSTP1 gene promoter and then inhibit the transcription of the mRNA and protein expression. GSTP1 gene plays an important role in arsenicism or carcinogenic process.

The mycotoxin patulin (PAT) can occur in many moldy fruits, grains and other foods, but the major sources of patulin are moldy apples and apple products. Recently, we have shown the mutagenic potential of PAT in the hprt locus of V79 cells at concentrations as low as 0.6 μM. The mutagenicity of PAT at such low concentrations is in contrast with the assumption that the intracellular excess of glutathione (GSH) ascents the complete inactivation of small reactive electrophiles such as PAT. Furthermore, DNA-DNA crosslinks rather than DNA strand breaks or oxidative DNA base modifications contributed to the PAT-induced mutagenicity, indicating the reaction of PAT with DNA bases. Thus, cell-free reactions of PAT with adenine in the presence and absence of equimolar concentrations of GSH were carried out. Reaction products were separated by means of HPLC and their structure was elucidated by MS/MS. Besides already known PAT-GSH adducts, at least two PAT-adenine adducts were detected in the presence and absence of GSH. In addition, at least one novel product exhibiting a mass to charge ratio and fragmentation pattern identifying a mixed PAT-GSH-adenine adduct was observed. Moreover, one hitherto unknown PAT-GSH adduct was identified. Since its fragmentation pattern correlated with the PAT-GSH-adenine adduct before its linkage to adenine, it might represent the precursor of the mixed adduct, suggesting the activation of PAT by reaction with GSH. Based on our observations, we propose a structure of the mixed PAT-GSH-adenine adduct and the putative mechanism of its formation. These findings are in contrast with the assumption that the conjugation of the major intracellular nucleophile GSH induces the complete inactivation of PAT. In fact, the formation of a reactive PAT-GSH monoadduct and its potential to associate with DNA bases could contribute to the mutagenicity of PAT.

The role of smoking in breast cancer (BC) remains controversial, BC is hormone dependent and cigarette smoking may have antieestrogen effects in women (Vogel 2006). The association between smoking and DNA repair capacity (DRC) is largely unknown. A low DRC is an important risk factor in BC (Ramos et al. 2004). The focus of this study was to test the hypothesis that smoking was associated with changes in DRC level and BC risk. We conducted in Puerto Rico a 5 year IRB approved study with 385 women with recently diagnosed BC and 606 controls. BC risk factors in relation to smoking and other epidemiological variables were solicited and DRC was measured in lymphocytes utilizing a host cell reactivation
assay. Multiple logistic regression was used to calculate odds ratio (OR) to assess the association of DRC, smoking and BC after adjusting for all confounders simultaneously. Compared to the controls, women with BC (smokers and non-smokers) showed an average decrease of 55% in their DRC levels (p<0.001). Women with low DRC (< 3.185) were 16.5 times more likely to have BC (OR=16.5, 95%CI 11.6, 23.5; p<0.001). Smokers had 60% more odds of having BC (OR = 1.6, 95%CI 1.01, 2.59; p=0.045) and 50% more odds of having a low DRC (OR=1.5, 95%CI 1.00, 2.40; p=0.085) although the latter was borderline significant. Every year of smoking increased 2% the odds of having BC (OR=1.02 95%CI 1.0, 1.04; p=0.189), and 7% the odds to have a low DRC (OR=1.07, 95% CI 1.01, 1.13; p=0.025). However, the former was not statistically significant after adjusting for age, BMI, family history of BC, menopause, alcohol consumption, marital status, eating saturated fat and history of pregnancy. This study provides the first evidence of the potential association of smoking with a diminished DRC and increased BC risk. Supported by grants 506 GM008239-20 and 1SC1AI57250-2 from the NCI Center to Reduce Health Disparities and NIH-MBRS Program (NIGMS).

645 NANOFLUIDS FOR DRUG DELIVERY: HOW TO ASSESS DANGER FOR MITOCHONDRIA?
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Recently, we reported that Eudragit® RS (ERS) empty nanoparticles, prepared by nanoprecipitation (NP) or double emulsion (DE) techniques, induced cytotoxicity in NR8383 macrophages. As drug delivery nanoparticles are currently used in human therapies, we investigated both morphological and biochemical effects on macrophages exposed to ERS nanoparticles (15 and 100 micrograms/mL; diameter = 56 nm). Damaged cells did not display an apoptotic cell death whereas ERS induced another autophagy. Transmission electron microscopy showed that ERS nanoparticles were internalized per unit by macrophages and entered mitochondria that lost their shape and integrity. By the way, a p*, a gene marker of mitochondria cytotoxicity, was downregulated. Ultrastructural studies showed typical autophagosome vesicles, autophagy markers with upregulation of atg16l1 and map1lc3 genes, as evidenced at both mRNA and protein level. In this study, we reported for the first time that such polymeric drug nanovectors enter mitochondria and act as autophagy activators, like viruses. Thus, nanoveccors, whatever their type, should be assayed on macrophages in order to determine potential induction of autophagy and mitochondria damage for toxicological safety.

646 SIZE-DEPENDENT AGGRAVATING-EFFECT OF AMORPHOUS SILICA NANOPARTICLES ON ATOPIC DERMATITIS.
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Nanomaterials (NMs) have been becoming essentials in daily life. However, because of unique physicochemical properties and innovative functions of NMs, it is a concern that NMs can exhibit unknown harmful effects. In this regards, to make safe NMs and enjoy their many benefits, it is essential to evaluate the hazard associated with NMs on human health. However, since NMs do not behave in solution whatever their type, should be assayed on macrophages in order to determine potential induction of autophagy and mitochondria damage for toxicological safety.

647 MICRONUCLEI IN TK6 CELLS ARE INCREASED BY SILVER NANOPARTICLES IN A DOSE, SIZE AND SURFACE COATING-DEPENDENT MANNER.
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Nanoparticles have unique physical and chemical attributes, such as size, surface charge, and surface coating, and these different physicochemical properties could have different biological consequences. In the present study, we evaluated the effects of the size and surface coating of nanoparticles on micronucleus (MN) induction in human lymphoblastoid TK6 cells. To test the effects of surface coating, cells were treated with different concentrations of 5 nm uncoated, polyvinylpyrrolidone (PVP)-coated and tannic acid (TA)-coated silver nanoparticles (AgNPs). Size effects were evaluated by treating cells with different sizes of citrate coated AgNPs (Ag-10 nm, Ag-20 nm, Ag-50 nm and Ag-100 nm). Additional cells were treated with water, 0.75 Gy X-ray as well as with MN frequencies in AgNPs was increased significantly and in a dose-dependent manner by the 5 nm coated and uncoated AgNP treatment. Treatment of TK6 cells with 1.5 μg/mL PVP-coated, 3.0 μg/mL TA-coated and 30 μg/mL uncoated AgNPs (concentrations resulting in around 40% relative population doubling) resulted in 4.12, 2.81, and 3.1 fold-changes in MN frequency, respectively. The surface charges and aggregation natures of the nanoparticles are considered responsible for the genotoxicity differences. Different sizes of citrate AgNPs generated weak, but dose-dependent increases in MN frequency and more experiments are being conducted to confirm this finding. The data indicate that the AgNPs induce micronuclei in TK6 cells, and the MN induction is dose-, surface coating- and size-dependent.

648 IN VITRO PULMONARY TOXICITY OF METAL OXIDE NANOPARTICLES.
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Nanomaterials (NMs) encompass a diversity of materials with unique physicochemical characteristics which raise concerns about their potential risk to human health. Rapid predictive testing methods are needed to characterize NMs health effects as well as to screen and prioritize NMs for comprehensive toxicological assessments. BEAS2B human bronchial epithelial cells were employed to assess the in vitro pulmonary toxicity of 4 TiO2 and 4 CeO2 particles varying in size (6 - 1288 nm), morphology and crystalline structure. Exposures were conducted over several concentrations for each endpoint examined. No BEAS2B cytotoxicity was observed for any particle following a 24 hr exposure to concentrations up to 100 μg/mL. The ability of TiO2 and CeO2 particles to induce inflammation and oxidative stress was assessed by gene induction using RT-PCR. At 50 μg/mL maximal IL-8 and IL-6 gene induction by 6nm TiO2 and 8nm CeO2 NMs was observed at 6hr and 24hr post-exposure, respectively, with 8nm CeO2 NMs producing the greatest induction of cytokine mRNA levels. Smaller TiO2 and CeO2 NMs produced greater induction of cytokine- and IL-6 mRNA levels compared to their larger sized counterparts. At 50 μg/mL all TiO2 and CeO2 particles induced similar increases in HO-1 mRNA levels at 6hr and 24hr post-exposure, respectively. The pattern of HO-1 gene induction was inconsistent with a role of oxidative stress in metal oxide induced BEAS2B cytotoxic gene expression. Pretreatment of BEAS2B cells with 10 nM inhibition III BMI-S345/41 completely inhibit 25nm TiO2 and 60nm CeO2 NM induction of IL-8, IL-6 and HO-1 gene expression indicating a role of NFκB in these responses. A cell-based ELISA for NfκB p65 phosphorylation revealed rapid Ser536 phosphorylation in BEAS2B cells following exposure to 50 μg/mL of TiO2 and CeO2 NMs with sizes ≥25nm. Results demonstrate the ability to employ in vitro methods to assess NM induced pulmonary toxicity. Some responses were found to be totally dependent NM size/surface area indicating composition and surface properties play a role in mediating NM toxicity. This abstract does not reflect EPA policy.

649 THE ROLE OF ROS AND NFκB ON CYTOTOXIC AND PRO-INFLAMMATORY EFFECTS OF BLACK TONER POWDER.
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Major components of black toner powders are styrene-acrylate copolymer (SAC), magnetite and Carbon Black (CB). Recent reports have suggested possible significant adverse health effects from toner dust inhalation. Moreover, in a previous
study, we observed genotoxic effects in A549 human lung cells exposed to three commercially available black toner powders. The aim of this study was to unravel the underlying mechanisms of toxicity by comparing the cytotoxic and pro-inflammatory potential of these three toner powders and the influence of ROS and NF-κB induction. Toner powders A, B and C consist mainly of SAC (40–60 wt%), silica (1–5 wt%) and CB (<1 wt%). The three black toner powders and their suspensions were examined by SEM. The toner powders consist of C-bearing, rounded to slightly elongated particles with typical diameters of 2 to 8 μm. The particle surface is somewhat rough and is covered by rounded nanoparticules (30–200 nm). In all the experiments, A549 cells were exposed for 24 h to various concentrations of the three toner powder suspensions. Cytotoxicity was assessed by the WST-1 and NR assay and ROS induction by the DCFH-DA assay. Cytokine release was quantified using FlowCytomixTM Technology. Nuclear NF-κB was detected by the electrophoretic mobility shift assay (EMSA). Slight cytotoxic effects were found for the three toner powders. A concentration-dependent induction was observed with the three toner powder suspensio ns. Exposure to toner powder enhanced IL-6 and IL-8 production. Moreover, EMSA results exhibit release of the pro-inflammatory transcription factor NF-κB at the mRNA level in human T cells in vitro, whereas larger AgNPs (110 nm) induce (i) DNA damage, cell cycle arrest and apoptosis in T cells (PMID 20932003), and (ii) cytotoxicity, ROS production, and IL-8 secretion in macrophages (PMID 21390403). In this study we assessed whether different sizes of AgNPs have specific effects on the function of T cells in vitro. Genome wide expression patterns were used as functional readout. Methods and Results: Human Jurkat T cells (clone E6.1, ATCC) were exposed for 6 hours to three sizes of AgNPs (10, 30, 110 nm), or to a supernatant of 30 nm AgNPs, that were subjected to ultracentrifugation, without or with addition of AgNO3 (50 μM), 1 μM, respectively. mRNA molecules were extracted and transcriptomes were generated (Affymetrix U133A, plus 2.0) from N=4 independent experiments. We found, by unsupervised hierarchical clustering of the transcriptome profiles, that metallothioneins were selectively induced by 10 nm and 30 nm AgNPs, whereas 110 nm AgNPs and ionic Ag had no effects. This finding was verified by qRT-PCR (r=2.2-2.65 fold, p≤0.05). Cytokines: Small nanosized AgNPs (10-30 nm) selectively induce MTs at the mRNA level in human T cells in vitro, whereas larger AgNPs (100 nm) and ionic silver do not have this property. Currently experiments are on-going to verify whether this induction of MTs (i) is also observed at the protein level, (ii) is mediated by the transcription factor MTF-1, and (iii) has specific functional consequences for the immune system.

650 THE INDUCTION OF METALLOTHIONEINS BY SILVER NANOPARTICLES AS A NOVEL PUTATIVE IMMUNOMODULATORY MECHANISM IN T CELLS.

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Introduction: Silver nanoparticles (AgNPs) are used in numerous consumer and health care products, mainly for their antimicrobial properties. Recent in vitro studies suggest that AgNPs can affect the immune system. For instance, AgNPs can induce (i) DNA damage, cell cycle arrest and apoptosis in T cells (PMID 20932003), and (ii) cytotoxicity, ROS production, and IL-8 secretion in macrophages (PMID 21390403). In this study we assessed whether different sizes of AgNPs have specific effects on the function of T cells in vitro. Genome wide expression patterns were used as functional readout. Methods and Results: Human Jurkat T cells (clone E6.1, ATCC) were exposed for 6 hours to three sizes of AgNPs (10, 30, 110 nm), or to a supernatant of 30 nm AgNPs, that were subjected to ultracentrifugation, without or with addition of AgNO3 (50 μM), 1 μM, respectively. mRNA molecules were extracted and transcriptomes were generated (Affymetrix U133A, plus 2.0) from N=4 independent experiments. We found, by unsupervised hierarchical clustering of the transcriptome profiles, that metallothioneins were selectively induced by 10 nm and 30 nm AgNPs, whereas 110 nm AgNPs and ionic Ag had no effects. This finding was verified by qRT-PCR (r=2.2-2.65 fold, p≤0.05). Cytokines: Small nanosized AgNPs (10-30 nm) selectively induce MTs at the mRNA level in human T cells in vitro, whereas larger AgNPs (100 nm) and ionic silver do not have this property. Currently experiments are on-going to verify whether this induction of MTs (i) is also observed at the protein level, (ii) is mediated by the transcription factor MTF-1, and (iii) has specific functional consequences for the immune system.

651 PULMONARY TOXICITY FOLLOWING REPEATED INTRATRACHEAL INSTILLATION OF DISPERSED SILVER NANOPARTICLES IN RATS.

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Silver nanoparticles (AgNPs) are one of the fastest growing categories of nanomaterials, etc., and can be readily found in soil and airborne. With the growing use of nanoparticles in industrial and environmental applications, the toxicological effects of these materials increasingly have come into question. Nanoparticles are thought to enter cells through active transport mechanisms such as receptor-mediated endocytosis, which has led to a growing concern over their long-term health effects. Polychlorinated biphenyls (PCBs), which are common environmental pollutants, have well-known toxicology. PCBs can adsorb onto nanoparticles, though, primarily due to hydrophobic effects, and potentially enter cells using nanoparticles as carriers.

Various environmentally prevalent and/or toxicologically relevant PCBs, including PCB-77, -118, -126, and -153, were adsorbed onto alumina and polystyrene nanoparticles, and adsorption was confirmed using GC-MS and energy-dispersive}{
X-ray spectroscopy. Dynamic light scattering and transmission electron microscopy were used to confirm that particles were of a relevant size for entry into cells and cell viability of A549 lung epithelial cells, MH-S alveolar macrophages, and primary porcine endothelial cells was subsequently studied to determine if nanoparticle-PCB complexes cause increased cytotoxicity as compared to nanoparticles and PCBs alone. Treatment of MH-S alveolar macrophages with PCB/nanoparticle complexes mixtures caused marked cell viability decrease for both polystyrene and aluminum oxide nanoparticle carriers, while these complexes result in limited additional epithelial and endothelial cell line toxicity.

**654 COMPARISON OF SYSTEMIC INFLAMMATION BETWEEN INTRAVENOUSLY DELIVERED MULTIWALLED CARBON NOBUTES AND GRAPHENE NANOSHEETS.**

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Nanotechnology is a rapidly growing industry that has potential applications as biomedical devices and carriers for drug delivery. Carbon-based nanomaterials, such as multiwalled carbon nanotubes (MWCNTs) and graphene nanosheets (GNS), have been proposed for these purposes. However, our understanding of potential toxicity of these carbon-based nanomaterials is lacking. We have previously demonstrated that oropharyngeal instillation of MWCNTs in C57BL/6 mice leads to a Th2 type immune response with increased numbers of inflammatory cells and increased Th2 cytokines in the lung. In this study, we compared MWCNTs (~25 nm diameter, -113 m²/g surface area and ~5 wt% Fe catalyst) with GNS (~2 - 5 nm thickness, -2630 m²/g surface area and no metal catalyst). We hypothesized that intravenous administration of MWCNTs but not GNS would result in a systemic inflammatory Th2 type response due to differences in physicochemical properties. We intravenously administered 0.01, 0.1, and 1 mg/kg MWCNTs or 1 mg/kg GNS and measured spleen weight, inflammatory cell recruitment, CD4⁺ T lymphocytes and Th2 cytokines in lung and spleen at 24h and 7d following injection. We observed an increased spleen weight in GNS administration groups but not in MWCNTs groups. CD4⁺ T lymphocytes were increased in the spleen of MWCNTs groups at 24h and 7d following injection, while an increase in CD4⁺ T lymphocytes following GNS administration was only observed at 7d. In conclusion, the use of MWCNTs and GNS as nano-carriers for drug delivery may result in potential adverse effects. Therefore, additional modifications to these materials may be warranted before their routine use as drug delivery systems. This work supported by NIH RO1 ES019311 (JMB).

**655 IN VITRO CYTOTOXICITY OF SILVER NANOMATERIALS IN MURINE MACROPHAGES.**

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Silver nanomaterials are increasingly used as antimicrobial agents in a variety of products. Although there is considerable potential for human exposure to these nanomaterials, little is known about the health risks associated with their use. Macrophages are prominent immune cells that clear pathogens, cellular debris, and foreign particles during inflammatory responses. As phagocytes, macrophages might readily ingest silver nanoparticles. The cytotoxic effects of short-term exposure to ionic silver (silver nitrate) was compared to PVP-coated silver nanoparticles (median hydrodynamic diameter = 13 nm) using an in vitro cytotoxicity assay that measured reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in murine macrophage J774A.1 cell line. Cells were exposed to ionic silver or PVP-coated silver nanoparticles for up to 24 hours before assessment of cellular status. Both ionic silver and PVP-coated silver nanoparticles diminished MTT reduction capacity of J774A.1 cells with 50% reductions in activity seen in the low parts per million of silver concentration range. Given that cytotoxic responses were similar after exposure to ionic silver and silver nanoparticle, it is likely that the kinetics of silver uptake and accumulation were similar in both exposure scenarios. However, characterization of the kinetic behavior of silver in macrophages exposed to ionic silver or silver nanoparticles is still needed to elucidate the dose-response relationships for these two forms of silver as cytotoxicants. (This abstract does not reflect the policies of the US EPA).

**656 HYPERTHERMIA-INDUCED CANCER CELL DEATH BY TARGETED MAGNETIC NANOPARTICLES.**

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Magnetic nanoparticle (MNP)-based hyperthermia offers an efficient method of heat delivery for selective killing of tumor cells. Binding of luteinizing hormone-releasing hormone (LHRH) to MNPs such as superparamagnetic iron oxide nanoparticles (SPIONs) coated with gold (SPIONs@Au) can have additional advantages in terms of targeted delivery of MNPs to tumor cells of the reproductive system, particularly those that express the receptors for LHRH. To understand the effects of hyperthermia generated in situ, we synthesized LHRH bound to SPIONs@Au (SPIONs@Au-LHRH; linker: cysteamine) and exposed them to LHRH receptor over-expressing non-cancerous (murine GT1-7 hypotalamic neurons) and cancerous (human LnCap prostate carcinoma) cells. When exposed to SPIONs@Au-LHRH (25-500 μg/mL) for 24 h, there was no significant decrease in the viability of the two cell types. However, when cells were pretreated with SPIONs@Au-LHRH (25-500 μg/mL) for 24 h and then exposed to a magnetic field of 44 Hz for 15 min for LnCap cells or 44 Hz for 2x15 min for GT1-7 neurons, both cell types showed a significant increase in cell death. The cell death in GT1-7 neurons appeared to be late apoptosis or early necrosis, while necrosis was prominent in LnCap cells. LnCap cells exposed to 44 Hz for 15 min had a significant drop in mitochondrial transmembrane potential (ΔΨm); however, no such change in ΔΨm was evident in GT1-7 neurons. In LnCap cells, but not GT1-7 neurons, the cell death was found to be mediated through the caspase-3 pathway. There was an increased expression of heat-shock protein-70 (HSP-70) in GT1-7 neurons but not in LnCap cells. Based on differences in ΔΨm, caspase-3 activity and HSP-70 expression, we believe that LaCap cells are more susceptible to the heat-induced destruction. These findings are new and important and advance our understanding of targeted MNPs for useful therapeutic applications. [Funding support from NSF (HRD1043316) and the US Department of Education (P031R04040) is acknowledged. Corresponding author: rao_uppu@subr.edu].

**657 GRAPHENE PHYTOTOXICITY IN THE SEEDLING STAGE OF CABBAGE, TOMATO, RED SPINACH, AND LETTUCE.**


Graphene, the most recently discovered carbon allotrope, is a two-dimensional building block of atomic thickness that can be stacked into three-dimensional graphite, rolled into one-dimensional nanotubes, or wrapped into zero-dimensional fullerenes. The effects of graphene on root and shoot growth, biomass, shape, cell death, and reactive oxygen species (ROS) of cabbage, tomato, red spinach, and lettuce, were investigated using a concentration range from 500 to 2000 mg/L. The results of the combined morphological and physiological analyses indicate that after 20 days of exposure under our experimental conditions, graphene significantly inhibited plant growth and biomass compared to a control. The number and size of leaves of the graphene-treated plants were reduced in a dose-dependent manner. Significant effects also were detected showing a concentration-dependent increase in ROS and cell death as well as visible symptoms of necrotic lesions, indicating graphene-induced adverse effects on cabbage, tomato, and red spinach mediated by oxidative stress necrosis. Little or no significant toxic effect was observed with lettuce seedlings under the same conditions. The potential effect of graphene largely depends on dose, exposure time, and plant species and deserves further attention.

**658 SUBACUTE AND SUBCHRONIC INHALATION TOXICITY AND DERMAL ABSORPTION OF THE NANOSCALED ZINC OXIDE Z.COTE® HP1 IN THE RAT.**

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This investigation on ZnO nanoparticles was conducted at Fraunhofer ITEM as the European Chemical Industry Council (CEFIC)-funded contribution to the OECD Sponsorship Programme for the Testing of Manufactured Nanomaterials. A
14-day and a subsequent 90-day nose-only inhalation test were conducted with the nanoscaled test item Z-COTE® HP1 and a microsized reference ZnO. OECD guidelines 412 and 413 were expanded by additional endpoints addressing nanoparticle-specific toxicity: i.) bronchoalveolar lavage (BAL) to analyse cytokine release and inflammation; ii.) chemical analysis to follow toxicokinetics of the test item (retention and TEM in lungs and various organs; solubility in physiological fluids); iii.) genotoxicity: micronucleus test (MN); 8-OH-dG in lung tissue; iv.) cell proliferation (Br-DU). After inhalative uptake and phagocytosis Z-COTE® HP1 shows a rapid dissolution under acid conditions in lysosomes and thus has no potential to accumulate in lungs. Chemical toxicokinetics resulted in very small Zn amounts detectable in lungs only 1 day after end of exposure. TEM analysis on particles was negative. Lung toxicity was characterized by an acute inflammation in the high dose group that showed a rapid and complete recovery within 14/28 days (BAL). Histopathological examination revealed a slight degeneration of the olfactory epithelium as the only adverse effect. MN test and 8-OH-dG analysis were negative. The NOAEL values derived from these 14-day and 90-day inhalation tests were 2 and 1.5 mg/m³, respectively. Microscaled ZnO showed tendentially stronger effects than Z-COTE® HP1, probably because of the longer dissolution time needed. In a dermal absorption test in rats (OECD guideline 427) Z-COTE® HP1 was treated by neutron activation to use the Zn-65 gamma tag for a highly sensitive analysis. Zn-65-Z-COTE® HP1 particles or Zn-65 ions were not absorbed via skin.

659 INVESTIGATION OF NANOZEOLITES CYTOTOXICITY.


Nanoseized zeolite particles are important materials for many applications in the field of nanotechnology. The possible adverse effects of these nanomaterials on human health have been scarcely investigated and remain largely unknown. In this study, nanoseized Y and A have been synthesized with particle sizes of 25-50 nm and 45-100 nm, respectively. Adequate colloidal stability for in vitro cytotoxicity experiments has been confirmed. The cytotoxic response of human macrophages (differenitated TPH-1), epithelial (A549) and endothelial (EA.hy926) cells to these nanocrystals was assessed in the absence and presence of fetal calf serum (FCS) by determining mitochondrial activity (MTT assay) and cell membrane integrity (LDH leakage assay). After 24h of exposure, no significant cytotoxic activity was detected for both nanozelotes at doses up to 500 μg/ml, in the presence of FCS or not. Compared to 60 nm amorphous silica nanoparticles, these crystalline nanozelotes-induced only limited toxicity despite an approximately 10-fold greater specific surface area. Low cytotoxic responses were consistent with the absence of intracellular particles in both epithelial and endothelial cells, as confirmed by TEM. In TPH-1 cells, consistent with their phagocytic activity, a limited number of intracellular particles was detected by TEM. Addition of FCS in the cell culture media during exposure to nanozelotes did not increase particle uptake by any cell type. These results may contribute to the application of safe nanozelites for purposes such as medical imaging, sensing materials, low-k films and molecular separation processes. Work was partially financed by the Belgian Ministry of Scientific Policy in the frame of S2NANO project (contract number SD/HE/02A).

660 MEASUREMENT OF DIAMETER OF DIFFERENT NANOPARTICLE AGGLOMERATES AFTER CONTACT WITH A549 CELLS REVEALED DIFFERENT SIZE BEHAVIOR.


Multiwall carbon nanotubes (MWCNT) are discussed to exhibit a fiber-like toxicological potential. For this reason, potential adverse biological effects in vivo (rat) and in vitro (M¢T-5A human mesothelial cells) of MWCNT are investigated in a project, funded by the Federal Ministry of Education and Research (BMBF contract No. 03X0109A), in which MWCNT data will finally be compared with long amosite asbestites as positive control and more particle-like carbon material (Baytubes® and milled MWCNT).

Method: It is hypothesized that only well dispersed MWCNT with a certain fiber length are able to induce mesothelioma. To examine this hypothesis, it is crucial to develop appropriate dispersion methods for MWCNT in a lung-like milieu and to produce and compare MWCNT with different length and diameter characteristics. To reach the planned CNT characteristic: 1) mean length 5-10 μm, D=40 nm; 2) mean length 10-20 μm, D=40 nm; 3) mean length 5-10 μm, D=60 nm) different custom-made MWCNT were synthesized and a dispersion method, using artificial lung medium (Porter et al. 2008) and sonication twice for 5 min with a sonotrode was developed and optimized to enable separation of individual MWCNT fibers. Suspension and MWCNT size distribution were monitored by SEM.

Results: In the present project, it was possible to produce MWCNT with the desired characteristics. The optimized suspension method enabled separation of both MWCNT and long amosite asbestos fibers, but not of Baytubes®. However, for in vitro use dispersion had to be modified, because of some cytotoxicity of the artificial lung medium. Conclusion: These suspensions with custom-made MWCNT will now be used to investigate lung retention, translocation to the pleural cavity after in vivo inhalation, and their potential carcinogenic effect after i.p. injection. For in vivo/in vitro correlation influence of MWCNT on cell viability, proliferation, and DNA-integrity in M¢T-5A cells are investigated.
Silver nanoparticles (SNP) belong to the most commercialized nanomaterials. Here we monitored the biological effects of SNP of different sizes (20 nm, 40 nm) and coatings (citrate, peptide) in human macrophages in vitro. We used THP-1 derived macrophages as a model. The cellular uptake was analyzed by confocal Raman microscopy, TEM or Laser postionization secondary neutral mass spectrometry (Laser-SNMS). Cellular responses upon SNP treatment were studied by time-of-flight secondary ion mass spectrometry (TOF-SIMS) and several biological end-points were evaluated, i.e., cytotoxicity, protein carbonyl formation and induction of heme oxygenase-1 (HO-1). Toxicity of SNP was dependent on exposure time, dose and particle coating. Nanogold proved mainly inert. All kinds of nanoparticles were efficiently taken up. Aggregates and single particles could be detected throughout whole cells, including nuclei and lysosomes. With Laser-SNMS we visualized intracellular SNP and with TOF-SIMS we detected significant changes in the membrane lipid pattern indicating oxidative stress and fluidity changes. We measured strong induction of HO-1 and formation of protein carbonyls with different time patterns. Each type of SNP induced a characteristic carbonyl pattern as resolved by 2D gel electrophoresis. SNP but not nanogold significantly affected the phagocytic activity of macrophages. Some of the particle-mediated effects could be reversed depending on the time and doses applied. Conclusion: SNP exert adverse effects in human macrophages already at subcytotoxic doses. Different kinds of SNP induce distinguishable effects at cellular and biochemical levels.

Important components in estimating risks associated with exposure to nanomaterials include understanding the uptake, distribution, and elimination of nanoparticles. Our project investigates the absorption, distribution, metabolism, and excretion of fullerene C60 and forms of multi-walled carbon nanotubes in nonpregnant, pregnant, and lactating rats and mice. Our initial studies have focused on female rats and mice dosed via tail vein injection with an aqueous suspension of [14C]C60 in polyvinylpyrrolidone (PVP). Urine and feces were collected daily, and tissues were collected at 1, 5, 14 days following 5 consecutive days of i.v. dose. Tissues were also collected at 14 days following 5 consecutive days of i.v. dosing. The largest portions of the recovered radioactivity were detected in the liver (50%), lung (15%), and spleen (9%). Similar distribution profiles for each time-point were determined for female rats and mice administered the single or 5 consecutive doses. Radioactivity levels were above background for many additional tissues, including blood, lymph nodes, GI tract, pancreas, muscle, reproductive tract, mammary tissue, fat, heart, and brain. Only a small portion of the radioactivity recovered (~2%) was eliminated in urine and feces over the weekly collection periods. Transmission electron microscopy (TEM) analysis indicated the presence of electron dense material in lysosomes of Kupffer cells, hepatocytes, and in lung macrophages. Electron dense material was associated with the surface of lipid bodies in adipose tissue. Subcellular localization of the nanomaterials continues via TEM, as well as determination of plasma cytokine levels (markers of inflammation), urinary 8-hydroxydeoxyguanosine (marker of oxidative stress), and quantitative whole body autoradiography. (Supported by NIEHS U19 ES019525.)
Carbonaceous nanoparticles (CNP) have distinctive physical and chemical properties that make them useful in a wide range of applications. As the production of these particles increases, there is a growing need to explore their potentially harmful effects due to environmental and occupational exposure. Mounting evidence indicates that toxicological outcomes of CNP exposure may vary depending on surface properties, size, shape, and functionalization. In the current study, we evaluated pulmonary inflammation and systemic immune responses in mice after pulmonary exposure to structurally different CNPs: pristine C60 fullerene; TRIS-functionalized C60 (C60-TRIS) and graphene oxide (GO). The inflammatory potential of the tested CNPs was found to be as follows: GO > C60-TRIS > C60, as evidenced by accumulation of PMNs, macrophages, and lymphocytes as well as changes in lung permeability and inflammatory cytokine profiles in the lungs on days 1 and 7 post exposure. Further, GO and fullerenols were found to induce reactive oxygen species production by RAW 264.7 macrophages in vitro. To investigate if pulmonary exposure to CNPs altered systemic immune activity, we tested the proliferative response of spleen T cells exposed to GO and C60. Cell proliferation was decreased; however, it was increased in fullerenol-exposed animals. Co-incubation of OVA-specific B3Z hybridoma T cells with OVA-loaded dendritic cells (DC) exposed to GO or fullerenes resulted in altered IL-2 production by B3Z cells, suggesting that modified T cells responses seen in vivo can be partially attributed to a direct modulation of DC functions by GO. Overall, our study shows the potential of fullerenes and GO to induce pulmonary inflammation.
Silver nanoparticles (AgNP) are widely used in a variety of consumer products and biological applications. Intentional and unintentional exposure to AgNP is on the rise, causing health concerns. The toxicity and cell uptake of citrate BioPure™ Ag, (19.2nm), and silica-coated Ag, (40.5nm), were studied in human epidermal keratinocytes (HEK). The AgNP was characterized by diluting in cell culture medium at the highest concentration tested and the size determined by dynamic light scattering at 0, 8, and 24h which increased with time. HEK were exposed to a range of AgNP from 6.25 to 400μg/ml (n=12 wells per concentration). Citrate Ag caused a significant increase in viability at 12.5μg/ml and significant decrease at 50, 100, 200, and 400μg/ml, while silica-coated Ag caused a significant increase at 12.5μg/ml and significant decrease at 400μg/ml. NP controls, serial dilutions of the AgNP in alamar Blue (aB) medium at concentrations and conditions identical to the viability assay, showed silica-coated Ag interacted with aB dye from 12.5 to 400μg/ml. Normalized IL-8 was significantly greater than control in citrate Ag at 6.25, 12.5, and 500μg/ml and significantly less from 100 to 400μg/ml. For silica-coated Ag, IL-8 was significantly greater at 25μg/ml and significantly less at 400μg/ml. Spiked IL-8 controls showed that NP interactions with the cytokine assay suppressed the actual IL-8 concentration by 57.3% in citrate Ag and 41.8% in silica-coated Ag. TEM found agglomerates of electron-dense citrate Ag and silica-coated Ag within cytoplasmic vacuoles of the cells. High concentrations of AgNP can affect the viability and cytokine data thereby causing inaccurate results, so data must be critically analyzed to discern the actual effect from NP interaction. These results show that AgNP do interact with viability and cytokine assays, but citrate Ag and silica-coated Ag causes cell death at the concentrations tested. (Supported by NIH RO1 ES051638)

**Biochemical and Histopathological Evaluation of Functionalized Single-Walled Carbon Nanotubes in Swiss-Webster Mice.**

With their unique physiochemical properties, single-walled carbon nanotubes (SWCNTs) have many potential new applications in medicine and industry. A biomedical application of single-wall carbon nanotubes such as drug delivery requires a fundamental understanding of their fate and toxicological profile after administration. However, the toxicity of SWCNT is barely known when they are introduced into the blood circulation, which is especially vital for their biomedical applications. The aim of this study was to assess the effects, after intraperitoneal injection, of functionalized SWCNTs (carboxyl groups) on reactive oxygen species (ROS) induction and various hepatotoxicity markers (ALT, AST, ALP, LPO and morphology of liver) in the mouse model. We exposed mice to three different concentrations of functionalized SWCNTs and two controls (negative and positive). Samples were collected twenty four hours after the last treatment following standard procedures. Exposure to functionalized SWCNT induced ROS generation and enhanced the activities of serum-amino-transferases (ALT/AST) and alkaline phosphatases (ALP) and the concentration of lipid hydroperoxide compared with control. Histopathology of the exposed liver showed a statistically significant effect in the morphological alterations of the tissue compared with controls. The cellular findings reported here do suggest that purified carboxyl functionalized SWCNT has the potential to induce hepatotoxicity in Swiss-Webster mice through activation of the mechanisms of oxidative stress, which is of sufficient significance to warrant in vivo animal exposure studies. However, more studies to clarify the role of functionalization in the in vivo toxicity of SWCNTs are required and parallel comparison is preferred.

**The Comparison of Mesoporous Silica Nanoparticles and Colloidal Silica on Inflammation and Apoptosis.**

Mesoporous silica (MPS), synthesized via the supramolecular polymer templating method, is one of the most attractive nanomaterials for biomedical applications, such as drug delivery systems, labeling, and tissue engineering. The significant dif-
676 THE CYTOTOXIC EFFECT OF ETOPOSIDE AND DEXAMETHASONE INDIVIDUALLY AND IN COMBINATION WITH SILVER, GOLD, AND SINGLE-WALLED CARBON NANOTUBES ON CANCER CELLS.

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Our laboratory has been focused on the synthesis and development of various nanomaterials for the purpose of delivering chemotherapeutic agents to cancer cells with the intention of selectively killing cancer cells while leaving normal cells intact. Initially we evaluated the ability of known apoptotic agents, such as dexamethasone (D) and etoposide (E) individually and in combination with gold and silver nanoparticles (Au-NPs, Ag-NPs) and single-walled carbon nanotubes (SW-CNTs) to kill HeLa cells and HEK293 cells. We first tested various concentrations of these agents alone and in combination as determined by the MTT assay and FACScan analysis. MPS nanoparticles showed significantly less cytotoxicity and apoptotic cell death than colloidal silica nanoparticles. MPS nanoparticles induced lower expression of pro-inflammatory cytokines, such as tumor necrosis factor-α, interleukin (IL)-6, and IL-1β, in macrophages. The reduced inflammatory response and apoptosis elicited by MPS nanoparticles were resulting from the reduction of mitogen-activated protein kinases, nuclear factor-kB, and caspase 3. In addition, using the local lymph node assay, a standalone in vivo method for hazard identification of contact hypersensitivity, we showed that colloidal silica nanoparticles act as an immunogenic sensitizer and induce contact hypersensitivity but not MPS nanoparticles. In conclusion, the pore architecture of silica nanoparticles greatly influences their biocompatibility and should be carefully designed. The MPS nanoparticles exhibit better biocompatibility than colloidal silica and promise excellent potential usage in the field of biomediatric and biotechnological applications.

677 MICELLAR ENCAPSULATED SULFUR DONORS TO COMBAT CYANIDE INTOXICATION.

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Cyanide poses a threat to human populations worldwide as a chemical warfare agent. After penetrating the mitochondrial membrane, it inhibits cytochrome c oxidase in the electron transport chain of cellular respiration, essentially halting the metabolism of the organism. To combat the cyanide, sulfur donors convert HCN to thiocyanate, which is less toxic and easily excreted in urine. This study is focused on the synthesis and development of various micelle encapsulated sulfur donors for the treatment of specific cancers. We first studied the influence of pore structural conditions of silica nanoparticles on both inflammation and apoptosis, in vitro and in vivo, by comparing MPS and colloidal silica, and defined underlying mechanisms of action. The specific surface area of colloidal silica and MPS was different, respectively, while other conditions, including particle size (100 nm) and shape (spherical) were kept constant. In both MTT assay and FACScan analysis, MPS nanoparticles showed significantly less cytotoxicity and apoptotic cell death than colloidal silica nanoparticles. MPS nanoparticles induced lower expression of pro-inflammatory cytokines, such as tumor necrosis factor-α, interleukin (IL)-6, and IL-1β, in macrophages. The reduced inflammatory response and apoptosis elicited by MPS nanoparticles were resulting from the reduction of mitogen-activated protein kinases, nuclear factor-kB, and caspase 3. In addition, using the local lymph node assay, a standalone in vivo method for hazard identification of contact hypersensitivity, we showed that colloidal silica nanoparticles act as an immunogenic sensitizer and induce contact hypersensitivity but not MPS nanoparticles. In conclusion, the pore architecture of silica nanoparticles greatly influences their biocompatibility and should be carefully designed. The MPS nanoparticles exhibit better biocompatibility than colloidal silica and promise excellent potential usage in the field of biomediatric and biotechnological applications.

678 THE RESPONSES TO DIFFERENT PARTICLES OF ALUMINUM OXIDE IN A549 AND THP-1 CELL.


Background: The increased applications of nanoparticles in various fields of industry raise the concern about toxicological potentiality to human. There are some reports that show the toxicity of nanoparticles depending on many factors including size, shape, chemical composition, surface area, surface charge and others. We compared the toxicity of size (micro-sized and nano-sized particles) and shape (sphere and needle particles) of Al2O3 in cultured cells. In this study, the release of the cytokines IL-6 or IL-8 and LDH were measured at 4, 24 and 48 hours after treatment, and cells were incubated at concentrations of 10, 100,1000 and 3000 μg/mL nanoparticles. Human lung carcinoma epithelial cells (A549) and human acute monocytic leukemia cell line (THP-1) cells were adopted on the assumption of inhalation exposure. Results: The LDH response to nano-sized needle particles was more potent than that to nano-sized sphere particles in both cells. On the other hand, there were no evident differences between nano-seized particles and micro-sized particles at the induction of IL-6, IL-8 and LDH secretion in the in vitro models used in this study. Conclusion: This discovery raises the possibility that Al2O3 has potency to induce LDH secretion in A549 and THP-1 cells depending on their shape.

679 90-DAY INHALATION TOXICITY STUDY IN RATS WITH CARBON NANOFIBERS: CELL PROLIFERATION AND HISTOPATHOLOGY ASSESSMENTS DEMONSTRATE LUNG PARENCHYMAL BUT NOT PLEURAL OR MESOTHELIAL EFFECTS.

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Recent studies have reported that pulmonary or intraperitoneal exposures to carbon nanotubes in rodents have produced pleural or mesothelial effects resembling asbestos exposure. This study investigated the potential adverse pleural effects following a subchronic exposure to VGCF™-H carbon nanofibers (CNF) in male and female SD rats. Groups of rats were exposed nose-only, 6 h/d, 5 d/wk to 0.54, (4.9 f/cc), 2.5 (56 f/cc) or 25 (252 f/cc) mg/m3 VGCF™-H over a 90-day period. Groups of animals from the high and control group were allowed a 90 day recovery period to determine the reversibility of effects observed at the end of the exposure period. Rats were evaluated by standard histopathology, bronchoalveolar lavage and BrdU cell proliferation (CP) methods in selected regions of the respiratory tract (i.e., airway, lung parenchymal, subpleural/mesothelial regions). Minimal and slight inflammation of the terminal bronchiole (TB) and alveolar duct (AD) areas of the lungs were noted in rats exposed to 2.5 and 25 mg/m3, respectively wherein fibrillar-laden alveolar macrophages had accumulated. CNF did not translocate from sites of alveolar deposition to the mesothelial surfaces but were noted within macrophages in the distal alveolar duct/subpleural regions. Greater than 90% BAL-recovered macrophages from animals exposed to 2.5 and 25 mg/m3 VGCF™-H contained CNF, while ~60% of macrophages recovered from rats exposed to 0.5 mg/m3 contained CNF. No adverse histopathological effects of the pleural area were noted. CP studies demonstrated increased (vs. control) BrdU labeling of the TB, AD and subpleural but not mesothelial areas in male and female rats exposed only to 25 mg/m3 VGCF™-H. After 3 months of recovery, CP in the AD and subpleural areas were still increased; however effects in the TB area had reversed. Therefore, subchronic exposure to VGCF™-H resulted in a pulmonary inflammation that was not associated with adverse effects in the pleural or mesothelial areas.

680 INDOLES ALLEVIATE THE DEVELOPMENT OF EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE) BY ACTIVATION OF ARYL HYDROCARBON AND ESTROGEN RECEPTORS LEADING TO ALTERED MICRO-RNA REGULATION AND SUPPRESSION OF TH17 ACTIVATION.

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Diindolylmethane (DIM) and its precursor, Indole-3-carbinol (I3C) are examples of dietary indoles and can be found at relatively high levels in cruciferous vegetables. Recent studies have shown that these indoles may possess therapeutic potential...
based on their inhibitory effects on numerous types of cancer. Data also suggested that DIM and I3C may elicit their effects by acting as ligands for both aryl hydrocarbon receptor (AhR), similar to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and the estrogen receptor (ER). Based on this, we hypothesized that DIM and I3C may regulate the immune response through the activation of AhR and ER to mitigate the development of experimental autoimmune encephalomyelitis (EAE) in a transgenic model of multiple sclerosis (MS). Our study demonstrated that DIM and I3C treatment in EAE-induced mice not only delayed the clinical symptoms of paralysis, but also curtailed the overall severity. We observed that treatment with 13C and DIM resulted in a dramatic decrease in infiltrating T-cells in the brain and spinal cord. In several in vivo and in vitro experiments, DIM and I3C dramatically reduced T cells through interaction with AhR and ER. Based on qPCR analysis, DIM and I3C treatment in EAE-induced mice led to the upregulation of miR-22, which represses target genes ER- 
alpha and IL-17. Together, these studies demonstrated that indoles may mediate their effects through miRNA regulation to suppress inflammation and EAE development.

**681 VITAMIN C RESCUES HYPEROXIA-COMPROMISED MACROPHAGE PHAGOCYTIC FUNCTION BY INHIBITING HMGB1 RELEASE.**

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Mechanical ventilation with supraphysiologic concentrations of oxygen (hyperoxia) is a life saving intervention in patients with respiratory distress. However, prolonged exposure to hyperoxia is associated with high susceptibility to lung injury and ventilator-associated pneumonia (VAP). Alveolar macrophages (AM) phagocytic function can be compromised on prolonged hyperoxia. Previous studies in our lab, have determined that prolonged exposure to hyperoxia leads to HMGB1 release, which can directly impair macrophage’s ability to phagocytose. Excessive ROS production, under hyperoxic conditions disturbs the ROS/Antioxidant balance leading to devastation of the antioxidant protection. Ascorbic acid an antioxidant, exhibits beneficial effect in various diseases mediated by ROS. The aim of this study was to examine whether Vitamin-C could inhibit hyperoxia-induced HMGB1 release and improve hyperoxia compromised macrophage function. RAW 264.7 cells (a macrophage like cell line) were exposed to different concentrations of Vitamin-C prior to 95% O2 exposure for 24h. At the end of 24h: ROS activity, phagocytosis, HMGB1 release and NF-kB translocation were analysed. We show here that Vitamin-C inhibited ROS activity and significantly improved hyperoxia- compromised macrophage ability to phagocytose. Vitamin-C also inhibited HMGB1 release which was associated with inhibition of NF-kB translocation to nuclei. These data indicate that Vitamin-C is beneficial in rescuing hyperoxia compromised macrophage function by inhibiting hyperoxia-induced HMGB1 release.

**682 INHIBITION OF PLATELET AGGREGATION WITH A SPELEN TYROSINE KINASE (SYK) INHIBITOR.**

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Platelets are a key component in hemostasis. Spleen Tyrosine Kinase (SYK) inhibitors have been shown to inhibit platelet activation through inhibition of platelet surface receptors. In a repeat-dose SYK inhibitor study in male rats, pathology findings included hemorrhage in multiple tissues, despite normal prothrombin and partial thromboplastin coagulation times. To identify potential mechanisms of the hemorrhage, we investigated effects of SYK inhibition on platelet function. However, the standard platelet inhibition/platelet aggregation assay is normally low throughput, requires large blood volumes and must be run on specialized equipment. Therefore, we developed a medium throughput, low blood volume standard kinetic absorbance microplate-based platelet aggregation assay using rat whole blood. The platelet aggregation assay was validated and optimized with multiple known platelet activator inhibitors and assay conditions with final selection of thrombin and 13CaCl2 at 95% O2 exposure for 24h. At the end of 24h: ROS activity, phagocytosis, HMGB1 release and NF-kB translocation were analysed. We show here that Vitamin-C inhibited ROS activity and significantly improved hyperoxia-compromised macrophage ability to phagocytose. Vitamin-C also inhibited HMGB1 release which was associated with inhibition of NF-kB translocation to nuclei. These data indicate that Vitamin-C is beneficial in rescuing hyperoxia compromised macrophage function by inhibiting hyperoxia-induced HMGB1 release.

**683 SELENIUM DEFICIENCY PROMOTES PROINFLAMMATORY RESPONSES TO BACTERIAL ENDOTOXIN.**

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Patients with advanced alcoholic liver disease and rodents chronically exposed to ethanol are selenium deficient and have high levels of hepatic inflammation. Selenium acts as an antioxidant, but only after it is co-translationally incorporated into a small number of antioxidant selenoproteins. Recently we showed that the selenium deficiency associated with dietary restriction of selenium resulted in down-regulation of the selenoprotein thioredoxin reductase (TrxR) in the livers of rats. In the present study we tested the hypothesis that selenium deficiency contributes to a pro-inflammatory environment via alterations in TrxR-dependent inflammatory signaling pathways. Pro- and anti-inflammatory cytokine production was measured in a monocyte/macroage cell line under selenium-deficient and selenium-sufficient conditions. Selenium deficiency was induced by culturing RAW 264.7 cells in DMEM supplemented with 10% fetal bovine serum. Although this is a typical culture medium, it supplies only 5 nM selenium (from the fetal bovine serum). This concentration is 10-fold lower than physiological levels. Therefore, selenium-sufficient cultured cells were exposed to 30 nM selenite (as sodium selenite) to the culture medium. Selenium-sufficient cultures had 50% more TrxR protein and activity (measured by western blotting and the insulin reduction assay, respectively) than selenium-deficient cultures. When exposed to bacterial endotoxin (an inflammatory stimulus relevant to the etiology of alcoholic liver disease), both cultures released the pro-inflammatory cytokine TNF-alpha, but the selenium-deficient cells released much less of the anti-inflammatory cytokine IL-10. Therefore, we propose that selenium deficiency contributes to a pro-inflammatory environment by inhibiting the expression of anti-inflammatory mediators such as IL-10.

**684 INHIBITION OF OSTEOSTATIC DIFFERENTIATION BY BROMOPROPANES.**

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1-Bromopropane and 2-Bromopropane was introduced as an alternative to ozone-depleting solvents. However, they were found to exhibit neurotoxicity, reproductive toxicity, and hepatotoxicity in rodents and neurotoxicity in human. However, the influence of bromopropanes on differentiation of osteoblast is unknown. Here we investigated whether bromopropanes has an inhibitory effect on osteoblastic differentiation using an osteoblastic cell line (C2C12). Bromopropanes inhibited the bone morphogenetic protein (BMP) 2-induced mRNA levels of alkaline phosphatase (ALP), collagen I (COL1), osteocalcin (OC) and Runx2, which are specific markers for osteoblastic differentiation, in a dose-dependent manner. Moreover, the addition of COL1 and ALP-based reporter genes, which are induced by BMP2, and the transcriptional activity of Runx2 on OC and ALP promoters were inhibited by bromopropanes. The amount and activity of ALP induced by BMP2 were also decreased by bromopropanes. These results suggest that bromopropanes have an inhibitory effect on osteoblastic differentiation.

**685 POSITIVE REGULATION OF MUCOSAL MICROsomAL PROSTAGLANDIN E SYNTHASE 1 BY ACTIVATED ONCOGENE RHOA GTPASE IN EPITHELIAL INFLAMMATION AND CARCINOGENESIS.**

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Oncogenic RhoA GTPase has been investigated as a mediator of pro-inflammatory responses and aggressive carcinogenesis. Among the various targets of RhoA-linked signals, pro-inflammatory prostaglandin E2 (PGE2), a major prostaglandin metabolite, was assessed in epithelial cells. RhoA activation increased PGE2 levels and gene expression of the rate-limiting PGE2 producing enzymes, cyclooxygenase-2 and microsomal prostaglandin E synthase 1 (mPGES-1). In particular, human mPGES-1 was induced by RhoA via transcriptional activation in epithelial cells. To address the involvement of potent signaling pathways in RhoA-activated mPGES-1 induction, various signaling inhibitors were screened for their effects on mPGES-1 promoter activity. RhoA activation increased basal and cytokine-activated nuclear factor-kB and extracellular signal-regulated kinase1/2 proteins, all of which were positively involved in RhoA-induced gene expression of mPGES-1. As one potent 

**686 INHIBITORY EFFECTS OF CELENDA IN HYPEROXIA-MEDIATED INFLAMMATORY DAMAGE IN AN IN VIVO HUMAN CHONDROCYTE MODEL **


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Here we investigated the effects of celenda, a novel substance, on the hyperoxia-mediated inflammatory damage in an in vivo human chondrocyte model. The chondrocyte model was established using human articular cartilage from the knee. The chondrocytes were cultured in high oxygen environment (25% O2) for 24 h, followed by celenda treatment (0-100 μM) for 24 h. The effects of celenda were assessed using matrix metalloproteinase (MMP)-13 and matrix metalloproteinase-8 (MMP-8) by western blotting and enzyme-linked immunosorbent assay. The results demonstrated that celenda inhibited the expression of MMP-13 and MMP-8 in a dose-dependent manner. These findings suggest that celenda may have potential therapeutic applications in the treatment of hyperoxia-mediated inflammatory damage.
687 1-BROMOPROPAINE INDUCES CYCLOOXYGENASE-2 GENE EXPRESSION IN MURINE MACROPHAGE RAW 264.7 CELLS.

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1-Bromopropene (1-BP) has been used in the workplace as an alternative to ozone-depleting solvents. This study examined the effect of 1-BP on cyclooxygenase-2 (COX-2) expression and analyzed the molecular mechanism of its activity in murine macrophage RAW 264.7 cells. Exposure to 1-BP markedly enhanced the production of prostaglandin E2 (PGE2), a major COX-2 metabolite in RAW 264.7 cells. Additionally, 1-BP dose dependently increased the levels of COX-2 mRNA and protein. Moreover, 1-BP enhanced luciferase activity of COX-2 regulation-related transcription factors including nuclear factor kB (NF-kB) and CCAAT-enhancer binding protein (C/EBP). Phosphorylation level of PI3K/Akt, extracellular signal-regulated kinase (ERK) and p38 MAPK phosphorylation were also significantly activated by 1-BP. These results suggest that 1-BP increases COX-2 expression by activation of NF-kB and C/EBP via PI3K/Akt, ERK and p38 MAPK.

688 ACTIVATION OF NICOTINIC PATHWAY INHIBITS HYPEROXIA-INDUCED HMGB1 RELEASE AND RESCUES MACROPHAGE PHAGOCYTIC FUNCTION.

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The vagus nerve, the longest cranial nerve of autonomous nervous system, regulates the levels of cytokines, including HMGB1, thus modulating inflammatory damage. This modulation is executed through α7 nicotinic acetylcholine receptor (α7nAChR), a nicotinic anti-inflammatory pathway. Macrophages play a very crucial role in inflammatory responses against invading pathogens. However, exposure to prolonged hyperoxia compromises their ability to phagocytose bacteria via inducing HMGB1 release into extracellular milieu. The aim of this study was to examine whether GTS-21, a selective α7nAChR agonist could inhibit hyperoxia-induced HMGB1 release from RAW 264.7 cells (a macrophage like cell line) and enhance macrophage function under hyperoxic condition. RAW cells were treated with different concentrations of GTS-21 prior to exposure to 95% O2. Phagocytosis assay was performed to determine macrophage function via phagocytosis microorganisms. HMGB1 release was assessed by Western blotting and immunofluorescence analysis. Nuclear factor kappa B (NFkB) has been shown to modulate endotoxin-induced HMGB1 release. Activation of NFkB was assessed by its translocation from cytoplasm to nuclei by immunofluorescence assay. We show here that GTS-21 dose dependently enhanced macrophage phagocytic function and inhibited HMGB1 release.

689 THE ROLE OF NO AND OXIDATIVE STRESS IN THE INTRATRACHEAL BLEOMYCIN MODEL OF LUNG INJURY AND INFLAMMATION.

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Pulmonary injury, and its consequent inflammation, has a pronounced effect on lung function, as determined by its elastance and resistance profiles. However, the mechanisms relating the effects of inflammation upon lung function remain unclear. In this study the effects of intratracheally-administered bleomycin (ITB) upon lung inflammation, and its amelioration by γ-tocopherol, were examined at the molecular and functional level. 10 week old C57/B16, wild-type, mice were administered 2 U/kg bleomycin sulfate, or buffered saline as control, and assessed on days 3 and 8 post injury. Mice were fed either a control or a 0.3% γ-tocopherol mixture diet from 2 weeks prior to, and throughout, ITB. Lung function was measured via Forced Oscillation Technique (FOT) at a range of Positive End Expiratory Pressures (PEEPs). Following functional assessment lungs were lavaged and tissue harvested for the measurement of inflammatory markers. ITB resulted in pronounced increases to both the low frequency resistance and elastance profiles; these effects were PEEP-dependent. These changes were accompanied by increases in inflammatory score, via H&E, and expression of biomarkers namely, iNOS and COX2 by IHC and rt-PCR. Pulmonary inflammation, and its amelioration by γ-tocopherol, were examined at the molecular and functional level. 10 week old C57/Bl6, wild-type, mice were administered 2 U/kg bleomycin sulfate, or buffered saline as control, and assessed on days 3 and 8 post injury. Mice were fed either a control or a 0.3% γ-tocopherol mixture diet from 2 weeks prior to, and throughout, ITB. Lung function was measured via Forced Oscillation Technique (FOT) at a range of Positive End Expiratory Pressures (PEEPs). Following functional assessment lungs were lavaged and tissue harvested for the measurement of inflammatory markers. ITB resulted in pronounced increases to both the low frequency resistance and elastance profiles; these effects were PEEP-dependent. These changes were accompanied by increases in inflammatory score, via H&E, and expression of biomarkers namely, iNOS and COX2 by IHC and rt-PCR.

690 MECHANISM OF ANTHRAX LETHAL TOXIN-INDUCED PULMONARY EDEMA.

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Anthrax is characterized by the development of severe vascular leak that has been linked to its lethality. This leak is believed to be mediated by the toxin produced by the bacillus anthracis bacteria, in particular anthrax lethal toxin (LeTx), which on its own can reproduce the vascular leak and lethality of anthrax infection. The active component of LeTx, referred to as lethal factor, is a protein specified by the gene coding for the biologically active form of the lethal factor (LF, a β-galactosidase enzyme with anthrax LeTx increases endothelial barrier permeability and gap formation between endothelial cells through disrupting p38 signaling. LeTx treatment increases MKK3b degradation and in turn decreases p38 activity at baseline as well as after activation of p38 signaling. Consequently LeTx treatment decreases activation of the p38 substrate kinase, MK2, and the phosphorylation of the latter's substrate, HSP27. LeTx treatment disrupted other signaling pathways leading to suppression of Erk-mediated signaling, but these effects do not correlate with LeTx-induced barrier compromise. Overexpressing phosphomimicking (pm)HSP27, which protected the endothelial...
The role of corepressor splice-variants in regulating lung inflammation.


The incidence of inflammatory lung diseases, such as asthma and COPD, is increasing dramatically worldwide. While many theories address the cause of this phenomenon, the most convincing argument is exposure of the population to inhaled oxidants. Tobacco smoke and many air pollutants cause oxidative stress, a major player in the inflammatory response. However, the mechanistic connections between oxidative stress and disease state remain largely unknown. Consequently, the majority of current treatments for asthma and COPD involve mediating symptoms rather than targeting inflammation. The need for novel therapies and the mechanistic disconnect between oxidants and disease make further investigation critically necessary. Emerging evidence indicates an anti-inflammatory role for nuclear receptors (ligand-regulated transcription factors) in lung disease. Some agonists for these receptors seem to mitigate the immune response, and may offer more specificity and efficacy than current drugs on the market. An accepted model for the anti-inflammatory effects of nuclear receptor agonists involves the recruitment of corepressor (CoR) proteins to the promoters of inflammatory genes, and subsequent suppression of the inflammatory response. However, CoRPs are subject to alternative mRNA splicing and different isoforms have been shown to possess unique behaviors. I propose that a subset of CoR splice-variants are particularly effective at suppressing the inflammatory gene expression caused by inhaled oxidants. Using immune cells as a model, functional reporter assays reveal differences in the abilities of CoR isoforms to suppress the inflammatory response. Furthermore, in vitro protein binding assays show preference of inflammatory transcription factors for certain CoR splice-variants over others. These results, in combination with ongoing chromatin immunoprecipitation experiments and inflammation models in CoR knockout mice, will determine which CoR isoforms are the best pharmaceutical targets and which nuclear receptor agonists are the best treatments for ameliorating inflammatory lung diseases.

Mitochondrial uncoupling protein 3 (UCP3) is a member of the highly conserved subfamily of mitochondrial carriers that regulates the efficiency of skeletal muscle (SKM) mitochondrial respiration through proton leak. Mounting evidence has implicated UCP3 in the protection of SKM from high fat diet-induced insulin resistance, yet the mechanisms by which UCP3 exerts these protective effects are not well understood. Δ3,5,Δ2,4 dienoyl-CoA isomerase (DCI) is an "auxiliary" fatty acid (FA) metabolizing enzyme that permits the complete oxidation of unsaturated fatty acids with double bonds in the odd position (e.g. oleate), through isomerization of the double bond from odd to even positions along the fatty acid chain. Although little is known about the physiological function of DCI, it has been postulated to prevent the accumulation of un-metabolizable unsaturated FA metabolites. Interestingly, DCI exhibits an overlapping protein expression profile with UCP3 in brown adipose tissue, skeletal muscle, and heart. Using a variety of biochemical approaches, we show that UCP3 interacts with DCI in the mitochondrial matrix via the central matrix UCP3 loop, and in a manner augmented by the presence of oleate in cells. The data also indicate that the DCI:UCP3 complex functions to metabolize unsaturated fats specifically. Studies to assess the potential importance of the DCI:UCP3 complex in lipid signaling and toxicity will likely yield valuable insights into the mechanisms of UCP3-regulated fat metabolism and protection from lipotoxicity.

Persistent neuroinflammation and microglial activation have been found to play an integral role in the pathogenesis of many neurodegenerative disorders including Parkinson's disease (PD). Here, we investigated the role of sodium channels (Nav) and Nav/H+ exchangers (NHE) in the activation of microglia like cells (BV-2) after lipopolysaccharide (LPS) exposure. LPS (10 and 100 ng/ml) caused a rapid dose- and time-dependent accumulation of intracellular sodium ([Na+]i) in activated BV-2 cells. Pre-treatment of cells with the Nav antagonist tetrodotoxin (TTX, 1μM) completely abolished Na+ influx but was unable to prevent the accumulation of [Na+]i observed at 6 and 24 hr after LPS exposure. NHE inhibitor cariporide (1μM) significantly reduced the accumulation of [Na+]i 24 hr after LPS exposure. Furthermore, LPS significantly decreased the mRNA expression of a number of Nav isoforms by 30-60% at the 6-24 hrs time points, which was prevented by TTX at LPS 10 ng/ml but not at 100 ng/ml. Furthermore, LPS increased the mRNA expression and protein level of NHE-1 in a dose- and time-dependent manner which was significantly reduced after co-treatment with cariporide. LPS increased TNF-α, IL-1β, IL-6, and IL6 mRNA in a dose- and time-dependent manner. The increased expression of these cytokines was significantly reduced by NHE inhibition. In addition, increased production of ROS and H2O2, and expression of grp94/psa was also observed following LPS treatment which was significantly reduced by TTX or the combination of TTX and cariporide. Collectively, these data suggest that down-regulation of Nav isoforms following LPS exposure may alter intracellular pH homeostasis, resulting in a compensatory up-regulation of NHE-1. Thus, the up-regulation of NHE-1 causes excessive accumulation of [Na+]i into BV2 cells, which may contribute to the inflammatory cascade and neurodegeneration. Supported by NIH ES015991 and ES070022.

Amelioration of lipopolysaccharide/D-galactosamine-induced acute liver toxicity by 3-diindolylmethane, a plant-derived ahr ligand.

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Lipopolysaccharide/D-Galactosamine (LPS/D-GalN) induced acute liver injury is widely used as an animal model for screening of potential hepatoprotective compounds. The mouse model is characterized by activation of macrophages, upregula-
SULFOPHANE RESTORES HISTONE DEACETYLASE ACTIVITY IN HUMAN EPITHELIAL CELLS EXPOSED TO CIGARETTE SMOKE RESULTING IN DECREASED CYTOKINE PRODUCTION.

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RATIONALE: Chronic obstructive pulmonary disease (COPD) is a major public health problem and currently the third leading cause of death in the US. Exposure to cigarette smoke (CS) is the primary factor associated with the development of COPD. CS exposure leads to oxidative stress in the lungs, which decrease histone deacetylase (HDAC) activity and promote the expression of pro-inflammatory cytokines. Decreased HDAC activity levels correlate with disease severity and increased cytokine production in COPD patients. Sulfophane (SFN), a phytochemical found in broccoli, is able to activate nuclear erythroid 2 like factor-2 (Nrf2), which is a basic leucine zipper transcription factor that regulates the expression of multiple genes involved in antioxidant and detoxification pathways. We hypothesize that SFN will activate Nrf2-dependent gene expression to increase HDAC activity levels thereby reducing CS-induced cytokine production in human epithelial cells.

RESULTS: Exposure to cigarette smoke extract (CSE) in BEAS-2B cells significantly decreased HDAC activity levels and concomitantly increased production of both IL-8 and MCP-1 protein in cell culture supernatants. HDAC activity levels were restored by activating Nrf2-dependent pathways in BEAS-2B cells with SFN prior to CSE exposure. Microarray analysis was conducted to determine which genes may be responsible for the rescue of HDAC activity levels.

CONCLUSIONS: Our results indicate that SFN can restore HDAC activity levels in human epithelial cells exposed to CSE. However, the cellular mechanisms, by which HDAC activity and the production of cytokines are altered by SFN treatment, remain to be elucidated. We hypothesize that SFN activates Nrf2-dependent pathways leading to the inhibition of pro-inflammatory pathways such as NF-kB or AP-1 possibly through chromatin remodeling of the promoter sequences.

FUNDING SOURCE: Funding for this research was provided by the Byron Dare Junior Faculty Award and the Faculty Development Grant from Fort Lewis College.

698 PERFLUOROOROCANIC ACID INDUCES MAST CELL-MEDIATED ALLERGIC INFLAMMATION: RELEASE OF HISTAMINE, INVOLVEMENT OF CALCIUM, CASPASE-1, COX-2 AND NUCLEAR FACTOR-XB.

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Perfluorooctanoic acid (PFOA) has become an increasing concern because of its immune response in immune system, regularly found in the blood of animals and humans worldwide. Effects of PFOA is widely known and studied in the field of toxicology because of its unique physical and chemical characteristics, water and oil repellency, thermal stability, and surfactant properties. The role of PFOA in the allergic inflammation is not well-known. To further extend the study on PFOA, we examined mast cell-mediated allergic inflammation by PFOA and studied the possible mechanism of action. PFOA dose dependently increased histamine release and the release of histamine was mediated by the modulation of intracellular calcium. In addition, PFOA induced gene expression and secretion of pro-inflammatory cytokines such as tumor necrosis factor (TNF-α), Interleukin (IL)-1β, IL-6 and IL-8 in human mast cells. The inducing effect of PFOA on these pro-inflammatory cytokines was nuclear factor-XB, p38 and caspase-1 dependent. Furthermore, the activation of cyclooxygenase (COX)–2 by PFOA suggests the involvement of allergic inflammatory mediators in human mast cells. Our findings provide evidence that PFOA, the known immunotoxic agent, induces mast cell-mediated allergic inflammatory reactions by histamine release and pro-inflammatory cytokine expression.

699 TCDD EXACERBATES CONCANAVALIN A–INDUCED LIVER INJURY AND REVEALS A CRITICAL ROLE FOR NK CELLS IN A MODEL OF IMMUNE-MEDIATED LIVER INJURY.

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For many liver diseases, including viral and autoimmune hepatitis, immune cells play an important role in the development and progression of liver injury. ConcanaValin A (con A) administration is used as a model of immune-mediated liver injury resembling autoimmune hepatitis. 2,3,7,8-tetrachlordibenzo-p-dioxin (TCDD) has been reported to affect the development and severity of some autoimmune diseases. We have shown previously that mice treated with TCDD have an exacerbated response to administration of a mild dose (6 mg/kg) of con A, resulting in increased hepatocellular damage. In the present study, we tested the hypothesis that natural killer (NK) cells and the inflammatory cytokine interferon gamma (IFNγ) play critical roles in the development of liver injury from TCDD/con A co-exposure. Mice were treated on day 0 with TCDD (30 μg/kg) or olive oil (vehicle) and four days later with either con A or saline. Plasma samples were collected at various times after treatment, and liver injury was assessed from the activity of alanine aminotransferase in the plasma. Plasma concentration of IFNγ was significantly increased by TCDD/con A treatment, and injury was attenuated in IFNγ KO mice. After treatment, intrahepatic NK cells were isolated, and cell activation was determined by flow cytometry. TCDD/con A treatment increased the percentage of NK cells expressing activation marker CD69 and the costimulatory receptor NKG2d, and they were a significant source of IFNγ. Depletion of NK cells prior to treatment resulted in significant reductions in plasma IFNγ and liver injury from TCDD/con A treatment. In summary, exposure to TCDD exacerbated the immune-mediated liver injury induced by con A. In addition, our findings suggest
Activated macrophages play a key role in the pathogenic response to toxic doses of APAP. However, their contribution depends on their phenotype and timing of appearance in the liver. Thus, whereas early on, classically activated macrophages (CAM) contribute to tissue injury by releasing proinflammatory/cytotoxic mediators, later, alternatively activated macrophages (AAM) suppress inflammation and initiate tissue repair. We speculated that the β-galactoside binding lectin, Gal-3, is important in regulating macrophage activation phenotype. Treatment of WT mice with APAP (300 mg/kg, i.p.) resulted in increases in serum transaminases, beginning within 6 h, and histological evidence of centrilobular necrosis, which persisted for 24-48 h. This was associated with an influx of proinflammatory Ly6C-positive macrophages into centrilobular regions of the liver, which were distinct from F4/80-positive resident macrophages. The appearance of the Ly6C-positive macrophages correlated with increased hepatic expression of the CAM markers, iNOS and IL-12, and chemokines CCL3, CCL4, and CCL20. Ly6C-positive CAM were also found to express Gal-3. Gal-3-/- mice were used to analyze the role of this protein in macrophage activation. APAP-induced expression of CAM markers and hepatotoxicity were significantly attenuated in Gal-3-/- mice. In contrast, expression of endothelial nitric oxide synthase and nitric oxide levels, as well as the production of various extracellular (ECM) proteins were determined. We also assessed retinal EC adhesion on various ECM proteins, their junctional organization, VEGF expression, and oxidative stress state after incubation with various inflammatory cytokines. Cell viability and proliferation of retinal EC incubated with TNF-α, IL-1β and MCP-1 for 24 h was not affected. Incubation with TNF-α and IL-1β, but not MCP-1, decreased retinal EC migration and their ability to undergo capillary morphogenesis. Incubation with TNF-α and IL-1β, but not MCP-1, for 48 h resulted in increased production of VEGF, increased oxidative stress, and abnormal junctional localization of VE-cadherin at sites of the cell-cell contact consistent with altered cellular permeability. Together our results demonstrate that the inflammatory cytokines have specific adverse effects on angiogenic properties of retinal EC which is associated with increased oxidative stress and altered vascular permeability. Thus, altered production of proinflammatory cytokines during diabetes has a significant impact on retinal vascular function and pathogenesis of DR.

Macrophages are a major immune constituent of the lung, where they are the first line of defense and are instrumental in the clearance of inhaled particulates. Both in vitro and in vivo experiments have shown that interaction of macrophages with certain particulates (silica, nanomaterials) can have profound effects. Some studies have focused on cytotoxicity, while others have investigated alterations in functions. In our silicosis model we have observed death, survival, and expansion of macrophages. This suggests that either macrophages react differently to the same particle, or these observations are the result of different types of macrophages. A growing area of research is the role of macrophage subsets in certain pathologies. Previous work in our laboratory has described a role for the Th2-associated M2a subset in the Balb/c model of silicosis. Data further suggests that the insulin-like growth factor (IGF)-1-Akt pathway plays a role in the survival of a subset of particle-exposed macrophages. In addition to this pathway, other Th2-associated components that appear to contribute to the generation of a fibrotic environment include IL-33 and myeloid suppressors. Because IL-33 is a product of inflammasome activation and a promoter of Th2 immunity, it represents a candidate for the switch between the initial particle-induced Th1 inflammation and Th2-guided fibrosis in these models; while the early rise in IL-4+ myeloid suppressors may induce early IGF-1 production. These data suggest the contribution of multiple components in the rise of Th2 immunity following pulmonary exposure to certain particulates and the generation of a fibrotic environment.

This work is supported by NIH grant RR-017670 and ES015294.
ity under inflammatory stress. In this study, we developed an in vitro model to study the roles of caspase activation and oxidative stress in TNF potentiation of AMD cytotoxicity. AMD caused apoptotic cell death in Hepa1c1c7 cells, and TNF co-treatment potentiated its toxicity. Activation of caspases 9 and 3/7 was observed in AMD/TNF-α co-treated cells, and caspase inhibitors provided minor protection from cytotoxicity. Cytotoxic and mitochondrial reactive oxygen species and lipid peroxidation were observed after treatment with AMD and were further elevated by TNF co-treatment. Adding water-soluble antioxidants (tropolox, N-acetylcysteine, glutathione or ascorbate) produced only minor attenuation of AMD/TNF-induced cytotoxicity and did not influence the effect of AMD alone. On the other hand, α-tocopherol prevented AMD toxicity and caused pronounced reduction in cytotoxicity from AMD/TNF co-treatment. α-Tocopherol also reduced pan-caspase inhibitor completely abolished AMD/TNF-induced cytotoxicity. In summary, activation of caspases and oxidative stress were observed after AMD/TNF co-treatment, and caspase inhibitors and a lipid-soluble free radical scavenger attenuated AMD/TNF-induced cytotoxicity. (Supported by NIH R01DK061315.)

705 MOLECULAR MARKERS AND EVALUATION OF PHENOTYPIC RESPONSES IN RAW 264.7 MACROPHAGES.
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The adopion of alveolar macrophage phenotype, either classical (M1) or alternative (M2) is critical to the balance between inflammation and repair in the lung. The establishment of a cellular model to examine the mechanisms involved in phenotype adoption would be invaluable. We used RAW 264.7 cells, the M1 activator, LPS, and the M2 activator, IL-4 to develop a model system to study macrophage phenotypic adoption. Further we have used this system to examine the role of the pulmonary collectin Surfactant Protein-D in phenotypic differentiation. Cells were treated with LPS or IL-4 at varying doses and after 24 hours later evaluated for morphology, NO production, surface marker expression, gene and protein expression, enzyme activity. Morphologic studies were conducted by microscopy and by assaying cell volume; NO release was measured as a function of NO2- content in the medium using Sievers NOA; mRNA expression was measured by rt-PCR assaying cell volume; NO release was measured as a function of NO2- content in the medium using Sievers NOA; mRNA expression was measured by rt-PCR. Our results confirm RAW 264.7 as a viable model of macrophage phenotype in response to treatment with classical activators and a valuable tool to assess the mechanism involved.

706 COMPARISON OF IN VITRO AND IN VIVO CYTOKINE RESPONSE IN CYTOMOLGUS MONKEYS AFTER TREATMENT WITH AN ANTI-CD3 MAB.
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Evaluation of test article-related effects on inflammatory markers requires a good understanding of the specificity and sensitivity of the various markers available in preclinical GLP studies. Markers of the inflammatory response includes cytokines, acute-phase proteins (CRP) and complement factors. In vitro stimulation with an anti-CD3 mAb was used to evaluate the correlation between release of acute-phase proteins, cytokines and complement proteins in large animals. Furthermore, use of in vivo cytokine release assays as predictive tools of in vivo cytokine release was also evaluated during this study. Animals were administered doses of anti-CD3 mAb by bolus IV injection. Samples were collected pre and post-treatment for immunophenotyping and for cytokine, complement factor and CRP analysis. For each animal, whole blood samples were collected pre-treatment and were incubated with each animal's expected post-treatment plasmatic concentration of anti-CD3 mAb. Plasma was then analyzed for cytokines to evaluate if the cytokine response observed in vitro was predictive of the cytokine release observed in vivo. In most animals, substantial decrease in lymphocyte counts was observed 24 hours after treatment at all doses. Lymphocytes were back to baseline level 5 days after anti-CD3 mAb injection. Dose dependent transient increases of TNF-α, IL-10, IL-2, IFN-γ, IL-12, IL-5, IL-6, G-CSF, IL-1RA, CRP and complement split products Bb and C3a were observed after treatment. For one mid dose male, these changes correlated with strong in vitro release of IL-2, IFN-γ, TNF-α and IL-1RA. The anti-CD3 mAb model was useful to demonstrate that analytical methods were suitably sensitive to detect biologically relevant changes for all the biomarkers measured in the study. Data also showed that in vitro cytokine release assays may be predictive of intensity of the cytokine release in vitro but not necessarily of the occurrence of cytokine release.

707 A SMALL MOLECULE INHIBITOR OF THE TGF-β1 TYPE 1 RECEPTOR SUPPRESSES UVB-INDUCED MOUSE SKIN CARCINOGENESIS.
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Ultraviolet radiation is a key environmental mutagen and chronic exposure to UVB is the leading cause of Skin Cancer. Transforming Growth Factor (TGF-β) represents an important immunoregulatory cytokine in the skin microenvironment with context dependent role in modulating tumor formation and progression. The immune system plays a critical role in containing the toxic damage caused by UVB; TGF-β has been known to play a key role in the activation and survival of immune cells. However, the specific role of TGF-β signaling in UVB induced skin cancers is not clear. To study this interplay between UVB, TGF-β and the immune system, we performed a UVB carcinogenesis study in Skin Hairless (SKH1) mice in the presence or absence of SB431542, a small molecule inhibitor of the TGF-β1 type I receptor. We observed a significant decrease in the number of papillomas with the inhibition of TGF-β1 pathway and a concomitant decrease in the tumor infiltration and activation status (IFNγ secretion) of CD4 and CD8 lymphocytes. In order to understand the mechanism of suppression of T cell response by SB431542, we analyzed the immediate upstream event, antigen presentation by dermal dendritic cells and langerhans cells. Preliminary data suggests that inhibition of the TGF-β1 pathway with SB431542 suppresses the migration of skin dendritic cell subsets to the lymph nodes in response to UV irradiation, in turn suppressing the T lymphocyte response. In summary, the TGF-β1 signaling pathway is important for the activation of the inflammatory response to UVB irradiation of the skin, which may mediated through Dendritic cells.

708 EXTRACT OF Ripe FRUITS OF RUBUS COREANUS INHIBITS MAST CELL-MEDIATED ALLERGIC INFLAMMATION.
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In this study, we investigated the effect of water extract of ripe fruits of Rubus coreanus Miq. (Rosaceae) (RFRC) on the mast cell-mediated allergic inflammation and studied the possible mechanism of action. Mast cell-mediated allergic disease is involved in many diseases such as anaphylaxis, rhinitis, asthma and atopic dermatitis. RFRC dose dependently inhibited compound 48/80-induced systemic anaphylaxis and serum histamine release in mice. RFRC reduced the immunoglobulin E mediated local allergic reaction, passive cutaneous anaphylaxis. RFRC attenuated histamine release from rat peritoneal mast cells and human mast cells by the reduction of intracellular calcium. RFRC decreased phorbol 12-myristate 13-acetate (PMA) and calcium ionophore A23187 (PMACI)-stimulated expression and secretion of pro-inflammatory cytokines in human mast cells. The inhibitory effect of RFRC on the cytokines production was nuclear factor (NF)-κB and mitogen-activated protein kinase (MAPK) dependent. In addition, RFRC suppressed the activation of caspase-1. Our findings provide evidence that RFRC inhibits mast cell-derived allergenic inflammatory reactions, and the involvement of calcium, NF-κB, MAPKs, and caspase-1 in these effects. Furthermore, in vivo and in vitro anti-allergic inflammatory effects of RFRC provide affirmative proof that a possible therapeutic application of this agent in allergic inflammatory diseases.

709 WHOLE-MOUNT IMAGE CAPTURE AND ANALYSIS OF IDIOPATHIC PULMONARY FIBROSIS IN BLEOMYCIN-INDUCED MOUSE MODELS USING MICRO-COMPUTED TOMOGRAPHY AND VIRTUAL HISTOLOGY.

Drug-induced idiopathic pulmonary fibrosis (IPF) is a known adverse side effect associated with regemented bleomycin therapy for remediation of a number of oncologic diseases. Preclinical animal models have been developed for comparative
analysis to clinical etiological pathways of IPF, but imaging methods to capture the heterogeneity of fibrotic distribution in whole-mount lung tissue have not been ade-
quate addressed. It is demonstrated here that contrast enhanced micro computed
tomography (microCT) scanning allows for whole-mount imaging and analysis
(Virtual Histology) of IPF in both intratracheal (IT) instillation and subcutaneous
(Sub Q) micropump dosing of bleomycin in mouse models. Virtual Histology is
compared with traditional histological methods including Masson’s trichrome and
modified Ashcroft scoring. The advantages of this non-destructive Virtual
Histology over traditional histology are reviewed. In addition to derivation of ex-
quise high-resolution planar (2-dimensional) and volumetric (3-dimensional) re-
formats, computational analysis reveals discrete attenuation curves associated with
naive, acute (IT), and chronic (Sub Q) dosing methods, facilitating more accurate
assessments of fibrotic lung tissue burden and distribution in preclinical drug
development pipelines.

**710 HEPATIC METALLOTHIONEIN INCREASES AFTER EXPERIMENTAL SURGERY: ROLE OF SOME CYTOKINES AND STRESS HORMONES.**

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Abdominal surgery as a localized inflammatory model has been used to study the
redistribution plasma/liver zinc redistribution and the induction of hepatic metal-
lothionein (MT) synthesis. In this work we explore the role of inflammatory cyto-
kines and stress hormones on the increases of liver MT in the early stages after an
abdominal surgery in rats. Wistar rats were surgically operated, receiving a 3-cm
ventral incision. Groups of five animals were killed by exsanguinations at 3, 6, 9
hours, and liver samples were obtained and stored at -70C. Plasma cyto-
kines (IL-1, -6 and TNF-ct) and hormones (adrenaline, noradrenalin and glucagon)
were determined by an ELISA assay. Hepatic MT protein was deter-
mined using a cadmium saturation method. Other groups of rats were adminis-
tered with inhibitors of IL-1 and -6 MT synthesis signaling pathways (A6490,
SB236093 and PDTC) and hormone inhibitors (propranolol and oestredose), pre-
nious surgery operation. Hepatic MT protein increased significantly 6h post-sur-
ger and peaked up to fivefold of control animals at 12h. IL-1 and -6 shown in-
treases starting at 3h after surgery, however, TNF-ct did not show changes as
compared with the control group. Adrenaline and glucagon shown significantly in-
tereases after surgery, increases start in the first hour post-surgery, keeping these lev-
el at the end of the evaluation, meanwhile, noradrenalin show a decrease. Signaling
pathways and hormone inhibitors decreased MT hepatic levels as compared with
the MT levels showed by the animals with surgery. Results obtained shown the role
of IL-1 and -6, adrenaline and glucagon as inducers of MT synthesis in the early stages
of experimental surgical trauma.

**711 PLUM POLYPHENOLICS AMELIORATE INFLAMMATION AND METABOLIC ALTERATIONS IN OBESE ZUCKER RATS.**

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Obesity is a risk factor leading to inflammation and chronic diseases and epidemiolo-
gical evidence has demonstrated protective effects of fruits and vegetables. We
investigated how peach and plum polyphenolics can prevent metabolic disorders in
obese Zucker rats. Experimental groups were feed with peach or plum juice ad libi-
tum, the placebo and lean Zucker groups received same amount of sugars as peach
and plum juices in drinking water. Body weights were recorded every week, and
blood serum was analyzed at baseline and after 11 weeks. Gene expression in heart
tissues was analyzed by RT-PCR. Total polyphenolic content was higher in plum
than in peach juice (1270 vs 430 mg gallic acid equiv./mL). Results showed that at
the concentration of 0.79 and 0.58- fold respectively). In conclusion, consumption of plum juice
maintained lower body mass index than Zucker rats against obesity-induced inflammation and metabolic
disorders at higher extent than peach juice and this was due to the higher content of polyphenolics.

**712 ODSH ENHANCES BACTERIAL CLEARANCE IN PSEUDOMONAS AERUGINOSA PNEUMONIA.**

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Nosocomial pneumonia (NP) is one of the most common infectious diseases ac-
quired in hospital and is the leading cause of morbidity and mortality in the inten-
sive care unit (ICU), despite recent advances in antimicrobial therapy.
Pseudomonas aeruginosa (PA) is one of the most common organisms associated
with NP. ODSH, 2-o, 3-o –desulfated heparin, has been shown to exhibit anti-in-
flammatory properties and can reduce lung injury in sterile inflammation, with a
minimal antiaggregation effect. The objective of this study was to determine whether
ODSH can improve bacterial clearance. C57BL6 mice were randomized to receive subcutaneous administration of either a series of concentrations of
ODSH or saline twice at 0h and 12h after intranasal inoculation with PA. Mice
were euthanized 24 h post-infection and lungs and BALF were harvested. Bacterial
load in the lungs and airways were measured by plating serial dilutions of lung ho-
modenate and BALF respectively. Our results show that ODSH significantly im-
poved bacterial clearance in both lungs and airways. These results suggest that
ODSH can effectively enhance the host’s ability to clear bacteria and may provide
a novel therapeutic approach in the treatment of nosocomial pneumonia.

**713 ODSH IMPROVES PSEUDOMONAS AERUGINOSA-INDUCED LUNG INJURY BY DECREASING INFILTRATION OF INFLAMMATORY CELLS.**

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Nosocomial Pneumonia (NP) or Hospital Acquired Pneumonia (HAP) is associ-
ated with infections originated from the hospital borne pathogens. Resistance
against antimicrobial agents is the most common feature of these infections which
results in high mortality rates and greater therapeutic costs. Due to involvement of
multidrug resistance bacteria, alternative or supportive therapies are always re-
quired. Heparin, a well-known anticoagulant, has been found to have anti-inflam-
matory properties independent of anticoagulant property. ODSH, a desulfated he-
parin, has been proven to have anti-inflammatory properties with minimal
antiaggregation effect. ODSH has been shown to reduce lung injury in sterile in-
flammation and reducing inflammatory lung damage. Pseudomonas aeruginosa,
agr negative opportunistic pathogen, is one of the prominent pathogens associ-
ated with NP. It has been shown that PA pneumonia increases the secretion of in-
flammatory cytokines, neutrophil infiltration and subsequent lung damage. In this
study, we sought to find whether ODSH can be effective in reducing lung damage
caused by Pseudomonas aeruginosa. It was found that ODSH reduces lung injury
caused by PA, marked as reduced total protein concentration in the BAL measured
by using BCA Assay. This attenuation in lung injury was accompanied with signifi-
cantly decreased total airway cell count and neutrophil infiltration in the lungs.
These data indicate that ODSH protects against PA induced lung injury and neu-
trophil infiltration.

**714 CYANOBACTERIAL LIPOPOLYSACCHARIDES (LPS) ELICIT RELEASE OF SUPEROXIDE ANION, THROMBOXANE B₂, AND TUMOR NECROSIS-α FROM RAT BRAIN MICROGLIA.**

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We have recently reported that the cyanobacterium Microcystis aeruginosa
lipopolysaccharide (LPS) elicited classical activation of rat microglia (B MGM) in vitro
and concomitant release of superoxide anion, thromboxane B₂, cytokines,
chemokines, and matrix metalloproteinase-9 (Toxicological Sciences, 121(1): 63-

715 DOES AFGHANISTAN SAND DUST INHALATION INFLUENCE MILD BLAST-INDUCED IMMUNE AND COGNITIVE RESPONSES?

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Soldiers have significant exposure to particulate matter, including when they are subjected to a blast injury. Evidence suggests that inhalation of sand induces an inflammatory response. The aim of the current study was to investigate the effect of blast trauma and sand inhalation on the expression of pro- and anti-inflammatory mediators, as well as on the number of immune cells in rats. The results showed that sand inhalation increased the expression of pro-inflammatory cytokines and decreased the expression of anti-inflammatory cytokines. Furthermore, blast trauma and sand inhalation together had a synergistic effect on the expression of these mediators. These findings suggest that sand inhalation may exacerbate the inflammatory response to blast trauma and contribute to the development of post-traumatic syndromes in humans. Additionally, there exists a possibility that inhaled dust containing potentially harmful metals and chemical components may enter the brain and contribute to blast-trauma induced damage and to the development of neurological disorders. Identifying relevant blast injury pathogenic pathways and neurobehavioral deficits is vital for development of mild injury diagnostic biomarkers. In this study, rats were exposed to shock wave pressure to simulate mild traumatic brain injury (mTBI) with and without Afghanistan sand pre-exposure by nose inhalation. Prior to sacrifice, neurobehavioral tests were performed on 7, 14 and 28 days post-trauma animals. MWM tests revealed no significant difference among groups for all time points studied. Flow cytometric analysis of spleenocytes immunophenotyped with lymphocyte antibodies demonstrated reduction in activation of cytotoxic and helper T-cells in blast/sand exposed rats 3 days after trauma. The ratio of cytotoxic and helper T-cells decreased which indicate the vulnerability of the animals in response to blast/sand dust exposure. At 7 days post injury, significant increases in numbers of B-cell lymphocytes by sand exposure, blast or both compared to control. However, mTBI or sand alone has no significant influence on cytotoxic T-cells, helper T-cells and NK cells. Further, blast trauma rats pre-exposed to sand did not influence oncocytes B-cell activation or subpopulations of immune cells. Thus, data on mTBI model clearly demonstrates that some immune cells are potentially modulated at early time points due to neuroinflammatory insult during blast injury in conjunction with sand dust.

716 DEFICIENCY IN CD44S ENHANCES DEXTRAN SODIUM SULPHATE (DSS)-INDUCED COLITIS AND INFLAMMATORY TOXICITY IN THE COLON.

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Inflammatory bowel disease (IBD) is a chronic, relapsing and remitting inflammatory condition. CD44 is a widely expressed family of adhesion receptors. The most common form of CD44, referred to as CD44 standard form (CD44s) does not contain differentially spliced exons. The objective of this study is to find out the role of CD44s form on inflammation associated with dextran sodium sulphate (DSS)-induced colitis in mice. To this end, CD44s knockout (KO) mice and wildtype C57BL/6 mice were used to induce colitis with 3% DSS in drinking water at 2-week intervals for 5 times. Hematological parameters, body weight, and survival were assessed. In addition, serum amyloid A (SAA) and inflammatory cytokines IL-6 were quantitated. Splenic and mesenteric lymph node cells were analyzed for expression of CD3, CD4, CD8, NK1.1, CD69, and CD19. In addition, CD11b+Gr-1+ MDCs were also enumerated. Our current study shows that CD44s KO mice exhibited increased DSS-induced acute colitis as reflected by high lethality, weight loss, and histological scores compared to wild type mice. Also, SAA level in CD44s KO mice were higher. In addition, IL-6 showed high levels of expression in CD44s KO DSS-treated group, whereas undetectable levels were found in other groups. The flow cytometry results demonstrated that the proportion of SAA+ cells, CD19+ cells, activated T cells (CD3+CD69+) in mesenteric lymph node were higher in CD44sKO DSS-treated group compared to wild type DSS-treated mice, whereas NKT (CD3+NK1.1+) cells were lower in CD44sKO DSS-exposed group when compared to wild-type mice. In conclusion, CD44s deficiency enhances inflammation in the colon thereby providing therapeutic targets to treat colitis. (Supported in part by VA Merit BX001357, NIH grants R01AT006888, R01ES09908, P01AT003961, and R01ES019313; Israel Government).

717 PULMONARY PATHOPHYSIOLOGY OF AMBIDENT PARTICULATE DUST FROM IRAQI MILITARY FIELDS.

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The pathological response in relation to physical/chemical and morphological properties of ambient particulates from Iraq has become an area of great interest. This study investigated the role of metal contaminants present in ambient dust in the induction of pulmonary injury and examined the potential pulmonary risks from exposure to Iraqi particulate matter. Adult male Sprague-Dawley [Hsd:SD] CVF rats were dosed via intratracheal instillation (IT) with phosphate buffered saline (PBS) as control, ambient dust collected from Camp Victory, Iraq (CV), or NIST sand 840D. U.S. urban particulate matter in ambient dust contained in vitro toxicity compared to both CV and NIST. Differences in lung cell proliferation were examined via BrdU assay. Histopathological analysis was investigated using Mason’s Trichrome, Alcian Blue PAS, and H & E grading. The results from the LDH, albumin, and TNF-α showed no significant differences from control in all exposures. However, a significant difference was found in macrophage and neutrophil response in NIST (10mg/kg) at 150 days and CV (10 mg/kg) at all exposures compared to control. CV (5 mg/kg) dose also showed a significant differences in macrophages and neutrophil response at the 150 day time point compared to control. Our results indicate that a high dose of CV and NIST can induce pulmonary inflammation, but to a lesser degree than a highly fibrogenic particle (Min-U-Sil) at the same time point and concentration as determined by a previous study.

Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the US Army or NIOSH.

718 ASIAN SAND DUST ENHANCES MURINE LUNG INFLAMMATION CAUSED BY KLEBSIELLA PNEUMONIAE.

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Concomitant inhalation of Asian sand dust (ASD) and pathogen may result in exacerbation of pneumonia by the pathogen. The exacting effect of ASD on pneumonia induced by Klebsiella Pneumoniae (KP) was investigated in ICR mice. The organic substances adsorbed onto ASD collected from the atmosphere of Okinawa in Japan were excluded by heat treatment at 360°C for 30 min. ICR mice were instilled intratracheally with ASD at doses of 0.05 mg or 0.2 mg/mouse four times at 2-week intervals and were administered with ASD in presence or absence of KP at the last instillation intratracheal. Pathologically, ASD caused exacerbation of pneumonia by KP as shown by increased inflammatory cells within the broncholar and the alveolar compartments. ASD enhanced dose dependently neutrophil number and expression of cytokines (IL-1β, IL-6, IL-12, IFN-γ, TNF-α), and chemokines (KC, MCP-1, MIP-1α) in BALF related to KP. In an in vitro study using RAW264.7 cells, the treatment of ASD and KP increased gene expression of IL-6, IFN-β, TNF-α, KC, MCP-1 and MIP-1α and increased the expression of IL-1β, TNF-α, MCP-1 and MIP-1α in culture medium compared with each alone treatment. The combined treatment tends to induce the gene expression of Toll-like receptor 2 (TLR2), and NALP3, ASC and caspase-1 compared with KP alone. These results suggest that the exacerbation of pneumonia by ASD + KP could be due to the enhanced production of pro-inflammatory mediators via activation of TLR2 and NALP3 in ammossome pathway in alveolar macrophages.
Acid sphingomyelinase (A-SMase) level in AM was determined because it has been
Inflammasome's role in IL-1β
A significant reduction in IL-1β
and MARCO-/- mice with LPS and cathepsin B inhibitor prior to silica exposure.

IL-1β Inflammasome activation was quantitated by the release of IL-1β (MARCO) as an important receptor for binding, uptake and clearance of crys-

zyme of silicosis, an irreversible, inflammatory and fibrotic pulmonary disease.

Confirm that the increase in IL-1β in MARCO-/- AM was due to the absence of MARCO receptor; MARCOAb was used to block MARCO in WT. Release of IL-1β following MARCOAb was similar to IL-1β release from MARCO-/- AM. Inflammasome's role in IL-1β release was confirmed by pretreating AM from WT and MARCO-/- mice with LPS and cathepsin B inhibitor prior to silica exposure. A significant reduction in IL-1β release was observed with cathepsin B inhibition. Acid sphingomyelinase (A-SMase) level in AM was determined because it has been indicated in acute lung injury. A-SMase level was higher in MARCO-/- AM. The results demonstrate inflammasome activation is exacerbated in absence of MARCO and may be linked with increased A-SMase and the variance in MACRO expression may explain variance in human susceptibility to silicosis. This work was supported by NIH grants P20 RR017670 and R01 ES15294.

Inflammasome is an enzyme that controls the release of pro-inflammatory cytokines from immune cells. Inflammasome activation is a critical step in the inflammatory response and plays a role in the development of certain diseases, including autoimmune disorders and inflammatory bowel disease.

Inflammasome activity is assessed by measuring the release of pro-inflammatory cytokines, such as IL-1β, from immune cells. One common method to study inflammasome activity is to use inhibitors to block the enzyme responsible for cleaving pro-IL-1β into its active form. In this study, the researchers used LPS and cathepsin B inhibitor to block inflammasome activation, and they observed a significant reduction in IL-1β release from MARCO-/- AM compared to WT AM. This finding suggests that inflammasome activation is exacerbated in the absence of MARCO, a receptor that binds and internalizes inflammasome components.

Inflammasome activation is also regulated by cell-specific factors. For example, the researchers used MARCOAb to block MARCO in WT AM and observed a similar increase in IL-1β release as seen in MARCO-/- AM. This suggests that the absence of MARCO receptor is responsible for the increased inflammasome activity.

The study also indicates that inflammasome activation is not only due to the lack of MARCO but also due to the increased level of A-SMase. This is supported by the observation of a significant increase in AEA levels, when compared to controls. These data suggest that inflammasome activation leads to increased secretion of ECs and that ECs may play a critical role during inflammation in specific organs such as the liver.

Inflammatoxylin (IBD), including Crohn's disease and ulcerative colitis, are chronic inflammatory diseases of the gastrointestinal tract. Recently we have demonstrated the anti-inflammatory effect of a novel nonapeptide, fragment of histone H2A, termed IIIM1 in a variety of animal models of autoimmune and inflamma-
tyre diseases. Transgenic mice constitutively expressing IIIM1 were used in this study. The objective of the present research was to test the vulnerability of these animals to chemical-induced colitis in comparison to control mice. Bacterial inflamma-

tion was induced by rectal administration of trinitrobenzenesulfonic acid and 7 days later the animals were sacrificed. The control mice developed severe inflamma-
tory response expressed by increased colon weight and extensive proinflammatory nerosis and associated transmural infiltration of numerous neutrophils. In contrast, colon of transgenic mice was normal, devoid of pathological changes with marked (68%) weight reduction. Suppressed inflammatory response in the transgenic mice was also expressed by reduction in serum IL17 (49%) and IFNy (42%). This notion was corroborated by the significant increase in FoxP3- and TGFβ-producing cells in spleen of transgenic mice. In conclusion, animals constitutively expressing IIIM1 peptide are more resistant to chemical-induced bowel irritation via reduction in inflamma-
tory responses. These findings indicate for the potential of IIIM1 peptide to serve as a drug for IBD, chronic inflammatory diseases and chemical-induced ir-

Lung inflammation plays a key role in CARBON NANOPARTICLE-INDUCED ADJUVANT ACTIVITY.

Chronic exposure to combustion derived particles has been associated with an increase in the incidence of asthma. As a possible mechanism for this pathological outcome, the adjuvant effect of lung inflammation induced by these particles has been hypothesized. The aim of our study was to investigate the causal link between nanoparticle-induced lung inflammation and modulations of immune cell populations during processes leading to sensitization, and allergic immune responses of the airways.

Mice were treated with ovalbumin (OVA) alone or in combination with carbon nanoparticles (CNP). The induction of inflammation and the immune adjuvant activity were studied in the lungs and lung draining peribronchial lymph nodes (PBLN); (i) at the level of sensitization, and (ii) at the level of the immune re-

Our data show a link between neutrophilic lung inflammation and adjuvant effects of CNP. A specific reduction of neutrophils by the application of ectoine attenuated this NP induced adjuvant effect, indicating that particle induced inflammation rather than the direct interaction of nanoparticles with immune cells is the critical step in environmentally modulated immune diseases.
twelve week old C3H/HeN male mice were intratracheally treated with vehicle (PBS) or 375 μg/kg E. coli LPS. After 12 hours, mice were exposed to room air (RA) or 60% O2 for 1 or 3 days. Cytokine and chemokine expression was measured by ELISA in bronchoalveolar lavage fluid. Pulmonary function tests were performed on anesthetized mice using the Flexivent. On day 1, TNF-α, IL-6, RAGE, and MCP-1 were significantly increased in LPS/RA and LPS/O2 compared to PBS/RA and PBS/O2 treated mice. Neutrophil chemokine receptors, KC and MIP-2, were significantly increased in LPS/O2 mice compared to other treatment groups. Central airway resistance was significantly increased on day 3 in LPS/O2 mice compared to PBS/RA mice. Our data show that the combination of LPS and hypoxia uniquely increases expression of chemokines that mediate of neutrophil infiltration and increases airway resistance. We speculate that LPS and hypoxia interact to activate unique mechanisms that may contribute to the development of LPS-induced ALI in adult mice.

724  BETA2-ADRENERGIC RECEPTOR ENGAGEMENT ON A B CELL REGULATES THE LEVEL OF IGE VIA CD23 CLEAVAGE BY ADAM10 ON B CELL-DERIVED EXOSOMES.

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Allergic asthma is a major problem in toxicology and relief of bronchoconstriction by beta2-adrenergic receptor (β2AR)- agonist administration is the most effective treatment. Severity of allergic asthma is associated with high serum Immunoglobulin E (IgE). We previously showed that β2AR engagement on a primed murine B cell increases the level of IgE produced per cell, without affecting isotype switching. β2AR agonists alleviate bronchoconstriction by targeting the β2AR expressed on bronchiolar smooth muscle cells, triggering bronchodilation. These drugs also target the β2AR on primed B cells to increase IgE and soluble CD23 (sCD23), a positive regulator of IgE. Primed B cells exposed to a β2AR agonist show CD23 and sCD23 increases, while surface CD23 remains constant. Thus, β2AR engagement may worsen bronchoconstriction over time by increasing sCD23 and subsequently IgE, albeit relieving bronchoconstriction in the short term. CD23 cleavage by ADAM10 was recently found to occur primarily on exosomes as opposed to the cell surface. We found that β2AR engagement on a primed B cell did not alter ADAM10 production or surface expression compared to primed B cells alone, but increased the amount of exosome-localized ADAM10 with CD23 as determined by Western blot and FACs. In addition, only the exosomes expressing high ADAM10 and CD23 were decreased upon β2AR stimulation, indicating that CD23 was being cleaved and converted to exosomes expressing low CD23. Additionally, β2AR stimulation on a β2AR-deficient primed B cell failed to upregulate ADAM10 and CD23 expression on B cell-derived exosomes, indicating specificity of the response. Thus, ADAM10 expression on B cell-derived exosomes may serve as a molecular target to therapeutically prevent the β2AR-associated increase in sCD23 and IgE that worsens asthma, while still allowing for induction of the β2AR agonist-induced bronchodilation.

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725  INCREASED PHOSPHODIESTERASE 4B (PDE4B) AND DECREASED CELLULAR CAMP REGULATE LPS-INDUCIBLE TNF-α IN GLUCOSE-PRIMED MONOCYTES.

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Diabetes experience increased gut permeability leading to increased levels of serum endotoxin derived from intestinal Gram-negative bacteria. Consequently, diabetes suffers from chronic low-grade inflammation. Therefore, lipopolysaccharide (LPS)-inducible cytokines are highly relevant in this disease contributing to the development of diabetic complications. Specifically, Tumor Necrosis Factor-α (TNF-α), plays a major role in the evolution of multi-organ dysfunction and complications associated with diabetes. Our earlier work demonstrated that in monocyte/macrophage, PDE4B regulated cAMP plays a key role in regulating LPS-inducible TNF-α expression. Accordingly, the potential role of PDE4 in the regulation of high glucose exposure and LPS-mediated TNF-α expression was investigated in human monocytes. The data obtained demonstrates that human monocytes exposed to high glucose (HG;25mM) exhibit a “primed phenotype”, wherein in comparison to normal glucose exposure, they express elevated levels of TNF-α following LPS stimulation. The data obtained shows for the first time that

726  FOUR CASES OF NEUROPSYCHOLOGICAL ABNORMAL FINDING IN CHILDREN WITH HIGH BLOOD METHYL-MERCURY CONCENTRATION.

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Methyl-mercury easily passes the blood-brain barrier and accumulates in central nervous system, causing neurological symptoms. We report four cases of neuropsychological abnormal finding in children with high blood methyl-mercury concentration.

Cases Presentation: Four children admitted because of having a high blood mercury concentration through the national survey of mercury. Case 1 was a 9-year-old female with 16.6 μg/l blood mercury concentration on survey and on admission, the blood, hair mercury and blood methyl-mercury concentration (mercury indices) were 21.4 μg/l, 7.2 μg/g and 20.1 μg/l. In neuropsychological examination, cognitive impairment and attention deficit were observed. She had diet habit with 2-3 time fish intake per week and had been diagnosed with epilepsy at 3 year-old. Case 2 was a 12-year-old male with 15.4 μg/l on survey and the mercury indices were 12.7 μg/l, 5.7 μg/g and 11.8 μg/l. He was observed the finding of attention deficit/hyperactivity disorder. Case 3 was a 10-year-old male child with 17.4 μg/l on survey and the mercury indices were 21.6 μg/l, 7.5 μg/g and 21.5 μg/l. The finding of mild attention deficit was observed. Case 4 was a 9-year-old male with 20.6 μg/l on survey and the mercury indices were 18.9 μg/l, 8.3 μg/g and 14.4 μg/l. Mild attention difficulty was observed.

Discussion: We considered that fish consumption may be the main source of methyl-mercury exposure, and it could be influence neuropsychological finding in these cases.

727  A FEASIBILITY ASSESSMENT OF CONTINUOUS BLOOD PRESSURE AND HEART RATE ASSESSMENTS IN JUVENILE RATS.


It is reported that 90% of medicines used in pediatric care have only undergone preclinical safety evaluation in adult animals. However there are known differences in organ function between juvenile and adult animals which could potentially lead to both pharmacological and toxicological adverse findings not identified in traditional adult animal toxicity studies. The EMEA and the FDA have issued guidance requiring standalone pediatric studies when human safety and adult animal preclinical data are considered insufficient to support pediatric use.

In light of these requirements, the purpose of this study was to evaluate the feasibility of assessing heart rate and blood pressure in juvenile rats commencing 22 days post partum (pp). Rat pups were implanted at 21 days pp with a PA C10 transmitter traditionally used in mouse telemetry studies. The surgical procedure involved a femoral artery cannulation and placement of the transmitter on the flank. Intermittent assessments of heart rate and blood pressure were conducted on Days 22, 28, 40 and 70 pp, to verify transmitter patency. On Day 28 and Day 70 animals were treated with MK 801, a NMDA receptor antagonist, which was expected to induce hypertension and tachycardia.

Assessment of blood pressure and heart rates on Day 22 pp, confirmed accurate placement of the PA C10 implant with stable heart rate ranges of approximately 469 to 486 beats per minute and systolic and diastolic blood pressure ranges of 92 to 146 mmHg and 58 to 98 mmHg, respectively. Over the duration of the assessments baseline blood pressure levels remained stable with average heart rates decreasing in the older animals to approximately 305 to 362 bpm. Administration of MK-801 resulted in a profound hypertension and tachycardia.
The preliminary data demonstrated confirm that surgical placement of telemetry transmitters in juvenile rats for the measurement of continuous blood pressure and heart rate is feasible and therefore cardiovascular assessments can be incorporated into juvenile toxicity studies.

**728 PRECLINICAL DEVELOPMENT OF ALOPPREGANONOLE (ALLO) FOR TREATMENT OF NIEMANN-PICK TYPE-C, A RARE NEONATAL AND PEDIATRIC DISEASE.**

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Niemann-Pick Type-C (NP-C) is a rare autosomal recessive neurodegenerative disease caused by genetic mutations in NPC1 (95%) or NPC2 (5%), resulting in lysosomal accumulation of unesterified cholesterol and glycolipids. NP-C: clinical manifestations include severe infant and pediatric neurodegenerative disorders including early death. Treatment of neonatal NIH NPC1 mice with a single subcutaneous dose of ALLO resulted in a doubling of life span, substantial delay in onset of neurological symptoms, survival of cerebellar Purkinje and granule cell neurons, and reduction in cholesterol and ganglioside accumulation. Range-finding in neonatal 2-week old Beagle dogs with a slow bolus (2.5 min) intravenous infusion of 3.5 mg/kg ALLO in 20% 2-hydroxypropyl-beta-cyclodextrin in water induced sleep followed by death. Twice weekly treatment in the GLP 4-week definitive toxicity study with 0.25, 0.5, or 1 mg/kg ALLO transiently induced sleep indicating neurological activity. There were no dose-related changes in clinical signs, body weight, food consumption, hematology, clinical chemistry, urinalysis, ophthalmology, gross or microscopic pathology. On Days 1 and 14, plasma and brain ALLO levels (<300 and <340 ng/ml, respectively) declined quickly after the first time point and were not detectable beyond 1 hr post dose (lower limit of quantitation was 2.5 ng/ml). In the brain, total ALLO exposure was similar to plasma, but brain-to-plasma concentration ratios (0.5-2.5) showed variability with time. Rapid ALLO elimination and lack of accumulation were reflected in the short half-life (<0.45 hr), ample volume of distribution (1500-5000 ml/kg), and high rate of clearance (≤8700 ml/hr/kg). In conclusion, intravenously administered ALLO to 2-week old Beagle dogs resulted in rapid penetration to the brain. ALLO was well tolerated in juvenile dogs after 4 weeks twice weekly dose administration. This work was supported by NIH Grant U01-NS049462.

**729 MITOCHONDRIAL BASIS OF ADVERSE PERINATAL OUTCOME IN HIV-TREATED PREGNANT WOMEN.**

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Background: HIV and antiretrovirals cause mitochondrial DNA (mtDNA) depletion which can lead to mitochondrial dysfunction and oxidative stress (OxS). Secondary effects of HIV infection have been associated to mitochondrial injury. HIV pregnant women present increased rates of adverse obstetric and perinatal events of unknown aetiology. We wonder if mitochondrial abnormalities may underlie adverse perinatal outcome in HIV-pregnancies. Methods: We studied obstetric results and mitochondrial status in placenta in 48 pregnant women: 21 non-smokers and 27 smokers (19 moderate and 7 heavy smokers). Tobacco consumption was measured by the number of smoked cigarettes/day and plasmatic cotinine (ng/ml). Oxidative stress lipid peroxidation was measured by spectrophotometry (µM MDA+HAE/mg prot), mitochondrial DNA (mtDNA) content by nPCR (mtND2/nRNApolII) and mitochondrial number and tissular hypoxia by Western-Blot (V-DAC or HIF1a/B-actine). Results, expressed as mean±SEM, were analyzed by non-parametrical statistics. Results: Smoke-exposed newborn showed fetal growth restriction (OR=4.13 [0.44-38.49]), reduced weight (3136.15±31.34 vs. 3452.75±98.66; p<0.05) and decreased growth percentile (35.08±26.21 vs. 54.20±5.87; p<0.05). Fetal cotinine positively correlated the number of cigarettes consumed (p=0.05) and negatively correlated newborn weight (<0.001). Placenta of smoking mothers showed increased oxidative stress, especially heavy smokers (19.55±17.6 vs. 14.9±11.11; p<0.05), which indeed presented higher mitochondrial number manifested as increased mtDNA (1.37±0.14 vs. 1.26±0.06; p<NS) and V-DAC levels (0.78±0.09 vs. 0.44±0.08; p<0.05). Placenta of moderate smokers showed higher response to hypoxia (HIF1a expression: 2.76±0.47 vs. 2.38±0.45; p<NS). Conclusions: Tobacco increases placental oxidative stress, mtDNA levels, mitochondrial amount and hypoxia-response; CO-mediated mechanisms which may underlie intrauterine growth restriction. Funding: FIS 0229/08 and CIBERER (ISCIII).

**730 TOBACCADO-INDUCED MITOCHONDRIAL TOXICITY IN REDUCED INTRAUTERINE GROWTH.**

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Background: Maternal smoking is associated with reduced birth weight. Tobacco contains carbon monoxide (CO), considered particularly harmful due to its capacity to bind hemoglobin and mitochondrial complex IV leading, respectively, to tissular and cell hypoxia. We wonder if mitochondrial dysfunction or hypoxic response may underlie obstetric and perinatal adverse outcome of smoking pregnant women. Methods: We studied perinatal results and mitochondrial status in placentas of 48 pregnant women: 21 non-smokers and 27 smokers (19 moderate and 7 heavy smokers). Tobacco consumption was measured by the number of smoked cigarettes/day and plasmatic cotinine (ng/ml). Oxidative stress lipid peroxidation was measured by spectrophotometry (µM MDA+HAE/mg prot), mitochondrial DNA (mtDNA) content by nPCR (mtND2/nRNApolII) and mitochondrial number and tissular hypoxia by Western-Blot (V-DAC or HIF1a/B-actine). Results, expressed as mean±SEM, were analyzed by non-parametrical statistics. Results: Smoke-exposed newborn showed fetal growth restriction (OR=4.13 [0.44-38.49]), reduced weight (3136.15±31.34 vs. 3452.75±98.66; p<0.05) and decreased growth percentile (35.08±26.21 vs. 54.20±5.87; p<0.05). Fetal cotinine positively correlated the number of cigarettes consumed (p<0.05) and negatively correlated newborn weight (<0.001). Placenta of smoking mothers showed increased oxidative stress, especially heavy smokers (19.55±17.6 vs. 14.9±11.11; p<0.05), which indeed presented higher mitochondrial number manifested as increased mtDNA (1.37±0.14 vs. 1.26±0.06; p<NS) and V-DAC levels (0.78±0.09 vs. 0.44±0.08; p<0.05). Placenta of moderate smokers showed higher response to hypoxia (HIF1a expression: 2.76±0.47 vs. 2.38±0.45; p<NS). Conclusions: Tobacco increases placental oxidative stress, mtDNA levels, mitochondrial amount and hypoxia-response; CO-mediated mechanisms which may underlie intrauterine growth restriction. Funding: FIS 0229/08 and CIBERER (ISCIII).

**731 LONGITUDINAL STUDY OF MITOCHONDRIAL TOXICITY IN HIV-INFECTED CHILDREN RECEIVING MILD OR STRONG NUCLEOSIDE ANALOGUES.**

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Background: Adverse effects of Highly-Active-Antiretroviral Therapy (HAART) and HIV, similar to those described in primary mitochondrial genetic diseases, have become a major issue, especially in children. Currently, the main goal is to reduce the risk of virologic and mitochondrial failure while maximizing cost-effectiveness. Methods: We followed, within 2 years, the clinical and mitochondrial status of 38 HIV-infected children on HAART that either included strong nucleoside-reverse-transcriptase-inhibitors (NRTIs; didanosine, zidovudine or stavudine; n=15), or mild NRTIs (the rest of drugs; n=13); and compared them to an HIV-infected untreated group (n=10) and to uninfected control reference values. We measured the viral load (rt-PCR), lactate levels (spectrophotometry) and T-lymphocyte CD4+ content (flow-cytometry); we assessed mtDNA content (rt-PCR), mitochondrial protein synthesis (western blot); mitochondrial number, electron transport chain function and oxidative stress (spectrophotometry) in blood mononuclear cells. Results: Lactate levels became significantly higher in children on strong regimens compared with patients on mild NRTIs after 2 years (1.0±0.071 vs. 1.28±0.083; p<0.02). We found a significant decrease of cytochrome-c-oxidase activity in untreated patients and in those on strong NRTIs groups along time. Mitochondrial DNA content was similar among all HIV-infected groups and significantly lower compared to controls at baseline. Oxidative stress tended to increase along time in all groups. Conclusions: HIV itself and the use of strong NRTIs cause higher mito-
Anidulafungin and voriconazole are potent antifungal agents that may provide a powerful therapeutic option over current therapies when co-administered. A non-clinical combination toxicity study was completed as part of the voriconazole Paediatric Investigation Plan to enable clinical development of the combination in children. Juvenile Fisher rats received anidulafungin or voriconazole alone or in combination once daily from postnatal day (PND) 21-56 with a recovery period to PND 84. Doses were chosen to target exposures at or above the adult rat no observed adverse effect levels (NOAELs). Toxicokinetics were determined using a validated dual-analyte bioanalytical method. Systemic exposure at juvenile rat NOAELs was comparable to those found with adult rats in previous studies as well as anidulafungin exposure alone in a previous juvenile study. Transient and reversible reductions in body weight, hematology, serum chemistry, liver weight and minimal liver changes were associated with anidulafungin. Voriconazole caused an increase in gamma-glutamyltransferase in female rats only. Juvenile rats were not more sensitive to each drug dosed alone compared with adult rat data on the single drugs. No novel, additive or synergistic toxicities were noted when dosed in combination. This study supported pediatric clinical trials co-administering voriconazole and anidulafungin.

In the Northern Rocky Mountains, wood stoves are a popular heat source in homes during the cold winter months. These wood stoves contribute considerable levels of fine particulate matter (PM2.5) to the indoor environment. Residential exposure to biomass PM2.5 has the potential to exacerbate respiratory diseases, such as asthma. The primary aim of the University of Montana’s ARTIS study (A Randomized Trial of Indoor Air) is to assess the quality of life among asthmatic children following interventions that reduce in-home wood smoke PM exposures. Our recent studies focus on evaluating multiple inflammatory cytokines in the exhaled breath condensate (EBC) of asthmatic children enrolled in the ARTIS study. Rubes (Respiratory Research Inc., Charlottesville, VA) were used to collect EBC samples from 42 subjects during their first (baseline) winter. Sample collection was performed on two consecutive mornings during two wintertime sampling visits, with 41 subjects successfully completing sample collection during the first visit and 40 during the second visit. Data presented here is from the first visit only. Ten cytokines (GM-CSF, IL-1β, -2, -4, -5, -6, -8, -10, IFN-γ and TNF-α) were simultaneously quantitated in EBC samples using an ultrasensitive multiplex bead immunoassay analyzed with a Luminex 100 and StarStation software v. 2.0 (Applied Cytometry, Sacramento, CA). Briefly, TNF-α was detected in 88% of the samples with quantitated samples ranging from 0.12 to 3.44 pg/mL, with an average (± SD) concentration of 0.88 (± 0.57) pg/mL. The IL-4/IFN-γ ratio was on average 1.27 (± 1.33) with one sample below the LOD for IL-4 and no samples below the LOD for IFN-γ. Future analyses include evaluating these inflammatory biomarkers following home interventions designed to reduce children’s exposure to indoor PM2.5. This data will ultimately allow us to gain a better understanding of the health effects of reducing residential PM2.5 in wood stoves homes of asthmatic children.

The obesity epidemic affects at least 14.6% of children in the US with an even higher proportion among the underprivileged. This latter population is also exposed to higher levels of environmental lead (Pb) due to its persistence in old homes and inner-city residences. Previous findings have independently identified both Pb and obesity as etiological factors in the development of osteoporosis.
Interestingly, Pb-treated mice and those fed a high-fat diet share a number of other characteristics, including depression of bone formation and bone marrow stenosis. Increased adiposity of the bone marrow is itself associated with bone loss, and this is evident in cases of osteoarthritis, immobilization, and glucocorticoid treatment, as well as in osteoporotic postmenopausal women. Additionally, we have found that Pb treatment up-regulates the expression of sclerostin, a potent inhibitor of Wnt/beta-catenin signaling, which is an important molecular switch governing bone homeostasis. From these findings we hypothesize that Pb exposure and obesity both act through depression of Wnt signaling in mesenchymal cells to promote their differentiation into adipocytes, subsequently decreasing number of osteoblasts, resulting in bone loss. Using concomitant exposure to Pb (50 ppm in the drinking water) and high-fat diet (60% kcal from fat) in mice we found that they additively reduce bone mass, increase bone marrow adiposity, and reduce beta-catenin levels in stromal cells. This correlated with suppression of the osteogenic transcription factor Runx2 and induced expression of the adipogenic transcription factor Pparg. Pb and high-fat diet also act in vitro to promote adipogenic potential of mesenchymal cells by increasing lipid droplet formation, increasing activity of Pparg, and decreasing beta-catenin activity. These results provide mechanistic insight for targeted therapeutic intervention in bone diseases resulting from exposure to Pb, obesity, and a combination thereof.

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**PS 737 POLYMORPHISMS OF PHASE I AND II XENOBIOTIC ENZYMES INVOLVED IN ASTHMA CONDITION IN CHILDREN EXPOSED TO TOBACCO SMOKE.**

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Bronchial asthma is a common disorder which affects 12% of Mexican population and tends to increase in the next ten years. Although several environmental factors influence on asthma development such as air pollution or common allergens; one of the most important is exposure to environment tobacco smoke (ETS). The metabolism of some toxic compounds of ETS is through a complex network of enzymes and reactions of hydrolysis, reduction or oxidation (phase I) and conjugation (phase II). The role of polymorphisms of enzymes involved in xenobiotic metabolism may explain why some children are more susceptible to develop asthma. In the present study, we analyzed the association of several polymorphic markers including CYP1A1 (rs1048943), CYP2E1 (rs2070676), CYP2A6 (rs1801272), CYP1B1 (rs1056836), and GSTT1 or GSTM1 null variants with risk of asthma together with ETS exposure in a Mexican children population. The cytochrome SNPs were in linkage disequilibrium when they were associated with asthma. However, when population was divided according sex, male children with AA variant of CYP2A6 presented a significant risk to develop asthma (p=0.034). In addition, this association was also detected when exposure to ETS was included (p=0.021) According to the obtained results, CYP2A6 may be an indicative of susceptibility to develop asthma in male children exposed to ETS. Support Conacyt 104316 and 106463.

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**PS 738 LUNG FUNCTION IN CHILDREN CHRONICALLY EXPOSED TO ARSENIC IN DRINKING WATER.**


Introduction: Intense over-exploitation of the aquifers in the region known as the Comarca Lagunera has caused a gradual descent of water levels which in turn has given rise to a serious health issue, hydroarsenicism. Arsenic concentrations in drinking water have surpassed the permitted levels by the WHO (10μg/l) and the Mexican Official Norms (25μg/l). Arsenic exposure through drinking water has been associated with different types of neoplasia. Although, some of the most important negative effects of this metalloid over human health are lung function disturbances. Recent experimental animal studies animal have reported pathological changes in pulmonary development as well as bronchial hyperresponsiveness. Disturbances have also been reported in pulmonary repairing tissue proteins. Also clinical human studies have shown as well, diminishment in spirometric values and higher bronchialasthmas and chronic obstructive pulmonary disease frequencies. These findings have been reported in adult populations but not in children. Objective: To evaluate the relationship between lung function and inflammatory biomarkers with arsenic levels and with the polymorphisms of GSTO, AS3MT, GSTM1 and GSTT1 in children chronically exposed to arsenic in drinking water. Preliminary Results: At present a total of 58 children have been included in the study from two rural communities [Sofía de Arriba (58.3ppb) and El Porvenir (112 ppb)]. The mean children age is 9.6 years and most of them have living in the community all life (mean 9.3 years) and 62.1 % of them were conceived at those communities. 13.8% of the studied population reported chronic cough for more than 2 years and 8.6% for 7-12 years. In addition, 6.9% have been treated for repeated bronchiolitis. In all subjects the spirometric values such as FVC,FEV1 and FEV1/FVC ratio were lower with respect to the reference values for spirometry volumes. Conclusion: A high incidence of lung diseases and a reduction in the lung function was recorded in children chronically exposed to high arsenic levels in drinking water.

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**PS 739 GENETIC AND EPIGENETIC BIOMARKERS OF SUSCEPTIBILITY TO OP PESTICIDES AND OBESITY IN CHILDREN.**


Children’s susceptibility to environmental toxicants may depend on their genotype, levels of exposure and their interactions. In the longitudinal birth cohort study of low-income Mexican-American farmworker families in California (CHAMACOS) we found that in utero exposures to pesticides were associated with abnormal birth and neurodevelopmental outcomes at different ages (Eskanazi et al, 2004, 2007, 2010; Young et al, 2005; Bouchard et al, 2011). We also demonstrated that these health effects depend on phenotype and genotype of paraoxonase (PON1), a multifunctional enzyme involved in detoxification of organophosphate (OP) pesticides also oxidative stress (Hollander et al, 2006; Huen et al, 2009;2010 Eskanazi et al, 2010; Harley et al, 2011). CHAMACOS population has a high prevalence of obesity in mothers and children that exceeds national averages for Mexican-Americans. In this study of 383 CHAMACOS children, we observed significant associations of child PON1 genotypes and PON1 status (PON1192 genotype and PON1 enzyme levels) with both body mass index (BMI) Z-score and obesity status at age 2. Compared to children with the PON1192RR genotype, the odds of obesity were 5.2 and 9.6 fold higher in PON1192QR and PON1192QQ children, respectively. Additionally, we genotyped ancestry informative markers (AIMs) to estimate proportional ancestry for each individual and found little evidence of genetic confounding among two year old CHAMACOS children. In addition to genetic polymorphisms, epigenetic mechanisms such as DNA methylation in PON promoter region (assessed by Illumina 450K Methylation BeadChip), were also associated with PON1 expression in an allele-specific manner. Epigenetic and genetic influences on PON1 may affect susceptibility to OP pesticides and conditions related to oxidative stress including obesity. Possible contribution of environmental exposures such as prenatal pesticide exposures, to the genetic, epigenetic and behavioral mechanisms of obesity warrants additional attention. Supported by NIEHS and EPA grants.

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**PS 740 FUNCTIONAL DEVELOPMENT IN YOUNG RATS: BASIC DATA FOR THE PERFORMANCE OF JUVENILE ANIMAL STUDIES.**


Juvenile animal studies play an important role in the registration process of pharmaceuticals and chemicals, and functional parameters form an important part of such studies. As these parameters are evolving during the first days to weeks of life, laboratory data must be interpreted in relation to developmental changes. Therefore, generation of data sets for juvenile animal studies is a fundamental need. The normal development of some haematological, immunological and lung function characteristics, as well as of the auditory stearle reflex (ASR) was assessed in Wistar (WU) rats. For the hematopoietic system it was shown that blood counts of newborn rats revealed high numbers of immature progenitor cells and the red blood count showed frequent abnormalities that typically point to anaemia or RBC defects. Assessment of immune system development proved feasible starting at day 2 pp. Major developmental effects were seen in immunoglobulin secretion in peripheral blood and in lymphocyte surface marker expression in peripheral blood, spleen and bone marow. These methods have been developed to noninvasively assess juvenile rat lung function starting at a neonatal stage. A plethysmographic method has been modified and longitudinal data are presented which describe the functional development of
the lungs in male and female rats at an age of 2 days. Furthermore, the possibility of nose-only inhalation treatment in juvenile rats as young as day 4 pp has been proven. ASR data show the practicability of this test as early as day 10 pp with a coordinated animal response showing a significant pre-pulse inhibition around day 15 pp. The development of a gender-specific response starts around day 40 pp in coincidence with sexual maturation. Adult-like patterns of habitation can be assessed by day 60 pp.

The described techniques permit new insights into human neonatal risk assessment and therefore these animal models are suitable for regulatory studies. The presented data are important for correct planning and interpretation of juvenile animal studies.

741 SKIN-SENSITIZING POTENCY OF HALOGENATED PLATINUM SALTS.

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The relationship between occupational exposure to halogenated platinum (Pt) salts and Pt-specific allergic sensitization is well-established. Although human case reports and clinical studies demonstrate that Pt salts are potent skin sensitizers, no studies have been published that investigate whether there are differences in potencies of halogenated Pt salts. In this study, we evaluated ammonium hexachloroplatinate (AHCP), ammonium tetrachloroplatinate (ATCP) and cis-dichlorodiammine (CDDP) using the local lymph node assay. For 3 consecutive days, BALB/c mice were dosed topically on the dorsum of both ears with vehicle, 25% trimellitic anhydride (TMA; positive control) or one of 3 concentrations of Pt salt. On day 5, lymph nodes were harvested and single-cell suspensions were labeled ex vivo with 51Methyl thymidine. Lymphocyte proliferation was determined by scintillation counting. Concentration-dependent increases in ear thickness and lymphocyte proliferation were observed for all 3 Pt salts. None of the doses tested resulted in a skin irritant response since erythema was minimal and the maximum increase in ear thickness was less than 25% (n = 12). The EC3 values for AHCP, ATCP and CDDP were determined to be 0.58, 0.23 and 0.33%, respectively (n = 12). In complementary studies, lymph node cells were labeled ex vivo with bromodeoxyuridine (BrdU) to investigate an alternative to radioisotopic labeling. Incorporation of BrdU was determined by ELISA. In this case, the EC2 values for AHCP, ATCP and CDDP were determined to be 0.12, 0.95 and 0.17%, respectively (n = 6). The stimulation index of TMA was 4.6% (+/-1.2) and 2.9% (+/-0.3) in the radioisotope- and BrdU-labeling procedures, respectively. We conclude that AHCP, ATCP and CDDP are categorized as strong sensitizers according to the Globally Harmonized System for Classification and Labeling of Chemicals. Furthermore, these data suggest that BrdU labeling may not be as sensitive a procedure as labeling with radioisotope. This abstract does not reflect EPA policy.

743 REACTIVITY PROFILE OF CONTACT AND RESPIRATORY LOW MOLECULAR WEIGHT ALLERGENS IN A COMPETITIVE PEPTIDE REACTIVITY ASSAY.

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It is well established that certain low molecular weight chemicals cause allergic diseases of the skin and respiratory tract. Individual chemicals are typically associated primarily with one or other form of disease, generating selective Th1 or Th2 type responses. The reasons for this divergence are unclear; however selective modification of specific amino acids may play a role. The reactivity of chemical allergens to single nucleophile peptides is increasingly well-described with standardized assays for use in hazard assessment; adapting these methodologies to evaluate competitive binding of multiple nucleophiles may assist in defining preferential modifications. Using a peptide reactivity model, the reactivity of reference respiratory allergens (phthalic anhydride [PA], maleic anhydride [MA]) and skin allergens (dinitrochlorobenzene [DNCB], dinitrolu啰benzene [DNFB]) were investigated. One set of assays was conducted by reacting peptides containing either cysteine (Cys) or lysine (Lys) alone with an excess concentration of test chemical. To evaluate the effect of competition, assays were conducted by preparing reaction mixtures of these same peptides at various concentrations relative to the other. The ratios utilized were 1:1, 3:1, 6.1:1 and 9:1; in each case the total peptide constant was constant. When incubated with single peptides PA and MA (respiratory allergens) were observed to have increased reactivity to Lys, while DNCB and DNFB (contact allergens) preferentially reacted to Cys. These preferences were conserved or enhanced under competitive conditions. Under such conditions PA and DNCB were observed to deplete exclusively Lys and Cys, respectively. MA and DNFB both maintained the preferences observed with individual peptides. The selective reactivity demonstrated here may have important mechanistic relevance for the ability of these chemicals to induce divergent immune responses.

744 CHANGES IN THE FREQUENCY OF B220+ LYMPHOCYTES FOLLOWING TOPICAL EXPOSURE TO LINALOOL.

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Linalool is a terpene alcohol that is known to undergo autodissociation forming a variety of hydroperoxides and other degradation products. Linalool is not considered to be a skin sensitizer; however, the products formed during autodissociation are known to be contact allergens. In the murine local lymph node assay (LLNA), high purity samples of linalool have been reported to have a weak sensitization potential. As with other predictive test methods, false-positives are known to occur in the LLNA, particularly to subsets of certain classes of skin irritants. While these have not typically presented interpretive difficulties, strategies have been developed to assist in eliminating false positives in the LLNA. One approach is to align the LLNA with monitoring the frequency of B220+ lymphocytes in skin draining lymph nodes (LN). In the present study, assays were conducted by topically treating CBA/Ca mice with high purity linalool (50% or 100%; both with and without an antioxidant), dinitrochlorobenzene (DNBC; 0.25%; contact allergen control), benzalkonium chloride (BZC; 2%; irritant control) or with vehicle (acetone) alone using the standard LLNA dosing regimen. Draining LN were isolated and the frequency of B220+ lymphocytes was determined at various concentrations relative to the other. The ratios utilized were 1:1, 3:1, 6.1:1 and 9:1; in each case the total peptide constant was constant. When incubated with single peptides PA and MA (respiratory allergens) were observed to have increased reactivity to Lys, while DNCB and DNFB (contact allergens) preferentially reacted to Cys. These preferences were conserved or enhanced under competitive conditions. Under such conditions PA and DNCB were observed to deplete exclusively Lys and Cys, respectively. MA and DNFB both maintained the preferences observed with individual peptides. The selective reactivity demonstrated here may have important mechanistic relevance for the ability of these chemicals to induce divergent immune responses.
745 THE CONTRIBUTION OF PEANUT PROTEINS ARA H1, 2, 3 AND 6 IN PEANUT ALLERGY.
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Peanut is a major allergenic food product, and contains a large number of potentially allergenic proteins. The present study investigated the relative contribution of, and cross-reactivity between the major allergens Arah1-6 in a mouse model for peanut allergy. Mice were immunized by gavage with peanut protein extract or purified allergens Arah1-6. Hereafter, mice were challenged with the individual allergens and blood was collected to measure allergen-specific antibodies and the degree of mast cell degranulation (MMCP-1 and histamine). Spleens were isolated to measure allergen-specific T-cell reactivity. Sensitisation with whole peanut extract induced Arah1-6-specific IgE, IgG1 and IgG2a. In addition, sensitisation with the individual peanut allergens elicited antibody responses with specificity to the allergen used, but limited cross-reactivity between the allergens was observed. T-cell cultures showed Th1 and Th2 type cytokine production upon re-stimulation with individual peanut allergens elicited antibody responses with specificity to the allergen used and cross-reactivity between the allergens was observed. In future studies, the mechanism behind the functional differences of individual peanut allergens and the protein cross-reactivity on T-cell and antibody level will be investigated in vitro.

746 CHARACTERIZATION OF THE ALLERGENICITY OF DIFFERENT FORMS OF LACTOFERRIN.
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With the advent of genetically modified crop plants there is an increased interest in the development of methods for the identification of novel proteins as potential allergens. Current methods include assessment of resistance to digestion by simulated gastric fluid (SGF) and homology searches. There is a need for more holistic methods for the characterization of potential protein allergens and particularly the impact of post translocation modifications such as glycosylation. The allergenicity (IgE inducing) and immunogenicity (IgG inducing) properties of wild type native (milk) human lactoferrin (NFLF) and recombinant LF (rLF) produced in rice have been assessed. These forms of LF have identical amino acid sequences, but different glycosylation patterns: NFLF has a complex glycoprofile including stalic acid and Lewis (Le) x structures, whereas the rLF form is far simpler and rich in mannose residues. Both proteins are very labile to digestion by SGF, with intact protein disappearing after 1min of incubation in the presence of pepsin at pH1.2. Antibody responses induced in BALB/c strain mice by intraperitoneal exposure to NFLF and rLF were characterized. Serum were analysed for protein specific IgG and IgE by enzyme-linked immunosorbant assay (ELISA) and homologous passive cutaneous anaphylaxis assay (PCA), respectively. Immunisation with NFLF stimulated vigorous IgG and IgE antibody responses, whereas rLF was 40-fold less immunogenic and 200-fold less allergenic, irrespective of endotoxin content. The glycosans did not contribute to epitope formation, with equivalent IgE and IgG binding recorded for high titer anti-NLF anti sera whether the immunising NFLF or the rLF was used a substrate in the ELISA or PCA analysis. Similarly, identical low IgG and IgE anti-LF titres were recorded when either the immunising rLF or the NFLF was used in the analysis of anti-rLF antisera. These data demonstrate that differential glycosylation profiles can have a profound impact on protein allergenicity.

747 EXPOSURE TO TRICLOSAN AND BISPHENOL A AUGMENT ALLERGIC RESPONSES IN A MURINE MODEL OF ASTHMA.
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During the past several decades there has been a remarkable and unexplained increase in the prevalence of asthma. While the hygiene hypothesis provides one potential explanation, individuals in industrial societies are also inadvertently exposed to an increasing number of chemicals. While many chemicals are known to directly induce asthma there is also the potential for non-sensitizing chemicals to augment the immune response induced by other allergens. Our lab has previously demonstrated that dermal application of the environmentally relevant chemical, perfluorooctanoic acid, simultaneously with exposure to a single concentration of protein allergen (OVA) was found to augment the allergic response to that allergen. Triclosan and bisphenol-A (BPA) are commonly used in both occupational and public environments which have recently been associated with increases in allergy and asthma. BPA, considered to be non-sensitizing, is a substrate of polycarbonate plastics and has been produced in increasingly large quantities since the 1950s. BPA is used to form plastic bottles, as a lining for food and beverage cans, and as a flame retardant. Triclosan is an antibacterial compound that has been used in consumer products for 40 years and is currently found in many hand sanitizers and lotions. The tolerability and safety of triclosan has been evaluated in human volunteers with little indication of toxicity or sensitization. For these studies a murine model of asthma was used to evaluate the immunomodulatory effect of co-exposure to BPA or triclosan with OVA, Co-exposure to each of these chemicals individually (as low as 30% BPA and 1.5% triclosan) with OVA resulted in an at least 2-fold increase in OVA-specific IgE and an augmentation of the airway hyper-reactivity response to methacholine challenge (as low as 7% BPA and 0.75% triclosan) as compared to OVA exposure alone. Understanding the mechanisms by which mixed exposures influence and augment asthma and asthma-like symptoms may lead to better prevention strategies for those at risk for asthma.

748 IMPACT OF DOSING FREQUENCY ON ANAPHYLAXIS IN CYNOMOLGUS MONKEYS ADMINISTERED TRU-015, AN ANTI-CD20 SMIP™ THERAPEUTIC.
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TRU-015, a chimeric (mouse and human) anti-CD20 SMIP™ (mono-specific protein therapeutic), was administered intravenously (IV) to male and female cynomolgus monkeys at 0 (vehicle), 15, 50, and 150 mg/kg/cycle (4 to 7 animals/group) for 6 cycles (1 dose/2 wks) to evaluate its toxicity. Immuno reactions consistent with anaphylaxis occurred within 1 hr of dosing starting with 1 male at 15 mg/kg after the 3rd dose. By the 5th dose, animals at all dose levels were affected with an inverse dose relationship in the severity of reactions. Diphenhydramine pretreatment initiated at the 6th dose had no apparent impact on anaphylaxis, leading to dosing termination. A total of 9 of 36 TRU-015-dosed animals were affected, with elective euthanasia of 2 animals at 15 mg/kg. Of the 9 animals affected, 5 had anti-drug antibodies (ADA) and 4 had evidence of complement activation. The infusion reactions, onset of reactions relative to dosing, presence of ADA, and signs of complement activation were consistent with anaphylaxis secondary to an immune response to TRU-015. In an effort to successfully conduct a chronic toxicity study, and based on shorter term toxicity studies in which weekly dosing did not cause infusion reactions, TRU-015 was administered IV at 0 (vehicle), 15, and 150 mg/kg/cycle (4 animals/group) for 13 cycles (1 dose/wk). With this regimen, only 1 animal at 15 mg/kg out of 16 TRU-015-dosed animals had an infusion reaction consistent with anaphylaxis. The reaction occurred within 1 hr of the 7th dose and led to this animal’s death. This animal also had ADA and evidence of complement activation. Therefore, increasing the dosing frequency successfully reduced the incidence of anaphylaxis related to TRU-015 administration. This new dosing regimen permitted the conduct of longer duration toxicity studies in NHPs. Similar immune reactions have not been observed in humans following extensive clinical experience.

749 A CUTANEOUS HYPERSENSITIVITY REACTION TO A THYROID HORMONE SUPPLEMENT IN A DOG.
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A dog with clinical signs of hypothyroidism (an autoimmune disease common to dogs and humans) and low thyroid hormones (T4 and its T3 metabolite) received a generic T4 supplement. After 2 weeks, the dog developed a severe pruritus and pyoderma on its neck and back with a widespread erythema. The dog responded to the thyroid hormone supplementation with little indication of toxicity or sensitization. For these studies a murine model of asthma was used to evaluate the immunomodulatory effect of co-exposure to BPA or triclosan with OVA, Co-exposure to each of these chemicals individually (as low as 30% BPA and 1.5% triclosan) with OVA resulted in an at least 2-fold increase in OVA-specific IgE and an augmentation of the airway hyper-reactivity response to methacholine challenge (as low as 7% BPA and 0.75% triclosan) as compared to OVA exposure alone. Understanding the mechanisms by which mixed exposures influence and augment asthma and asthma-like symptoms may lead to better prevention strategies for those at risk for asthma.
Because the T3 hormone is a natural metabolite of T4, and because drug hypersensitivities can become life threatening with re-exposure to the drug, we ran a few in vitro tests before prescribing a T3 supplement. A thyroid autoantibody panel showed high levels of anti-thyroglobulin antibodies, low levels of anti-T3 antibodies, and no anti-T4 antibodies. This was compatible with a diagnosis of hypothyroidism, but probably not related to this dog’s drug hypersensitivity. With the owner’s consent, we also injected some peripheral blood mononuclear cells from the dog’s blood, and incubated them with T4 or T3 for 4 and 7 days. The cells proliferated in a dose- and time-dependent manner with T3; their response to T4 was lower and only after 7 days. This suggested that the skin reactions were indeed an immune reaction against the thyroid supplements, and more specifically against the T3 metabolite rather than T4 directly. To our knowledge, this is the first report of delayed hypersensitivity to a thyroid supplement where a specific cellular immunity against the active ingredient and its metabolite (T4/T3) is demonstrated. This clinical case provides a new example of parallels between drug allergies and autoimmune diseases.


In the context of the 7th amendment to the Cosmetic Directive, the cosmetic industry is concerned by the challenge of finding non-animal approaches to assess the sensitizing potential of chemicals in order to manage risk assessment. Using the in vitro model U937 cell line differentiated into activated macrophages, we have previously shown that sensitizers have the capacity to specifically inhibit the production of prostanooids (Del Bufalo et al. 2011). The aim of the present work is to evaluate if this new biochemical property could be a hallmark of all sensitizers and if this “anti-inflammatory” signature could be correlated with the sensitization potential of chemicals. For that purpose we tested 160 chemicals (64 non sensitizers and 96 sensitizers) in the U937 cells differentiated with phorbol myristate acetate (PMA) and stimulated with lipopolysaccharides (LPS) and evaluated the production of the inflammatory mediator PGE2. Statistical analysis tools as box plot and ROC (Receiver Operating Characteristic) curve allowed us to define the test parameters that discriminate sensitizers from non sensitizers. Our results show that the criteria “inhibition of the PGE2 production” at 24h is a good parameter to predict sensitizers and the performances of the assay are: 78 % concordance, 83% sensitivity and 71 % specificity (obtained on a validation data set). However there is no correlation between the magnitude of inhibition for a given molecule and its sensitization potential.

Contact sensitizers are reactive molecules (hapten) that have the ability to modify skin proteins to form an antigen which will be recognized by specific T cells activated during the sensitization process. In addition to the haptenation mechanism, contact sensitizers induce several phenotypic and functional changes of dendritic cells (DC) either directly or indirectly through intercellular signaling pathways implicating keratinocytes, fibroblasts and other skin cells. This rather complex and still not fully unravelled maturation process of DC induced by contact sensitizers, allow them, to migrate to the lymphnode, present antigen and prime efficiently hapten-specific T cells.

Due to the complexity of this process it is now agreed that alternative hazard identification and risk assessment need to be addressed by combining a battery of methods. The aim of this study was to combine in silico and in vitro tools, from cheminformatics and toxicology, to facilitate the development of an integrated testing strategy for the evaluation of skin sensitization. For this purpose we used a full data set on 165 chemicals composed of different variables, representing the results from in silico predictions (Dock, TIMES, Tostree), from DPRA, MUSST, Nrf-2 and PGE-2 assays as well as numerous physico chemical experimental or calculated parameters. In order to avoid the any bias brought by the use of one single statistical method, we chose the Stacking model allowing us to integrate several statistical methods (Boosting, Bayesian, SVM, Sparse PLS-DA and Scoring). This model provides a probability for a chemical to be a sensitizer. The performances of the optimal model are: 85 % concordance, 90% sensitivity and 75 % specificity (obtained on a validation data set). This kind of alternative prediction will ultimately contribute to the risk assessment decision making in a Weight of Evidence approach.

Z. El Ali1, C. Gerbeix2, P. Esser1, P. Robert1, J. Legrand2, S. Martin1, M. Pallardy1 and S. Kerdine-Römer2.
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Chemical sensitizers, inducing contact hypersensitivity (CHS), are known to induce reactive oxygen species (ROS). The Nrf2/Keap1 pathway is central for detoxification. Nrf2 is a redox-sensitive basic leucine zipper transcription factor involved in the transcriptional regulation of many antioxidant genes. Nrf2 plays a central role in protecting cells from ROS and electrophiles. We have demonstrated that contact sensitizers induce, in vitro, the accumulation of Nrf2 in human dendritic cells. In order to elucidate the role of Nrf2 in CHS, we used the Local Lymph Node Assay (LLNA) for studying the sensitization phase and the Mouse Ear Swelling Test (MEST) for studying the elicitation phase during the CHS. These studies were performed, in nrf2 knock out (nrf2-/-) and in wild type (nrf2+/+) mice, in response to dinortrichlorobenzene (DNCCB). The MEST results showed that (DNCCB) (1%) induced a higher increase of ear thickness in nrf2-/- mice than in nrf2+/+ mice. Furthermore, the swelling increase was time dependent after challenge. Sensitization of mice with DNCCB (0.5%) induced a high significant response in nrf2-/- mice compared to nrf2+/+ mice where no swelling was observed after challenge. On the other hand, results obtained in LLNA showed that DNCCB induced an increase of lymphocyte proliferation in nrf2-/- and nrf2+/+ mice. However, the stimulation index (SI) for a same concentration was higher in nrf2-/- mice than in WT nrf2+/+ mice. These results underline the crucial role of Nrf2 in CHS. Nrf2 seems to control the inflammation response and the lymphocyte proliferation, involved in allergic response to chemical sensitizers.

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EVALUATION OF SENS-IS®, AN EPISKIN® BASED MODEL FOR IDENTIFYING CHEMICAL SENSITIZERS.

S. Teissier1, F. Tourneix1, A. Del Bufalo1, C. Gomes1, F. Cottrez2, H. Groux2

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In the context of the 2013 ban given by EU Cosmetics Directive, the ability to identify and classify the skin sensitization potential of chemicals without animals is of high importance for the cosmetic industry. A range of different in vitro chemistry-based (DPRA, GSH reactivity) and cell-based methods (MUST; ICLAT, Keratinosens) have been developed and we are currently evaluating some of them for their applicability to cosmetic ingredients and their physicochemical diversity. Although these assays appear to be promising for hazard identification, potency assessment is still limited. Immunosearch has developed SENS-IS, a new method, based on the quantitative analysis of specific biomarkers expressed in 3D reconstituted epidermis (Episkin®). This new assay provides a possible way to encompass the limitations of monolayer culture models (lack of skin bioavailability properties, different metabolism of the models compared to skin, inability to test water insoluble chemicals) and might therefore allow a better assessment of the sensitization potency of cosmetic ingredients. With the aim to evaluate the predictive capacity of this new assay, the skin sensitization assessment of 40 proprietary and public domain chemicals that were assessed by Immunosearch in a blinded manner. The results of this study show an overall performance of sensitizer/non sensitization prediction of 87% with a specificity of 83% and a sensitivity of 91%. The predictive capacity of potency categories of sensitizers (strong, moderate, weak) is however less performance (63% concordance) with 7 chemicals being overestimated and 8 chemicals being underestimated. Altogether, even if not yet sufficient for potency determination, SENS-IS is a promising new method for the skin sensitization evaluation of cosmetic ingredients.

INDIVIDUAL PREDICTIVE CAPACITIES OF IN SILICO AND IN VITRO METHODS FOR THE ALTERNATIVE ASSESSMENT OF SKIN SENSITIZATION: A COMPARATIVE STUDY ON A COMMON CHEMICAL SET.


Skin sensitization is a delayed type allergy consisting of a cellular immune reaction to small molecular weight chemicals. So, animal test methods such as the LLNA are used to predict the skin sensitization potential of unknown chemicals. In line with the 3Rs concept, ranges of in silico and in vitro alternative methods have been developed. While in silico methods are based on structure activity relationships, in vitro assays model the early events of the skin sensitization process: chemical reactivity assays (DPRA, GSH assay) reflect the haptenation mechanism, Nrf-2 based assays (Keratinosens, ADE-AD) analyze the induction of the cellular antioxidant pathway and DC-based assays (MUST; ICLAT) measure DC maturation markers (CD86, CD54). These assays were generally shown to have good predictive values for the hazard identification of skin sensitizers, but the correlation studies reported, differ in the nature of the reference that was used (animal or clinical references) as well as in the number of data analyzed.

In the present study, we show the data of 2 in silico methods (Derek, TIMES-SS) and 3 in vitro assays (DPRA, MUST, Nrf-2 reporter assay from InVitrogen) on a common chemical set composed of 165 compounds: 93 proprietary and 72 public domain references. We analyzed the individual performances of each method for the hazard identification of sensitizers. The results show that the accuracy varies from 73% to 92% with specificities between 66% and 78% and sensitivities between 76% and 100%. However, for some methods, often the more accurate ones, the number of inconclusive results can reach 40% of the chemicals, which reveals a restricted applicability domain for these methods. In conclusion we describe the complementarities of the 5 methods by showing how the combination of the results allows to overcome the limitations of restricted applicability domains (> 95% of chemicals classified) and optimize the accuracy of the prediction (> 85%).

RUTIN SUPPRESSES HOUSE DUST MITE EXTRACT AND 2, 4-DINITROCHLOROBENZENE-INDUCED ATOPIC DERMATITIS.

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Rutin has been used as a medication for blood vessel protection and as an ingredient of multivitamin preparations and herbal remedies. Rutin is known to have anti-inflammatory, anti-oxidative, and anti-cancer activities. However, the effect of rutin on atopic dermatitis (AD) has not been studied yet. We evaluated the efficacy of rutin in an allergic contact dermatitis (ACD) model on the ears of BALB/c mice using house dust mite extract (Dermatophagoides farinae extract, DFE) and 2,4-dinitrochlorobenzene (DNCB, 1%). We established an atopic dermatitis model in BALB/c mice by repeated local exposure of DFE/DNCB to the ears. Repeated alternative treatment of DFE/DNCB caused AD. Rutin reduced AD based on ear thickness and histopathological analysis, in addition to serum IgE levels. Rutin inhibited mast cell infiltration into the ear. Rutin suppressed DFE/DNCB-induced expression of IL-4, IL-5, IL-13, IL-31, IL-32, and INF-γ in the tissue. In addition, a local lymph node assay confirmed that rutin suppressed AD. Rutin also reduced ear thickness, serum IgE levels, and expression of IL-10, IL-17, and TNF-α in ACD ears. This study demonstrates that rutin inhibited AD and ACD, suggesting that rutin might be a candidate for the treatment of AD and ACD.

CONTINUOUS ACTIVATION OF RESPONDER T CELLS AND REGULATORY T CELLS CAUSED BY SILICA EXPOSURE INDUCE DYSREGULATION OF AUTOIMMUNITY.

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Patients with silicosis (SIL) are suffering from pulmonary fibrosis but also often autoimmune diseases such as RA, SSc and ANCA-related vasculitis/nephritis. Although it has been considered that silica can act as adjuvant, we had been reported that the inhibitory effects of peripheral CD4+25+ T cell fraction (should include CD4+25+Foxp3+ regulatory T cells, Treg) derived from SIL was reduced when compared with that derived from healthy donors (HD). To explore this phenomenon, the activation status of responder T cells (Tresp) and Treg had been analyzed. Regarding Tresp, silica stimulated Tresp in vitro when monitored by CD69 expression, as well as CD69 mRNA expression was higher in CD4+25- fraction from HD than those from SIL. The PD-1 mRNA as activation marker was higher both in CD4+25+ and 25- fraction from SIL than those from HD. These findings indicated silica can activate Tresp. On the other hand, Treg from SIL showed higher expression of CD95/Fas surface expression than that from HD and peripheral CD4+25- cells from SIL proceeded to apoptosis faster and stronger than that from HD, when these cells were cultured with Fas-stimulating MoAb. Furthermore, PBMCs from SIL or HD were cultured with silica, those from SIL showed higher loss of FoxP3+ true Treg than those from HD. Those findings indicate that silica can activate both Tresp and Treg resulting entry of activated Tresp into CD4+25+ fraction and early loss of true Treg caused by excess CD95/Fas expression as the phenotype of activation. Thereafter, these chronic and recurrent modifications of Tresp and Treg may induce the longer survival of Tresp including autoreactive T cell clones and also loss and recruitment of Treg with lesser function of inhibition for stimulated Tresp activity.

EFFECTS OF LIBBY AMPHIBOLE EXPOSURE ON THE DEVELOPMENT OF AUTOIMMUNITY IN THE RAT.


Epidemiological data suggest that exposure to the amphibole-containing vermiculite in Libby, MT was associated with increased risk (odds ratio of 2.14) for developing systemic autoimmune diseases (SAID). Elevated titers of antinuclear antibodies (ANA) were also found in Libby residents; however, increased ANA alone are not sufficient to induce SAID. Our goal was to establish a relationship between increased ANA titers in Libby amphibole (LA)-exposed rats with other hallmarks of SAID. Female Lewis rats were intratracheally instilled biweekly for 13-weeks with Libby amphibole or amosite. ANA was significantly increased in all asbestos dose groups, however no dose response was observed. ENA analysis determined that 94% of ENA-positive samples were specific for the Jo-1 antigen, a marker associated with interstitial lung disease and myositis. Urine protein concentration, anti-dsDNA titers and kidney histopathology were not affected by LA or amosite exposure. In addition, a NOAEL was not determined for ANA titers in LA-exposed rats. These data support...
TRICHLOROETHENE-INDUCED NITROSATIVE STRESS: PROTEOMIC IDENTIFICATION OF NITRATED PROTEINS IN THE LIVER OF MRL+/+ MICE.

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Exposure to trichloroethene (TCE), a common environmental contaminant, is associated with an autoimmune response, but the mechanisms remain largely unknown. Liver is one of the major organs affected by exposure. Previous studies from our laboratory have shown that TCE exposure in mice leads to increased nitrosative stress in livers, and suggested a potential role of the nitrosative stress in TCE-mediated autoimmunity. To further explore the role of nitrosative stress in TCE-mediated autoimmunity, the current study used proteomic approaches (2DE, gel, Western blot, MALDI TOF/TOF MS/MS, etc.), was conducted to characterize the nitrated proteins in the livers of TCE-exposed mice. Female MRL +/+ mice were treated with TCE (10 mmol/kg, i.p., every 4th day) for 6 weeks, and liver proteins were extracted for the identification of nitrated protein and INOS expression. A total of thirty nine proteins were identified in the livers of TCE-treated mice, whereas 21 proteins were found in the livers of both TCE-treated and control mice. The nitrated proteins were found in the molecular weight range of 19.1 to 128.3 kDa and PI range of 4.9 to 8.8. The identified nitrated proteins mainly represented skeletal proteins, enzymes, stress proteins and chaperones. Furthermore, TCE exposure led to significantly increased INOS protein expression in the livers, suggesting its contribution to increased formation of reactive nitrogen species and thus, contribution to increased nitrated proteins. The identified nitrated proteins provide a global map to further investigate alterations in their structural and functional properties, which will lead to a better understanding of the role of protein nitration in TCE-mediated autoimmunity. Supported by NIH ES016302.

INTERRELATIONSHIPS OF PEROXIDATION AND IMMUNOGENICITY OF CARDIOLIPIN: ANOXIDATIVE LIPIDOMICS APPROACH.

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The myriad of neoepitopes formed during lipid peroxidation trigger potent innate and adaptive immune responses. Experimental and clinical studies have been mostly focused on immunogenic properties of oxidized species of phosphatidylcholine (PCox) and phosphatidylethanolamine (PSox). Lately, a mitochondria-specific anionic phospholipid, cardiolipin (CL), particularly its peroxidized molecular species (CLox) attracted attention as innate and adaptive immunogens and predictors of clinical outcomes and novel therapeutic modalities for vaccination. Specific interrelationships between the emergence of individual molecular species of CLs/CLox in circulation and the appearance of the respective antibodies remain unknown. This is due to the lack of specific/sensitive analytical tools to characterize a huge diversity of individual CL and CLox. Using mass spectrometry based oxidative lipidomics, we focused on the analysis of molecular species of CLs and CLox in plasma of mice exposed to total body irradiation (9.5 Gy) or bacterial infection (Pseudomonas aeruginosa, PA103) as well as in plasma from ARDS patients. In the latter cases, we were able to detect the presence of both host and bacterial CLs in LC/MS spectra of plasma lipids. While relatively low molecular mass species (m/z 1376 and 1418) are typical of bacterial CLs, larger CL molecules (with m/z from 1448) dominate in MS of mammalian plasma CLs. Simultaneously, antiCL/CLox antibodies were detected in plasma. We also found that CL-containing liposomes and CL-coated mitochondria were recognized and taken-up by RAW264.7 macrophages. The data supported the context of possible roles that CLs/CLox play as anti-oxidants and anti-inflammatory responses. Supported by NIOSH OH008282; NIH U19 AI068021, HL70755, HL094488, ES020693, ES021068.

USE OF IN VITRO AND IN VIVO METHODS TO ASSESS THE SENSITIZATION POTENTIAL OF NOVEL AMINO ALCOHOLS.


The Local Lymph Node Assay (LLNA) is the principle preferred method for assessing the skin sensitization potential for EU REACH regulatory purposes. However, the LLNA has been found to overestimate sensitization potential for various compounds such as surfactants, fatty acids and alcohols. Amino Alcohols, as a class, have been shown to be non-sensitizers. We have discovered four alcohol amines; aminocyclohexanem (ACyH), 2-aminopropanol (2-AP), aminomethylcyclohexanel (ACyHM), and aminododecane (ADD), that tested positive in the LLNA. Supported positive in the LLNA.
To confirm the LLNA findings, we conducted the guinea pig maximization (GPMT) and protein reactivity tests. Only 2-AP and AMC/HOL tested positive in the GMPT. The protein reactivity assay failed to show any of these chemicals depleted free peptide by more than 15% through covalent bonding. QSAR (DERMWIN™ v2.01) predictions were used to estimate dermal absorption and ranged from 1% for 2-AP, 8% for AmCyHOL, 36% for AcYHM, and 100% for ADD. ADD may have high uptake in the stratum corneum but have a low transfer rate to the epidermis due to its high lipophilicity. Of note, amino alcohols are highly irritating and ACyHM and ADD were corrosive using the Epiderm™ skin model. Findings from these investigations will serve to train QSAR models for accurate predictions of skin sensitization for this class of chemicals. Additionally, it is important to determine which chemical classes or characteristics lead to overestimates in the LLNA.

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Incompatible database formats limit the value of the large amount of publicly available data on adverse events caused by drugs. The BioWisdom Safety Intelligence Program (SIP) is a harmonised database from Medline and other compound / safety databases that specifically links compounds to toxicities. We have combined physiological and pathological human observations from SIP with target profiles of drugs (BioPrint®, Cerep, France) to identify associations between pharmacological activities and adverse events. Compound-toxicity links were grouped into tissue categories and into a hierarchical system based on Medical Dictionary for Regulatory Activities (MedDRA). Fisher’s exact test (adjusted for false discovery) was used to identify significant associations between compounds in each tissue or MedDRA group and activity (<10 μM) against each target in BioPrint. 378 significant target tissue associations were identified, including known relationships such as cycloxygenase (COX) I/2 and the kidney and estrogen receptor alpha and the uterus. Less well known associations with targets were also identified, such as cytochrome P450 2C9 and the vasculature and neurokinin 2 and the heart. The MedDRA terminology enabled some target adverse event relationships to be tracked from the System Organ Class (SOC) to the mechanistically more informative Preferred Term (PT), for example HMG-CoA reductase to musculoskeletal disorder (SOC) and myositis (PT) and COX2 to renal and urinary disorders (SOC) and nephrotic syndrome (PT). In summary, we have validated an approach using harmonised adverse event data from the public domain to identify associations with pharmacological screening data; this could also be applied to other compound characteristics such as physicochemical properties and structural moieties.

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Comparative genomics and population genetics have led to better understanding of the genetic diversity in humans. The International HapMap and the 1000 Genomes projects have established human lymphoblast cell lines from racially and geographically diverse populations. These cells can be used as an in vitro toxicity testing model that takes advantage of the diverse genotyping and RNA sequencing data. We selected 1104 cell lines from 9 populations representing 5 continents: Utah residents with Northern & Western European ancestry; Han Chinese in Beijing, China; Japanese in Tokyo, Japan; Lutheran in Wépiong, Kenya; Mexican ancestry in Los Angeles, CA; Tuscan in Italy; Yoruban in Ibadan, Nigeria; British from England and Scotland; and Colombian in Medellin, Colombia. Of these, 1095 cell lines (99.2%) were screened in quantitative high-throughput screening format with 180 chemicals diverse environmental chemicals at 8 concentrations (0.3 nM-92 μM) in a cell viability (CellTiter-Glo®) assay. Duplicate 1356-well plates were screened for ~70% of the cell lines, revealing excellent intra- and inter-experiment reproducibility in both concentration-response curve class and relative cell viability (r=0.977 for EC50 values). Among the 180 substances screened, the variabil-
Accurate prediction of plasma protein binding (PPB) of small molecules is critical in pharmacokinetic research. Previous modeling studies of PPB were limited to small data sets. We have compiled and curated a set of 1244 compounds with known percent-scale PPB values (%PPB) from several publicly available depositaries. We compared two endpoints: (1) the standard %PPB and (2) lnKa calculated as a logarithm of “binding constant”-like parameter derived from %PPB values. Quantitative Structure-Activity Relationship (QSAR) models were derived using Dragon descriptors and three machine learning methods (k-nearest neighbors, support vector machines, and random forest). Based on the consensus prediction of the three modeling methods, five-fold external validation yielded correlation coefficient (R2) of 0.68 and the mean absolute error (MAE) of 16.4% for the %PPB models, and R2 of 0.66 and MAE of 13.6% for the lnKa models. Moreover, to additionally validate our QSAR models, we predicted a set of 236 chemicals with high-throughput screening %PPB values measured under the framework of US EPA ToxCast project. For those ToxCast chemicals, the MAE of the %PPB and lnKa models was 19.3% and 14.9%, respectively. Overall performance of lnKa QSAR models was only slightly better than those of %PPB models, but much better for high-affinity compounds. For example, 470 out of 1244 compounds had %PPB ≥ 90% and their external MAE was 15.7 and 7.8% for %PPB models and lnKa models, respectively. Likewise, the MAE of 158 high-affinity ToxCast chemicals was 18.1 and 11.5% for %PPB and lnKa models. For high-affinity compounds the experimental error is estimated to be 1-5%; MAE of lnKa models was found to be significantly lower than that of %PPB models (p < 0.01). In summary, we have developed and rigorously validated QSAR models for predicting PPB of chemicals (especially for strong binders) based on the largest publicly available PPB dataset. These models can be used to evaluate pharmaceuticokinetic endpoint as part of human health risk assessment.

Quantitative Structure-Activity Relationships for Cyclodiene Insecticides Inhibiting Gamma-Aminobutyric Acid Receptor Subtype A.

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Cyclodiene insecticides such as dieldrin and endosulfan act as non-competitive antagonists of GABA, an inhibitory neurotransmitter of the nervous system. It is well accepted that the site of action of these insecticides is the picrotoxinin (PTX) binding site on the GABAa receptor chloride channel complex. Other structurally similar GABA antagonists are believed to also act at the same or an overlapping site. Herein, a quantitative structure-activity relationship (QSAR) model using BHC, endosulfan and various bicyclophosphates, dioxatricyclododecenes and related compounds was developed to predict rat brain GABA receptor pIC50 (1 / log of the 50% inhibitory concentration of rat GABAa receptors). Chemical structures were drawn and three dimensionally optimized using the CHARMM force field in Advanced Chemistry Development (ACD) Labs, ChemSketch. Chemical descriptors were calculated using AMPAC and Codessa 2.51 (Semichem, Inc.) and heuristic regression was used to develop equations correlating structure with activity. The initial QSAR model developed yielded an R2 of 0.57. Separating the compounds into those with linear bridgehead substituents (series I) and non-linear substituents (series II) yielded R2 values of 0.80 and 0.65, respectively. The results emphasize the importance of classifying these compounds into two different series based upon their bridgehead substituent. This project received support from the Defense Threat Reduction Agency - Joint Science and Technology Office, Basic and Supporting Sciences Division. This abstract has been cleared for public release: 88ABW-2011-4758, 9/6/2011.
differential biological responses. Embryonic zebrafish exposed to 1.2 nm 3-MPA-AuNPs failed to respond to a touch on the caudal fin at 120 hours post fertilization, while those exposed to 1.5 nm MEEE-AuNPs had a normal touch response. To investigate the molecular mechanism underlying the differential touch response, whole animal RNA-seq was conducted. Total RNA was isolated from embryonic zebrafish exposed to the 100% effective concentration (EC100) for 1.2 nm 3-MPA- and 1.5 nm MEEE-AuNPs (10 ppm) at 48 hours post fertilization (hpf). While the core materials (Au) for both nanoparticles are identical, the surface functionalities caused unique gene expression changes. 57,095 transcripts were mapped to ensemble version 9. At 48 hpf, 810 transcripts were common between MPA- and MEEE-AuNP exposed groups, while 1,099 and 1,523 transcripts were unique and statistically significantly differentially expressed in MPA- and MEEE-exposed broys, respectively. Bioinformatic pathway analysis similarly identifies unique pathway perturbations following exposure to these AuNPs. This research is supported by NIEHS F31 ES019445-01, P30 ES002120, ES16886, and Air Force Research Laboratory #FA8650-05-1-5041.

773 DISCOVERY OF NOVEL RAT MICRORNAS USING NEXT-GENERATION SEQUENCING.
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MicroRNAs (miRNAs) are noncoding RNAs with 18–25 nucleotides in length. miRNAs can bind to their target mRNA to inhibit mRNA translation. Therefore, miRNAs are important regulators for gene expression. However, rat miRNAs have not been fully explored and currently only 679 have been reported in miRBase version 17. Since identification of novel miRNAs will provide valuable insights into their functions, we conducted this study to discover novel miRNAs in rat using the next-generation sequencing (NGS) technology. Eight rat kidney samples, 4 from untreated rat and 4 from rats treated with human carcinogen aristolochic acid (AA), were used for small RNA library construction. Application of the different animals treated with a carcinogen and its vehicle control could strengthen the discovery of possible novel miRNAs according to the common novel miRNAs existed in the different animals and the differentially expressed novel miRNAs due to the treatment. The NGS was conducted using Illumina Genome Analyzer II platform. NGS data was analyzed by miRAnalyzer. Totally, 14,358,136 sequence reads were obtained, among which 332 novel miRNAs candidates homolog to other species and 100 rat specific candidates were identified. Custom miRNA microarray containing 5,460 miRNAs from 32 vertebrate species and the 100 rat specific candidates were employed to validate those candidates. Nine novel rat miRNAs were discovered using a strict criteria (NGS reads >10 and miRNA array signal intensity >31 for every rat samples). One of the novel rat miRNA was statistically significantly dysregulated by the AA treatment (P <0.05). Since only one new rat miRNA was added from the samples). One of the novel rat miRNA was statistically significantly dysregulated by the AA treatment (P <0.05). Since only one new rat miRNA was added from the samples). One of the novel rat miRNA was statistically significantly dysregulated by the AA treatment (P <0.05).

774 GENE LOCI ASSOCIATED WITH METABOLISM OF INORGNIC ARSENIC IN MICE.
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To identify genetic loci responsible for inorganic As (IA) metabolism, a quantitative trait locus (QTL) analysis was performed using MXH/lpr recombinant inbred mouse strains exposed to inorganic As. We analyzed concentrations of arsenite (As(III)), arsenate (As(V)), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) in the liver of nine MXH/lpr strains. In all the strains, concentration of the sum of As compounds (SA) in the liver was negatively correlated with hepatic DMA composition (%DMA), whereas the opposite trend was observed for IA (As(III) + As(V)) composition (%IA). This result suggests that IA metabolic capacity significantly influences As accumulation in mice. Furthermore, significant differences in the concentrations and compositions of As compounds were found among these strains. QTL analysis showed that a significant linkage was identified on for %DMA with chromosomes 14 and 3. For %IA, a significant linkage was identified on chromosomes 14 and 5. Interestingly, the loci on the chromosomes 14 and 5 were correlated with %IA in an additive manner.

775 THE FUNCTION OF ETHYLENE IN SYNECHOCYSTIS SP. PCC 6803.
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Ethylene is a gaseous plant hormone that is important to many plant processes such as seed germination and ripening. Responses to ethylene are regulated by a set of receptors that are unique to each plant species. Bioinformatic analysis showed that ethylene binding like sequences have been found in cyanobacteria species. The purpose of our research is to investigate the role of ethylene signaling in cyanobacteria using Synechocystis sp. Based on the similarities between the DNA sequence of cyanobacteria and plant ethylene receptors, we hypothesized that lateral gene transfer may have occurred during endosymbiosis. To study ethylene binding in cyanobacteria, we determined if the presence of Synechocystis gene product, slr1212, in cyanobacteria is capable of binding ethylene and whether the knockout of Synechocystis gene disruption has a phototaxis response in this organism. slr1212, an evolutionary precursor to the ethylene signaling receptors found in other plants, was cloned into an E. coli expression vector and the amount of ethylene binding protein produced was measured using a radioligand binding assay. The Synechocystis slr1212 gene knockout was generated using homologous recombination. Analysis of slr1212 binding of ethylene in both whole cell and membrane preparation demonstrate that this protein has a high affinity for binding ethylene. Furthermore, when the Synechocystis gene was disrupted, we observed no significant change in phototactic response in cyanobacteria. Our preliminary data suggest that the role of slr1212 gene in cyanobacteria may be different from what is observed in other organisms.

776 IMPROVEMENT OF TOXICOGENOMICS PROFILE COMPARISON TO PREDICT PROTEIN-PROTEIN INTERACTIONS.
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We have developed in silico methodology for toxicogenomics data to compare a pair of probe sets to measure the similarity of gene expression profiles considering with their time courses and dose effects. This method can be applied to any kinds of gene expression data that is obtained in a systematic manner. The toxicogenomics project in Japan, started in 2002, produced genome-wide gene expression data of the liver of rat in vivo and vitro using 150 pharmaceutical drugs. The gene expression data has been obtained at 8 time points and 4 dose levels in vivo, and 3 time points and 4 dose levels in vitro. Previously, we showed an approach to find protein-inhibitor pairs using the above method. The gap of similarity score distribution between p53 and its specific inhibitor gene Mdm2 is good example to predict specific type of protein-protein interactions such as protein-inhibitor pairs. However, in this example, although the similarity score of p53 was highly ranked (p <0.01) in the score distribution between Mdm2 and all other genes, still over 50 genes were listed as candidate interaction partner genes. To narrow down to select the potential interaction partner genes and remove unrelated genes, we introduced co-expression clustering method. In our improved method, we employed k-means clustering and Euclidean distance converted from the scores. By clustering co-expressed genes, those multiple genes under same regulatory unit were merged into a cluster and the number of candidate genes were decreased. While Mdm2 does not have similar expression profile to the profile of p53, the specific type of protein-protein interaction is not likely to classify into same cluster. Our improved method reduced the number of unrelated genes and successfully narrow down the potential interaction partner genes. This method would be greatly helpful to reveal potential toxicological pathways and mechanisms.

777 HIGH-DIMENSIONAL PROFILING OF TRANSCRIPTION FACTOR ACTIVITY DIFFERENTIATES TOXCAST CHEMICAL GROUPS.
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The ToxCast™ project at the US EPA uses a diverse battery of high-throughput screening assays and informatics models to rapidly characterize the activity of chemicals. A central goal of the project is to provide empirical evidence to aid in the
prioritization of chemicals for additional toxicity testing. Chemicals can be differ-
entiated based on the observed activity profiles as reported by ToxCast assays. Here, we provide a statistical framework to quantify and visualize activity patterns across assays and chemicals. We demonstrate this method by compar-
ing broadly-defined chemical use groups amongst the 1060 unique compounds in
the combined ToxCast Phase I and II sets. The groups were obtained by query-
ing usage information across the ACToR database. The large chemical set represents a diverse landscape including groups such as pharmaceuticals, pesticides, food addi-
tives and fragrances. We used a set of 81 ToxCast assays that measure chemical in-
duced transcription factor activity in a human hepatic cell line. The multiplexed assay enables high-content, functional assessment of transcription factor activities, which are core components of cellular signaling networks. Using our statisti-
cal profiling framework, we highlight a subset of these assays that best differenti-
ate chemical use groups and report similarities amongst transcription factor activity profiles of chemicals used for radically different purposes. We then drill down into these profiles using a Bayesian approach to infer the degree of relatedness between all chemicals within a group—shedding light on informative subgroups having similar transcription factor activity patterns. Our methods provide a flexible framework for understanding high-dimensional toxicological data and are extensi-
bly to diverse definitions of “group”; e.g., alternate chemical usages, known in vitro toxicity endpoints, or structural classes. This abstract does not necessarily reflect US EPA policy.

778 DOSE-RESPONSE MODELING OF P53 OSCILLATION IN RESPONSE TO DNA DOUBLE STRAND BREAKS.

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The tumor suppressor p53 is a crucial guardian of genomic integrity in mammalian cells. In response to DNA damage, the p53 pathway is activated, leading to various cell fate decisions depending on damage severity and repair efficiency. Understanding p53 dynamics and its connection with toxicity endpoints such as cell proliferation, apoptosis, and mutagenesis is an important step toward quantita-
tive risk assessment for genotoxic chemicals. We have developed a computational model to simulate p53 oscillation in response to DNA double strand breaks (DSBs) that can be induced by ionizing radiation and radiomimetic chemicals. The model was formulated by incorporating a feedback circuit consisting of p53, Mdm2, ATM, and Wip1, ultra-sensitivity through experimentally justified multi-step sig-
naling and ATM autophosphorylation, and time delays through nuclear/cytoplas-
ic translocation. The deterministic version of the model is able to produce both
sustained and damped oscillations. When simulated stochastically with the Gillespie algorithm to take gene expression fluctuation into consideration, the model, which is parameterized to produce damped oscillations deterministically, exhibits sustained and noisy oscillations. The simulated p53 and Mdm2 protein levels oscillate with a large variance in amplitude and a relatively fixed period (7 hr) in individual cells. When averaged over a cell population, the oscillations become damped. As a result of ATM autophosphorylation, the amplitude and frequency of p53/mdm2 oscillations in individual cells are independent of the amount of DSBs. However, the number of cells oscillating increases as the irradiation dose increases. These simulation results are consistent with experimental observations reported in the literature. The p53 circuit developed here can be linked to cell cycle and apop-
tosis models to better understand the effects of DSBs on cell cycle arrest and cell death, and to predict mutagenesis rate.

779 USING POPULATION-BASED DOSE-RESPONSE CYTOTOXICITY DATA FOR IN SILICO PREDICTION
OF RAT ACUTE TOXICITY.

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Previously, we established that calculated chemical features combined with in vitro screening or toxicogenomics data afforded “hybrid” prediction models of toxicity with improved accuracy. Here, we explored the value of incorporating population diversity into hybrid modeling. We used quantitative high-throughput screening (qHTS) data on cytotoxicity and caspase-3/7 activation for 240 chemicals tested at 12 concentrations (62.5nM-400uM) in 81 densely genotyped human lymphoblast cell lines (CEPH panel, Mapmap). First, we used qHTS data as independent vari-
ables to model rat acute toxicity (119 chemicals with rat oral LD50 values catego-
rized as “toxic” or “non-toxic”) achieving the classification accuracy of 63.4% under 6-fold external cross-validation. Second, we developed both conventional quantitative structure-activity relationship (QSAR) and hybrid models (combining chemical descriptors and qHTS data across all cell lines). The accuracy of the hybrid models was significantly higher (70.3%) than that of conventional QSAR models (61.4%), in line with previous results. Model analysis indicated that
cytotoxicity measured at 15mM was the most frequently used in vitro predictor, and that cell line sensitivity varied based on the descriptors included. NA10748, NA12057, NA12156, and NA12753. We conclude that the “hybrid” modeling offers several advantages such as improvements in the model accuracy, en-
riched interpretation of the most predictive features, and expanded applicability domain for predicting larger variety of chemicals. Models developed in this study can identify GHS categories 1-3 of toxic compounds with reasonable accuracy and thus can be applied in prioritization of environmental compounds for risk assess-
mation. Future studies will integrate genotype-based descriptors to enrich current representation of compounds by their chemical structural features and by qHTS based biological descriptors.

780 PREDICTIVE IN SILICO MODELS OF TOXICITY USING C. ELEGANS GROWTH ASSAY ALONG WITH TOXCAST PHASE I SHORT-TERM ASSAY DATA.

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Development of predictive in silico models for chemical toxicity is a task of primary importance. The US EPA ToxCast Phase I data includes 615 in vitro and 76 in vitro assays for 320 substances (211 after curatiation). Here, we have explored the use of whole organism C. elegans toxicity data generated for ToxCast chemicals along with available in vitro data for toxicity predictions. Our results indicate that (i) the ToxCast data can be used to predict toxicity of molecules for C. elegans and (ii) the C. elegans growth assay can be used to help predict some of the in vivo toxicities in-
cluded in ToxCast data. QSAR approach was used to develop models for predic-
tions of nematode growth inhibition assays. When chemical properties alone were used as descriptors, no predictive models were generated; however, use of in vitro ass-
ays as descriptors yielded models with the correct classification rate (CCR) of 0.70. A hybrid approach that combines chemical and in vitro assays as descriptors gave models with the highest CCR of 0.71. Two in vitro assays were found to be similar to a subset of the C. elegans assay of 102 compounds which included 51 non-toxic and 51 toxic compounds. A hierarchical QSAR modeling approach in which chem-
icals are partitioned into two classes based on whether they test similarly or dissim-
ilarly in both C. elegans and in vitro assay was used to build models for these as-
says. The best model with CCR=0.67 was obtained for ‘CHR_Rat_LiverNecrosis’ assay. Our approach, which combines in vitro assays with chemical descriptors en-
hances predictive power of QSAR models and warrants further exploration of effec-
tive use of such cheminformatic approaches in predictive toxicology.

781 A COMPUTATIONAL ANALYSIS OF THE MECHANISTIC RELATIONSHIP BETWEEN CIGARETTE SMOKING AND RISK OF RHEUMATOID ARTHRITIS.

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Rheumatoid arthritis is a systemic autoimmune condition affecting the synovial joints. Cigarette smoking has been associated with increased risk rheumatoid arthri-
tis; however, the molecular mechanisms behind this phenomenon remain unclear. This study aimed to systematically analyze interactions between cigarette chemicals and genes important in rheumatoid arthritis pathogenesis. A North American Rheumatoid Arthritis Consortium genome-wide association study was used as the source of genes whose variants correlate with cases of rheumatoid arthritis. GeneGo’s MetaCore database was used to analyze these genes for interactions with chemicals in cigarette smoke and tobacco. In addition, this list of genes was com-
pared to genes differentially expressed in lymphocytes of smokers and non-smokers. Of the 1515 genes significant at 1E-3 in the genome-wide association study, 160 were found to interact with 122 chemical components of cigarettes. The majority of these interactions come from chemicals in tobacco, as opposed to cigarette smoke, and can be classified as inhibitory binding of a chemical to a gene product. Of the 37/6 genes significant at 1E-2, 76 were found to overlap with 303 genes dif-
fferentially expressed in human joints most significantly toqHTS descriptor. Gene ontology enrichment analysis revealed biological processes overrepresented in the above lists of genes. Examples include ion transport, immune response, cell proliferation, among others. Cigarette chemical interactions with genes grouped in these ontolo-
gies reveal that immune system genes are activated, whereas ion transport and anti-
apoptosis genes, among others, are inhibited. This study provides a broad look at the complex network of signaling events underpinning the relationship between cigarette smoking and rheumatoid arthritis and provides the groundwork for future studies examining important genetic-chemical interactions and gene ontologies.
AN EXAMINATION OF THE COMBINED EFFECTS OF ENVIRONMENTAL FACTORS AND SUSCEPTIBLE GENE ON INCREASING THE RISK OF ALZHEIMER'S DISEASE.

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There is increasing evidence that combinations of environmental and genetic factors are likely causes of the development of Alzheimer’s Disease (AD). This research was designed to visually interpret the interactions between known environmental and genetic risk factors. Using Genome-Wide Association Studies, key pathogenesis-related genes and gene products were identified: amyloid precursor protein (APP), phosphatidylinositol-binding clathrin assembly protein (PICALM), clus-terin (CLU), complement receptor 1 (CR1), sortilin receptor 1 (SORL1), and apolipoprotein E (ApoE). Potential environmental factors of interest were chosen based on publications implicating their potential roles in the cause and development of AD. These included: acrolein, 25-hydroxycholesterol, arsenic, copper, and aluminum. Using Genego Metacore and prior research, we mapped out cellular pathways that encompass both gene and environmental factor correlations. Results from this research indicate that the environmental factors supplement the formation of amyloid beta plaque formation in ways that differ from that of the genes of interest. While our genes and gene products were mainly focused on amyloid beta clearance and immunity, the environmental factors were shown to be involved in stabilization of amyloid beta oligomers. The combined effects of amyloid beta oligomer stabilization along with lack of clearance supports our hypothesis that exposure to certain environmental factors can increase the risk of development of AD in individuals who are already genetically susceptible. Since previous research has primarily dealt with examining the two factors separately, our results ultimately contribute to our understanding of the interactions between identified susceptible genes and environmental factors implicated in the cause of AD.

VIRTUAL LIVER: ESTIMATING Proliferation AND APOPTOSIS OF HEPATOCYTES EXPOSED TO ENVIRONMENTAL CHEMICALS USING TOXCAST DATA.


The US EPA’s ToxCast™ program has screened over a thousand chemicals for po- tential toxicity using hundreds of high-throughput, in vitro assays. The US EPA’s Virtual Liver (v-Liver™) is a cellular systems model of hepatic tissues that enables the estimation of in vitro effects using in vitro data by bridging multiple levels of bi- ological organization. A physiologically-based pharmacokinetic model links envi- ronmental exposures to liver dosimetry, while a microdosimetry model simulates the chemical transport through the sinusoids of the hepatic lobule to estimate cell- level concentrations of chemicals and other soluble factors. Finally, an agent-based model of hepatic cells is used to quantitatively estimate cellular decisions – e.g., apoptosis and proliferation – based on the perturbation of an intracellular signaling network by inputs from the microenvironment. Using the literature we recon- structed a putative crosstalk network to mechanistically relate hepatocyte G1/S pro- gression and apoptosis involving growth factors (e.g., EGF and HGF) and cy- tokines (e.g., TNFα). Next we related key proteins in this network to a subnet of assays in ToxCast including phosphorylation of H2AZ, H3, and c-Jun. We have developed a novel approach for modeling the dynamic response of individual cell- based agents, called Boolean network Ensembles with Asynchronous Threshold Logic (BEATL), to quantitatively estimate the response of hepatocyte populations using in vitro data. We used this framework to simulate the concentration depend- ent perturbations of key signaling molecules in cell proliferation and apoptosis based on ToxCast in vitro data for environmental chemicals (e.g., PFOA, Imazalil, Bisphenol A, Triclosan) as well as reference hepatotoxicants (e.g., acetaminophen). The model results were used to evaluate potential changes in hepatocyte fate based on chemical-induced concentration-dependent molecular perturbations. This ab- stract does not necessarily reflect US EPA policy.

BIOLoGICAL PROFILING OF THE TOXCAST PHASE II CHEMICAL LIBRARY IN PRIMARY HUMAN CELL COCULTURE SYSTEMS.


The US EPA’s ToxCast research project was developed to address the need for high- throughput testing of chemicals and a pathway-based approach to hazard screening. Phase I of ToxCast tested over 300 unique compounds (mostly pesticides and an- timicrobials). With the addition of Phase II, the library contains 1000 unique com- pounds, including 135 failed pharmaceuticals donated by industry partners, refer- ence compounds known to be endocrine disruptors, carcinogens or reproductive/developmental toxicants, high-production volume chemicals, food additives, cosmetic ingredients, and proposed alternatives to commonly used plasticizers and surfactants. The chemicals were tested in a panel of BioMAP® systems, using complex cocultures of primary human cells to characterize heterogeneous inter- actions underlying angiogenic and inflammatory processes. All compounds were assayed in duplicate at 4 concentrations in 8 cell systems using combinations of endo- thelial cells, peripheral blood mononuclear cells, bronchial epithelial cells, fi- broblasts, keratinocytes and coronary artery smooth muscle cells, under various en- dogenous stimuli. totaling 87 endpoints and over 450,000 data points. ToxCast compounds were classified based on their ability to cause overt cytotoxicity in vari- ous cell types and on their bioactivity profiles when compared to reference com- pounds. Chemicals were identified with activities similar to known inducers of mi- tochondrial dysfunction, microtubule disruptors, cAMP elevators and NFκB pathway inducers. Finally, using the ToxCAST 2.0 platform, we generated the corre- sponding-use compounds and in some cases found to have significantly lower bioactivity, or none at all, at the tested concentrations. In vitro signatures for disrup- tion of embryonic vasculogenesis and induction of tumor angiogenesis derived from ToxCast Phase I data were forward-validated for the Phase II compounds with in vivo developmental toxicity or carcinogenicity data. This abstract does not reflect US EPA policy.

TOXPI PRIORITIZATION AND PROFILING OF 1060 TOXCAST CHEMICALS ACROSS MULTIPLE SECTORS OF TOXICOLOGICAL CONCERN.

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The Toxological Prioritization Index (ToxFIT™) framework was developed as a de- cision-support tool to aid in the prioritization of chemicals for integrated toxicity testing. ToxPi consolidates information from multiple domains—including ToxCast™ bioactivity profiles (a diverse battery of over 700 high-throughput screening assays on multiple platforms), inferred toxicity pathways, exposure pre- dictions, and chemical descriptors—into comprehensive activity scores and multi- variate visualizations representing the contribution of each data domain to overall priority rankings. Here, we refine our methodology for aligning data with specific prioritization tasks, add formal quantification of ranking stability, and compare the ToxPi profiles of the combined Phase-I and Phase-II chemical sets. Considering the entire set of 1060 compounds, we explore prioritization tasks for three sectors of toxicological concern: cancer, developmental, and reproductive. We first link ToxRedDB in vivo data with ToxCast in vitro data to populate ToxPi slices for each sector. Second, multi-sector ToxPi profiles are estimated for the entire chemical set, and the priority (rank) order is estimated across and within sectors. Third, confi- dence intervals for all ranks are estimated. Finally, these priority ranks are evaluated in the context of sector-specific reference chemicals. Analogous to the logic under- lying standard curves or “spike-ins”, reference chemicals for each sector include compounds of low, medium, and high concern. Therefore, an appropriate ToxPi formulation would match the hypothesized reference chemical distribution, thereby giving context to the relative activity of test chemicals. The utility of such a prioritization scheme is that new test chemicals having unknown concern can be evaluated relative to known, reference, compounds, and their ToxPi profiles visual- ize activity in the context of a diverse set of compounds covering multiple chemical classes and proposed modes of action. This abstract does not necessarily reflect U.S. EPA policy.

A GLOBAL GENOMIC AND GENETIC STRATEGY TO PREDICT PATHWAY ACTIVATION OF XENOBIOTIC-RESPONSIVE TRANSCRIPTION FACTORS IN THE MOUSE LIVER.


Many drugs and environmentally-relevant chemicals activate xenobiotic-responsive transcription factors (TF). Identification of target genes of these factors would be useful in predicting pathway activation in vitro and in vivo chemical screening. Starting with a large compendium of Affymetrix files (>2000), we identified gene signatures by comparing the transcription profiles after exposure to TF activators in wild-type
and TF-null mice. The signatures included those regulated by PPARα, CAR, FXR, AhR, Nr2f2, FXR, and glucocorticoid receptor (GR). In addition, we identified sets of genes associated with phenomenon that are linked to liver toxicity/cancer including inflammation and cytotoxicity. Validation was carried out by characterizing the signature gene expression under independent conditions of chemical exposure or perturbation known to alter the TF. By using the sets of signature genes to query the compendium, a number of novel observations were made. 1) PPARα is activated in a number of nulligous mouse models that also lead to liver toxicity and may be linked to steatosis. 2) Nr2f2 is activated by a large number of conditions including environmentally-relevant chemicals, gene mutations, and bacterial infections. 3) GR is activated by a number of conditions including hepatoxic drugs, endoplasmic reticulum stress and systemic damage outside the liver. 4) Reexpression of male-specific signature genes occurred after castration, in GH-defective dwarf mice, during bacterial infections and chemical exposure. Our approach allows for creation of new gene signatures associated with other pathways or phenomenon in the mouse liver that can be tested using the compendium. We are presently using the signature sets to predict pathway activation after chemical exposure in mouse primary hepatocytes. (This abstract does not represent EPA policy.)

**Enhancing Mutagenicity Predictions Using Weight-of-Evidence Approaches.**

E. Ahlberg, C. Hasselgren, C. Yang, J. F. Rathman and S. Boyer.

Predicting the experimental outcome of the Ames test is done routinely within Pharmaceutical companies. In the early drug discovery phase, the high volumes of compounds are often handled using structural alerts or QSAR models. However, as drug development progresses, the need for reliable, more extensive information increases. At this later stage, it is also common practice to qualify impurities and degradants using SAR/QSAR methods. To ensure patient safety, highly reliable assessments are required using all available information in a weight-of-evidence (WoE) approach. The combination of diverse sources of evidence requires a mathematical structure that manages different types of probability expressions. One such example is Dempster-Shafer (DS) theory which deals with measures of “belief” as opposed to probability and can be viewed as an extension of Bayesian statistics. DS also introduces an explicit formulation for the unknown state which can be used to account for the amount of experimental error in an assay or model. In a classification data example, the confusion matrix for the training and/or test data can be used to calculate positive and negative predictive values to estimate probabilities for a prediction. In this study we present a DS WoE method where we combine QSAR modeling, the presence of structural alerts, number of experimentally tested structural near neighbors and their activities. For each prediction, the weights for the type of evidence are set dynamically. This enables an overall assessment based on all underlying information together with an uncertainty score which is unique for each compound. Application of the method on 300 internal compounds show an overall improvement compared to using a simple QSAR method. Specificity increased from 72 to 79%, sensitivity from 33 to 65%, false positive and negative rates decreased by ~10% when using the WoE method. Specificity was essentially unchanged and went from 85 to 84%.

**Computational Toxicology and Longitudinal Risk Assessment of Subchronic and Chronic Atorvastatin Calcium Administration.**

A. G. Ibrahim, F. E. Ibrahim and R. C. Shank.

As our wealth of scientific data becomes increasingly large, data mining algorithms and computational methods are used to discover new patterns of information to gain new knowledge about the progression of human disease, novel biomarkers, and toxicities of novel molecules with limited the extensive dependency on the use of animal models. In this study, computational mathematical algorithms are used to describe the kinetics of altered gene expression profiles of human disease and embryonic and adult stem cell differentiation in response to subchronic and extrapolated chronic administration of atorvastatin calcium (Lipitor®-B). High-resolution mRNA microarray data was obtained at 12 hours, 36 hours, 1 week, and 4 weeks from 11 male subjects with a mean age of 49.0 years +/- 12.5 years post administration of a daily dose of 20mg atorvastatin calcium. The data was used to generate independent differential equations for each microarray dataset for actual time points. Additional genetic profiles were extrapolated using two mathematical strategies: Discrete Random Kutta and Discrete Optimization. The extrapolated sets of genes regulated by atorvastatin were imported into the Ingenuity Pathway Analysis (IPA) software for data and toxicology interpretation.

The IPA Global Network is used as the data source for the construction of all biological pathways. Statistical and biological pathways for toxicity, altered biofunction, and disease were indentified. Using a System of Equations approach, we extrapolated new predicted gene expression data for 6 months, 1 year, and 5 years of chronic atorvastatin calcium administration independently with microarray signatures of the directed differentiation of H1 embryonic stem cell (hESC), active hepatitis C infection, and diabetes mellitus type II to address possible co-madmulation events.
Salmonella mutagenicity. The choice of chemical structures for an external valida-
tion set can greatly influence the performance statistics obtained with the set, po-
tentially leading to bias in the interpretation of and conclusions about a model's
suitability. Critical factors for consideration are numerous, but include the number
of chemicals, the ratio of positive to negative compounds, and the structural fea-
tures that are represented in the validation set compared to the model training set.
The first two criteria can be addressed relatively easily due to the abundance of data
for Salmonella mutagenicity; however, the latter requires a quantitative measure of
structural representation that can be applied to ensure that the test set is appropri-
ate for assessing the performance of a QSAR model based on its domain of applica-
ability. We characterized an established external validation set of over 2000 chemi-
cals using fingerprints for genotoxicity based upon known toxicophores and
assessed the overlap of these fingerprints with the domain of applicability of several
commercial QSAR models for Salmonella mutagenicity. Overall performance sta-
tistics for the models with this data set ranged from 59% to 82% sensitivity and
71% to 83% negative predictivity, and showed a 9% increase in sensitivity when
used in combination. However, within individual models, variation in performance
was observed across different toxicophores, providing a more detailed picture of
each model's strengths and weaknesses from a structural perspective.

Recent advances in systems biology and related scientific fields offer the potential
to fundamentally change the way that chemicals and drugs are tested for their risk
to humans. This new vision of toxicology testing will be based upon human rather
than animal biology and will involve a strong commitment to the 3Rs—replace-
ment, reduction, and refinement of animal use in research and testing. There are
many challenges to fully implementing this vision. Current formal approaches to
validation involve lengthy and expensive processes that require validating in vivo
data against in vitro data. This approach may not be relevant or even feasible for
the new pathways and endpoints being measured. Consequently, applying a one size
fits all approach to validation is not conducive to the rapid incorporation of emerg-
ing science or technology into the regulatory decision-making framework. As new
safety testing evolves, new approaches to demonstrating that a test is reliable and
valid for assessing the performance of a QSAR model based on its domain of applica-
tion advance, there's a concomitant need to demonstrate relevance and reliability
of each predictive model for its intended use such that regulatory agencies, the
regulated community, and the public have sufficient confidence in the decisions made
based on such approaches. Challenges and lessons learned from the application of
computational approaches under REACH will be discussed. Although traditional
method validation processes may not be practical let alone desirable for advanced
molecular screening methods, the OECD QSAR validation principles have helped
to provide a useful framework for characterizing models and their predictions,
thereby promoting/fostering regulatory acceptance for QSARs. Complementary
systematic evaluative procedures have been developed for selecting analogues for
read across of (eco)toxicological and environmental fate data. Recommendations
for adapting these principles and procedures to the Tox21 collaboration of EPA,
FDA, NIEHS/NTDP and NCGC will be presented.

In 2007, a National Academy of Sciences (NAS) report described a new vision and
strategy for toxicity testing in the 21st century based on human rather than animal
biology. This vision could be less expensive and time-consuming and would have
a strong commitment to replacement, reduction and or refinement of animal use in
testing. Unfortunately there seems to have been a general reluctance on the part of
industry and regulators to embrace this new vision and as a result the use of animal
tests continues to dominate regulatory toxicological testing practices. There are
many reasons for this including the long history of use of traditional animal meth-
ods, the familiarity, experience, and comfort level associated with their use, an
under confidence in non-animal methods and issues involved in validating these
methods. Historically, regulatory agencies have been slow to embrace new ideas as
seen by the reluctant adoption of genetic toxicology studies. As stated in the NAS
report "Change often involves a pivotal event that builds on previous history and
opens the door to a new era." The publication of the NAS report might be the "tip-
ping point" for a change but validation of these methods for regulatory use will be
a critical component in ensuring the vision's success. Using a one-size-fits-all ap-
proach to validation will deter the rapid incorporation of this emerging science into
the regulatory framework. As toxicology evolves, our approach to assessing these
methods should also evolve.

A biomarker is defined as a characteristic that is objectively measured and evaluated
as an indicator of normal biologic process, pathogenic process or biological re-
response to a therapeutic intervention. Qualification is a regulatory conclusion that,
within a stated context of use, the results of an assessment with a biomarker can be
relied upon to have a specific interpretation and application in drug development
and regulatory decisions. A biomarker is qualified by regulatory authorities if there
is appropriate data in support of its intended use. Once qualified, the biomarker
can be used without the regulatory agency's need to reconsider and reaffirm its ap-
plicability. The US FDA's CDER Biomarker Qualification Process involves a "fit-
for-purpose"- based evidentiary threshold approach that depends upon the un-
tended context of use in drug development. The biomarker qualification program
is an objective, science-based approach for evaluating the relevance, quality, and relia-
ability of a test based upon its intended use, for example as part of a screening pro-
gram or as a definitive surrogate endpoint in a pivotal clinical trial. It serves as a
model for the discussion of new validation approaches for the incorporation of al-
ternative test methods into a regulatory framework.

EPAs Office of Pesticide Programs (OPP) is building on the ambitious NAS vision
for Toxicity Testing in the 21st Century which calls for a shift toward the avoidance
of significant perturbations of normal cellular pathways in exposed populations by
using cell based assays to measure these perturbations, dose-response modeling or-
ganized around computational systems biology models of the circuity underlying
each toxicity pathway, and in vitro to in vivo extrapolations based on pharmacoki-
etic models to predict tissue concentrations under specific exposure conditions.
OPP's long-term goal is to move from a paradigm that involves requiring in vivo
testing for "every possible adverse outcome" toward a hypothesis-driven paradigm
where in vivo testing is targeted to the most likely hazards and risks of concern.
Thus, rather than taking a one size fits all approach to toxicity testing OPP pro-
poses a progressive, tiered-testing approach that starts with hazard-based hypothe-
ses about the plausible toxicological and fate potential of a pesticide or group of
pesticides based on their physical-chemical properties (e.g., using read-across and
structure activity relationships [SARs] to examine toxicological potential). Existing
exposure and toxicity information is then combined with refined exposure models,
computational toxicological models (e.g., quantitative SARs or QSARs ([Q]SARs]), and diagnostic in vitro assays to narrow requirements for in vivo.
Consistent with this view is the consideration of time and As the science evolves so
too must the evaluation process to "validate" regulatory test methods as the current
one size fits all approach is too costly and time consuming and which compares the
performance of a new test to an existing test which may not actually predict the ef-
ects of concern in human and non-humans well.
A multitude of non-animal assays are conducted before a drug is even given to an animal, much less humans. Lead compounds may be modified to eliminate unwanted effects found in various in vitro and in silico screens. All studies conducted with a drug candidate for human studies are submitted to the FDA: In silico, in vitro, and in vivo. Thus the knowledge base on a new drug candidate can be quite rich before humans have been exposed, in contrast to what is known about most high production volume chemicals. The use of exploratory clinical trials, as described in international harmonization guideline, ICHM3R2, can eliminate drugs that have poor human bioavailability or which don’t reach the intended target in humans. Such exploratory clinical trials are supported with much less animal data than normally needed for first in human studies.

Turning the promise of “Tox21” and “pathways of toxicity” into an animal-free health-protective reality is proving devilishly difficult, well more challenging than had been widely expected. One of the key issues is that while it’s possible to measure the expression of many genes and many biochemical endpoints, the right ones to measure often vary between compounds and model systems. Rather than trying for more discrimination and ever-higher definition, sometimes a lower-power, more overview-level analysis can actually be more helpful. Based on the hypothesis that different assays will have different strengths and complement each other, we have started to integrate data from the ECVM embryonic stem cell test and Zebrafish morphogenesis to give us an overall sense of the in vivo developmental toxicity of new drug candidates. Although still very much a work in progress, this presentation will discuss the current ways we balance the various developmental tox readouts with general cytotoxicity data. Our eventual goal is to develop a formalized and transportable process that can be used across chemical scaffolds and venues, and the only certainty is that the end method will look very different from what we’ve started with.

Despite decades of research into its causes and treatments, breast cancer remains the most common invasive cancer and the second leading cause of cancer mortality for women in the United States. Known risk factors include genetic mutations; birth weight and stature; timing of breast development, childbearing, lactation, and senescence; body fat; and physical inactivity. Some common external, environmental risk factors include radiation, hormone therapy, diet, alcohol, and perhaps smoking and shift work/circadian rhythm disruption. Several environmental chemicals have also been implicated in the etiology of breast cancer. Although toxicological studies indicate potential hazards through endocrine disruption and even some hormone independent mechanisms, strong epidemiological evidence is largely lacking. Animal bioassays for breast and other types of cancer typically assess each chemical independently, and exposure periods usually do not include potentially sensitive windows of susceptibility, such as in utero, the neonatal period, puberty, or menopause. Nevertheless, emerging epigenetic data support the concept that exposure during sensitive early life stages when the mammary gland is developing can result in developmental reprogramming of breast cells, thereby increasing breast cancer risk later in life. Similarly, stimulation of breast cell division later in life by endocrine-active agents may increase mutation rates or promote tumors at a life stage in which DNA repair is waning. The important thing is that this highlights the potential contributors to breast cancer risk in follow up to the 2011 report of the Institute of Medicine Committee on Breast Cancer and the Environment (funded by Komen for the Cure).
Compared to environmental chemicals such as Bisphenol-A, much less attention has focused on other disruptors of endocrine signaling that could significantly impact breast cancer risk. These include 1) zeranol, a myoestrogen used in livestock to enhance meat production, and 2) disruption of circadian rhythm by exposure to light at night. We conducted a cross-sectional analysis among 163 girls, aged 9 and 10 years, participating in the Jersey Girl Study to measure urinary myoestrogens and their possible impact on growth and development. We found that myoestrogens were detectable in urine in a substantial proportion of prepubertal girls (78.5%), and that urinary levels were predominantly associated with beef and popcorn intake. Urinary myoestrogen levels, however, may be associated with altered growth and lower early onset of breast development, factors related to decreased breast cancer risk. Disruption of circadian rhythm by shift work is associated with increased risks of breast and prostate cancer. Our studies in rats indicated that carcinogens also disrupt the expression of circadian-controlled genes (CCG), and that methylestenocysteine resets the expression levels and phase of CCG, including estrogen receptor β. MSC restores rhythmic fluctuations in histone acetylation on the Period 2 gene promoter, suggesting an epigenetic mechanism for MSC-mediated restoration of circadian rhythm. Changes in circadian gene and CCG expression in lymphocytes are being investigated as possible biomarkers of circadian disruption in shift workers. Agents that mediate their effects by epigenetic reprogramming of mammary cells may hence be amenable to chemopreventive agents that reverse these effects.

Nutritional and occupational factors that contribute to increased breast cancer risk during susceptible life stages such as in early life exposures, quantified exposures to multiple potential agents and factors, and focus on single agents. Epidemiological studies typically have not examined breast cancer, high dosing protocols that miss potential windows of susceptibility, endocrine disruption, genotoxicity and animal data, but little evidence confirms chemicals are suspected to pose breast cancer risks based on mechanistic (e.g., estrogen receptor β) effects at relatively low doses. In addition to using more refined techniques to quantify exposures over the life course, epidemiological studies could evaluate potential generic polymorphisms for specific agents (e.g., metabolic enzyme variants for alcohol or smoking) in combination with social or behavioral factors that may affect susceptibility to breast cancer.
The development of intracocular drugs to treat posterior segment disease, such as age-related macular degeneration, presents both advantages and challenges due to the unique aspects of ocular anatomy and physiology. While drug administration within the eye affords the promise of direct and local delivery, the reduction of systemic or local effects. Ocular implants have advantages over more conventional treatment modalities. Treatment approaches for ocular diseases affecting the posterior segment can be limited by the lack of effective delivery of drug. Conventional approaches for treating inflammatory diseases in the posterior segment include topical administration, periocular or intravitreal injections, and systemic administration of therapeutic agents. Several disadvantages to these treatment modalities include the typically low bioavailability following topical drug application, and the potential for increased systemic or local effects. Ocular implants have advantages over more conventional methods of drug administration, including delivery of a constant therapeutic drug level directly to the site of action. Retisert is a non-biodegradable intravitreal implant containing the steroid fluocinolone acetonide, for the treatment of chronic non-infectious uveitis affecting the posterior segment of the eye. The device releases active drug at a predictable rate for approximately 3 years following surgical placement of an implant into the vitreous. As a novel approach to therapy, regulatory testing expectations for non-biodegradable intravitreal implants have not been well established. To support the safety and registration of the Retisert implant, a comprehensive nonclinical program was conducted. This testing strategy was designed to characterize the release of the drug from the delivery system, and determine the chronic pharmacokinetic and toxicity profile of the implant. Additional testing was conducted to establish the biocompatibility of the implant material. Key considerations when developing such an implant include optimal selection of the drug, delivery characteristics and species selection for the nonclinical program. The unique drug delivery profile of this agent with a well-characterized adverse event profile, including cataract formation and increased intraocular pressure and glaucoma has resulted in complexities in regards to the global development and registration of the product. This presentation will highlight some of the key challenges and "lessons learned" with Retisert, and intravitreal implants in general.

S 811 REGULATORY PERSPECTIVES OF CONTEMPORARY ISSUES IN OCULAR TOXICOLOGY.


This presentation will provide an overview of regulatory considerations for the nonclinical development of drugs for ocular disease. Important nonclinical ocular findings will be discussed with respect to regulatory decision making and their implications for further preclinical and clinical evaluation.

S 812 MOLECULAR BASIS FOR PREVENTION OF CARDIOTOXICITY.

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Cardiac toxicity is an increasing concern for chemo, radiation, and gene targeting therapies. The overlapping molecular pathways of cardiac protection versus cancer cell growth, and cardiac energy metabolism versus underlying causes of diseases such as obesity or diabetes pose threats to new drug development against these diseases. Whereas anthracyclines, such as doxorubicin, are well known for inducing cardiotoxicity manifested by arrhythmia and chronic cardiomyopathy, the monoclonal antibody trastuzumab (Herceptin) targeting the epidermal growth factor receptor for cancer therapy induces apoptosis and hypertrophy of cardiomyocytes, leading to dilation cardiomyopathy. Small molecular kinase inhibitors, such as imatinib (Gleevec), cause cardiotoxicity due to induction of apoptosis of cardiomyocytes. While kinase inhibitors and gene targeting therapy have tremendous potential for pharmacological treatment of cancer and other diseases, finding the signaling pathways and genes that can protect against cardiotoxicity is an emerging issue. Thus, in exploration of this important topic, we will discuss the ways in which the heart can be protected from tissue injury by addressing signaling molecules, unique genes, and nutritional factors.

S 813 CARDIOTOXICITY OF CHEMOTHERAPEUTIC DRUGS: CLINIC PRESENTATION AND TREATMENT.

J. Alpert. Medicine, University of Arizona, Tucson, AZ. Sponsor: Q. Chen.

Cardiotoxicity of cancer chemotherapeutic agents has become an increasing concern for cardiologists. These agents can injure the myocardium or vascular system through a variety of mechanisms including: 1) direct toxic effects, such as induction of oxidative stress and apoptosis; 2) alteration of cellular signaling pathways and biochemical metabolism; 3) neurohormonal activation; 4) alteration of ion channels important for the contractile function; and 5) affecting components of coagulation. Notorious examples of these agents include anthracyclines, trastuzumab, imatinib, and 5-fluorouracil. While anthracyclines have been well studied for their cardiotoxic effects through generation of oxidants, small molecule or monoclonal antibody-based tyrosine kinase inhibitors can cause cardiotoxicity despite of the tremendous potential for cancer therapy. Alkylation agents, antimetabolics, antimitotuble agents, and proteasome inhibitors can also induce cardiotoxicity. The clinical presentation of cardiotoxicity differs between the drugs, and includes myocardial ischemia, infarction, hypertension, thromboembolism, arrhythmias, and congestive heart failure. Mechanisms of myocardial injury and the resulting clinical pathological entities produced will be discussed. Various biomarkers, hemodynamic, and clinical endpoints are used in clinical practice in order to monitor patients for cardiovascular complications associated with these therapies. A number of established and experimental preventive and/or therapeutic interventions are employed to combat cardiovascular complications involving these agents.

S 814 CARDIOTOXICITY OF KINASE-TARGETED THERAPEUTICS.


A decade ago, Hoshijima and Chien (JCI, 2002) drew largely theoretical parallels between the dysregulation of the signaling pathways driving cancer and those driving cardiac hypertrophy. On the surface, this statement appeared to stretch the limits of reason, given the fact that cancer cells are known for their proliferative capacity, and adult cardiomyocytes are, except under unusual circumstances, terminally differentiated and incapable of re-entering the cell cycle. However, on closer examination, there are numerous parallels between signaling pathways that drive tumorigenesis, and signaling pathways that regulate hypertrophic responses and survival in cardiomyocytes. Indeed, this issue appears to be at the core of the cardiotoxicity, often manifest as a dilated cardiomyopathy, that can result from treatment with agents typically referred to as “targeted therapeutics”, which target specific protein kinases that are dysregulated in cancer. Herein, we will examine the cardiotoxicity of targeted therapeutics, focusing on the underlying molecular mechanisms, thereby allowing an understanding of the problem, but also allowing the identification of novel, and sometimes surprising, roles played by protein kinases in the heart.
Mitochondrial aldehyde dehydrogenase 2 (ALDH2) is best known for its role in alcohol metabolism. Recent evidence suggests that ALDH2 serves as a natural shield from oxidative stress in the myocardium. We have identified Alda as selective aldehyde dehydrogenase activators. Protein crystallography studies identified the molecular basis of ALDH2 activation by Alda-1. We also found that Alda-1 corrects the structural defect in a mutant enzyme, a result of a single point mutation that is found in 40% of East Asians. We then show that Alda-1 treatment reduces oxidative stress and cardiac injury ex vivo and in vivo. Because of the critical role of ALDH2 in cardiac protection, we asked whether prolonged treatment of nitroglycerin, which inactivates ALDH2, may generate adverse effects in patients at the risk of myocardial infarction. Using an in vivo rat model of myocardial infarction (MI), we demonstrated that sustained nitroglycerin treatment results in increased cardiac dysfunctions following MI. We observed a lower fractional shortening and increased myocardial injury at 3 days and 2 weeks after MI in rats subjected to sustained nitroglycerin up to the ischemic event. Concomitant treatment with Alda-1 reduced nitroglycerin-induced increase in infarct size and rescued cardiac function after MI as measured up to 2 weeks after infarction. These data suggest that activation of ALDH2 could protect from nitroglycerin-induced increase in cardiac damage. The potential clinical implications of our data on the role of ALDH2 in acute and chronic oxidative stress of the myocardium will be discussed.

Oxidative stress plays an essential role in cardiotoxicity of chemotherapeutic agents. Whereas low to mild doses of oxidants provoke endogenous defense, high dose or prolonged oxidative stress induces death or hypertrophy of cardiomyocytes, contributing to the dilated cardiomyopathy and heart failure. Increasing endogenous antioxidant defense and inhibiting hypertrophy or apoptosis are expected to reduce cardiotoxicity. Nrf2, a transcription factor regulating the expression of a cluster of antioxidant and detoxification genes, can be activated by low to mild doses of oxidants and a number of natural products. In cardiomyocytes, oxidants cause rapid elevation of Nrf2 protein due to de novo protein translation. Recruitment of specific RNA binding proteins to 5'-Untranslated Region of Nrf2 mRNA contributes to stress induced de novo Nrf2 protein translation. Knocking out the Nrf2 gene results in a loss of cardiac protection in mice. Nrf2 overexpression prevents hypertrophy but not apoptosis of cardiomyocytes. Complementing the protective effect of Nrf2, we found that glucocorticoids inhibit apoptosis of cardiomyocytes and reduce cardiac injury in mice. A key molecule mediating the protective effect is Glucocorticoid Induced Leucine Zipper (GILZ), a cytosolic protein not capable of binding to DNA. When overexpressed in cultured cardiomyocytes, GILZ inhibited death induced by inducing apoptosis. Mechanistic studies found that GILZ interacts with HIF-1α and stabilizes bcl-xL protein. Since a number of dietary supplements induce Nrf2 and GILZ is inducible by steroids, our findings provide a hope for reactivation of HIF-1α and M. A. Dobrovolskaia. "SAIC-Frederick, Frederick, MD."

Nanotechnology holds great promise for targeted drug delivery. Unique chemical and physical properties of nanomaterials may lead to improvements in delivery technology in several ways. Examples include increasing the solubility of poorly soluble drugs, increasing efficacy and safety by delivering drugs directly to diseased tissues, and potentially decreasing costs by achieving therapeutic efficacy despite administration of lower doses of drugs. Many publications have discussed the toxicology of environmental and occupational nanomaterials, but more information is needed regarding nanoparticles designed for parenteral administration. These nanoparticles may present unique issues due to recognition by the immune system and downstream effects on adaptive immunity. Interactions with the immune system may result in premature clearance before payload delivery, disseminated intravascular coagulation-like toxicities, inflammation, anaphylaxis, and decreased resistance to infection or tumors. Nanoparticles are a very broad and diverse class of biomaterials, and there are significant gaps in our knowledge of the mechanisms by which nanoparticles interact with the immune system. Recognition by immune cells is influenced by many factors, including direct interaction of nanoparticles with red blood cell proteins as well as proteins of the complement and coagulation systems. Therefore nanomaterials may need to be screened for hematocompatibility and complement activation prior to preclinical in vivo studies. Investigation of nanomaterial effects on the function of immune cells, such as phagocytes and lymphocytes, is also important, and development of in vitro assays that are predictive of in vivo observations is critical. Further work is needed to elucidate which chemical and physical properties of nanoparticles are responsible for specific interactions with immune system proteins or cells, and understanding these interactions may allow exploitation of nanoparticle properties to ensure both safety and efficacy of nanomedicines.

Chemical and pathogenic stresses induce accumulation of the Hypoxia-Inducible Factor (HIF)-1α in the cells. When translocating to the nucleus, HIF-1α dimers with HIF-1α and interacts with cofactors to assemble the HIF-1 transcriptional complex. HIF-1 regulates myocardial cell function under hypoxic conditions via a series of shifts in metabolic pathways, such as increasing the expression of cytochrome c oxidase subunit COX-2, causing a switch from COX-1 to COX-2 and therefore adaptation of mitochondrial complex IV to the hypoxia conditions. The induction by HIF-1 of pyruvate dehydrogenase kinase 1 shunts pyruvate away from the mitochondria. HIF-1 triggers mitochondrial selective autophagy by induction of BNIP3. HIF-1 also induces microRNA-210, which blocks assembly of Fe/S clusters that are required for oxidative phosphorylation. These adaptive changes induced by HIF-1 protect the heart from the environmental and endogenous insults. HIF-1 is a rate-limiting step in the activation of gene, since it regulates the binding of HIF-1 to the HRE and formation of HIF-1 transcriptional complex. Copper loss from the heart occurs under cardiac hypertrophy and ischemic conditions, leading to accumulation of HIF-1α without activation of HIF-1 activity. The detrimental versus beneficial effect of HIF-1α accumulation during copper deficiency in cardiac diseases will be discussed.
a first step to investigate the use of LPNPs as novel drug delivery devices, their integration into a combined human immune system has been explored. The complement system is the most important biochemical cascade in the blood for the recognition, opsonization and killing of foreign materials. This presentation covers general knowledge on complement system to the state of the art on complement activation by other nanomaterials. The last part of the talk is focused on presenting the first systematic study of the blood biocompatibility properties of lipid-polymer nanoparticles functionalized with methoxyl (OCH3), amine (NH2), and carboxyl (COOH) functional groups. Our results show that LPNPs –OCH3 generate negligible levels of complement activation, while LPNP-NH2 induced the highest complement activation among carboxyl and methoxyl groups. None of these nanoparticles activated the coagulation cascade. In general, lipid-polymer nanoparticles functionalized with these three functional groups present good biocompatibility profiles in comparison to Zymosan, a well known activator of the complement system. The main contribution of this work is the creation of an effective and practical method to modulate the levels of activation of the complement system via the interaction of different functional groups on nanoparticle's surface.

821 RECOGNITION OF NANOPARTICLES BY MACROPHAGES—FROM PRINCIPLES TO CONSEQUENCES AND TOXICITY.

A. A. Shvedova1, 2, 1PPRB, NIOSH, Morgantown, WV and 2West Virginia University, Morgantown, WV.

Engineered nanomaterials (EN) have unique physico-chemical properties that make them promising for many biomedical applications. However, with the burgeoning capabilities to manipulate structures at the nano-scale, employing this machinery for safe and efficient drug delivery is not fully explored. To this end recognition of EN by the immune system, our primary defense against foreign invasion is a critical point. Recognition versus non-recognition of EN by the immune system not only determines the distribution of nanomaterials in the body but may also dictate their toxic potential. Recent studies showed that autophagy may have emerged as the initial and primordial defense of eukaryotic cells against microbes. It is therefore not surprising that immune-competent cells may respond to EN in a similar manner as to viruses/bacteria. Consequently, there are complex relationships between the infection process and inflammatory responses to EN resulting in potent effects of nanoparticles on pulmonary clearance of bacteria. Elucidation of how EN impact the conserved mechanism of autophagy, recognition and/or phagocytosis promise to be an interesting and fruitful area for better understanding of interactions of EN with the cells of innate immune system, particularly macrophages. It becomes clear that the presence of specific recognition patterns primarily defined by the EN size and charges are essential for their recognition and uptake by macrophages. Further, the presence of specific signals on the surface of EN, such as adsorbed lipids or proteins, confers additional features to the effectiveness of the recognition of nanoparticles by professions phagocytes. Finally, oxidative stress - known to act as an underlying mechanism that drives the toxicities of EN in vitro as well as in vivo – may be triggered as a macrophage response to recognized nanoparticles. Overall, the mechanisms of recognition, cellular internalization of EN by immunocompetent cells, particularly macrophages, represent an important new field of molecular nanotoxicology.

822 DEVELOPMENT OF CYT-6091 (AURIMUNE®): A MODEL CANCER NANOMEDICINE.


The use of nano-sized drug delivery systems to target potent, but toxic anticancer therapeutics to solid tumors is best accomplished by avoiding the drug’s uptake by the immune system and by limiting its biodistribution. Binding recombinant human tumor necrosis factor alpha (TNF) to the surface of 27 nm PEGylated colloidal gold particles (CYT-6091) meets these objectives. Each component serves a specific function. The gold nanoparticles limit biodistribution, while PEGylation prevents immune detection. TNF serves to localize the nanoparticle to the tumor and causes vascular disruption of the tumor blood supply. Clinically, an IV injection of 0.6 mg/m2 (>1 mg TNF per dose), and does traffic to tumors, limiting the drug’s biodistribution, reducing systemic toxicity and potentially bringing more drug to the site of disease. Further, since an intact tumor vasculature is critical for nanomedicines’ targeting tumors, the treatment of solid tumors should begin with a nanomedicine prior to surgery, even for resectable tumors.

823 CASE STUDY: INTERACTION OF DEXTRAN NANOMATERIALS WITH THE IMMUNE SYSTEM—IN VIVO AND IN VITRO STUDIES.


While nanomaterials made of biocompatible polymers hold great promise as drug delivery platforms, biological consequences resulting from interaction of nanomaterials with the immune system have not been fully elucidated. Dextran is well established as a biocompatible polymer with extensive use as a plasma expander. Hydrophobic derivatives of dextran that were developed to enable manufacture of drug-polymer nanoparticles, elicited severe toxicities in rat in vivo studies. Results of these studies suggested that the dextran derivatives were being recognized by the immune system and were capable of activating macrophages and the complement cascade. In vitro assays were used to screen for immune system activation and after further modifications to the polymer, dextran derivatives were identified that showed improved safety profiles in subsequent in vivo studies. These screening assays have the potential to facilitate selection of materials to minimize unwanted interaction with the immune system and to aid in the rational design of nanoparticle drug delivery systems.

824 TOXIC CELL-DEATH: SIGNALING PATHWAYS, CROSS-TALK, AND HIGH-THROUGHPUT ANALYSIS.

S. Orrenius1 and W. Slikker, Jr.1, 1Karolinska Institutet, Stockholm, Sweden and 2US FDA/NCIT, Jefferson, AR.

Cell death is the ultimate result of toxicity caused by damage to critical cell functions and/or activation of death signaling pathways. Toxicants can trigger multiple modes of cell death (apoptosis, necrosis, necroptosis, and autophagic cell death) with distinct morphological and biochemical characteristics. In fact, several cell death modalities may coexist within the same lesion with cross-talk between them. To address this important topic, we will begin by discussing the role that cell death plays in toxic insult and disease, and how improved knowledge of cell death signaling pathways and mechanisms will help us understand how toxicants might interfere with cell viability and function. After the description of various cell death modalities, and the possible cross-talk between them, mechanisms of apoptotic cell death will be described. Apoptotic cell death is a highly regulated process, which is involved in removal of dead cells in various developmental processes, and is crucial for maintaining tissue homeostasis. Further, the mechanism of action of certain chemotherapeutic agents and fungal toxins will be discussed to illustrate the role of sphingolipid signaling molecules in cell death and disease. Finally, a molecular epidemiology approach using novel technologies to assess cell death and environmental impact in individual cells and in human populations in a high-throughput manner will be presented. The program will cover important toxic mechanisms in multiple target organs and will hopefully contribute to a better understanding of the role of cell death mechanisms in toxic insult and disease.

825 MODES AND PATHWAYS OF TOXICANT-INDUCED CELL DEATH.

B. Zhivotovsky. Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.

Cell death is the ultimate result of toxicity. It is now apparent that during toxicity multiple cell death programs, i.e. apoptosis, necrosis, necroptosis and autophagy, can be activated. Some of them are shared by different tissues; others are tissue-specific and are linked to particular functions. Elucidating the signaling pathways regulating various modes of cell death, is highly relevant for our understanding of the
cellular targets and mechanisms of action of toxic agents. Such knowledge is also important in design of less toxic analogs. Further, using modern methodological approaches, (e.g., genomics and proteomics), knowledge of cell death mechanisms will help us understand how toxicants might interfere with cellular regulation of gene transcription and protein expression and function.

**PATHWAYS TO ANESTHETIC-INDUCED BRAIN CELL DEATH AND TO NEURONAL PROTECTION.**

**W. Slikker**, National Center for Toxicological Research/US FDA, Jefferson, AZ.

It is estimated that 2.3 million ambulatory anesthesia episodes of care were provided in the US to children younger than 15 years. Although comprehensive studies have yet to be reported in humans, data from five species including nonhuman primates indicate that prolonged anesthetic exposure of developing mammals at critical stages of development results in brain cell death and lasting cognitive deficits. One pathway to toxicity involves the N-methyl-D-aspartate (NMDA)-type (glutamate) receptor system. Blockade of the receptor by ketamine results in selective cell death via a pathway that involves a compensatory up-regulation of subunits of NMDA receptor. Specific subunits that are up-regulated as indicated by gene-expression studies and validated by Q-PCR include the NR1 and the NR2C. Initiation of a cell death cascade begins with Ca2+ influx and is postulated to produce an increase in reactive oxygen species. Several recent studies have indicated that reduction of oxidative stress may protect the developing animal from anesthetic-induced brain cell death. Evidence for the role of oxidative stress in anesthetic-induced neurotoxicity has been generated in studies that apply oxidative stress blockers, including L-carnitine (mitochondrial protector) and melatonin, in vitro, and specific antioxidants, in vitro, including the superoxide dismutase mimetic, M40403 and the NOS inhibitor, 7-nitroindazole. Recent gene expression assessments indicate that genes in the oxidative stress pathway are altered by anesthetic treatment of developing animals. Together, the application ofomics approaches along with traditional toxicological endpoints indicates that oxidative stress plays a critical role in the susceptibility of the developing brain to anesthetics. Prevention of neuronal death in studies with oxidative stress blockers have indicated that reduction of oxidative stress may provide protection from anesthetic-induced neuronal cell death during development.

**DIFFERENTIAL PATHWAYS OF CELL DEATH BY LEAD AND NEUROPROTECTION BY BCL-XL IN PHOTORECEPTOR SYNAPTIC AND NONSYNAPTIC MITOCHONDRIA.**

**D. A. Fox**, College of Optometry, University of Houston, Houston, TX.

Mitochondria are critically involved in the regulation of energy metabolism and apoptotic cell death in tissues, including the retina. Rod and cone photoreceptors have two mitochondrial compartments: inner segments (IS) that are a primary target for chemical-induced retinal degeneration, and synaptic terminals (ST). This talk will highlight the similarities and differences in these targets following postnatal lead exposure in wild-type and transgenic mice overexpressing Bcl-xL in photoreceptors. Lead produced rod-specific apoptotic activation by activating the cytochrome c-caspase cascade in rod ISs, which was blocked by Bcl-xL. In contrast, lead produced mitochondrial swelling and altered ultrastructure in both rod and cone STs that was not blocked by Bcl-xL. The results reveal the importance of compartmental analysis and differences between IS and ST mitochondria in rods and cones, and their differential sensitivity to apoptotic stimuli. They indicate that persistent structural- and visual functional- alterations of cone and rod ST remain even after rod apoptosis is blocked.

**CERAMIDE, SPHINGOID BASES, AND SPHINGOID BASE METABOLITES AS LIPID MEDIATORS IN SIGNALING PATHWAYS LEADING TO CELL DEATH AND DISEASE.**

**R. T. Riley**, Toxicology and Mycotoxin Research Unit, USDA-ARS-SAA, Athens, GA.

Increased ceramide generation de novo is a known to be involved in the mechanism of action of many chemotherapeutic agents and conditions which disrupt cell cycle progression and induce cell death. Conversely, the metabolism of ceramide to sphingoid bases and sphingoid base 1-phosphates has been implicated in cell survival and inhibition of cell death. Complicating this picture is the fact that fungal toxins that inhibit ceramide biosynthesis and induce elevated levels of sphingoid base 1-phosphates also induce increased cell death and cancer in animal models. In addition, elevated levels of sphingoid base 1-phosphates may disrupt signalling via disruption of sphingosine 1-phosphate (S1PR1-10) signaling. This presentation will provide an overview of sphingolipids as mediators of regulatory processes involved in cell growth, differentiation, cell migration, vascular regulation and cell death.

**MEASURING CELL DEATH AND GENOTOXICITY IN SINGLE CELLS AND HUMAN POPULATIONS USING LAB-ON-A-CHIP TECHNOLOGIES.**

**M. T. Smyth**, School of Public Health, University of California Berkeley, Berkeley, CA.

The Center for Exposure Biology at UC Berkeley has developed high-throughput single cell analyses using Lab-on-a-Chip (LOC) technologies. These methodologies, which include transcript translocations and point mutations linked to lymphoma. Recent work has led to the development and application of a novel microfluidic device that enables quantification of RNA transcripts and protein levels in parallel, expanding our analytical capability beyond DNA mutations. This device enables on-chip culture and analysis of gene expression, surface proteins and cell death in single cells. Use of novel geometry on the device has facilitated fluorescence detection of immunoassay results followed by real-time RT-PCR assay of mRNA for each individual cell. This scalable device performs multiple assays in parallel to achieve the necessary throughput for studies of the effects of environmental exposure on cell death and other parameters in single cells.

**CONCEPTS CRITICAL TO THE NEXT GENERATION OF HUMAN HEALTH AND ECOLOGICAL RISK ASSESSMENT.**

**R. Gentry** and **B. Lovsey**, ENVIRON International Corporation, Monroe, LA and ARCADIS, Novi, MI.

Through the collaborative effort between the SOT Risk Assessment Specialty Section and the SETAC Human Health Risk Assessment Advisory Group, this session was developed to highlight the challenges currently facing the next generation of risk assessors. With the release of the recent National Academy of Sciences reports on toxicity testing that present a vision for movement from in vivo testing to in vitro and in silico testing, as well as the most recent changes in risk assessment guidelines by regulatory agencies, risk assessor are faced with the challenge of integrating innovative data (e.g., genomics) into the current risk assessment paradigms or with the development of new paradigms or methods to address changing issues in risk assessment. In considering all the biological changes and scientific information, many of these new methods attempt to integrate all of the available scientific information of a kind of risk model that is able to inform both human health and ecological risk assessment/risk management decisions. Our panel of experts will provide information on new programs and approaches within regulatory agencies, as well as in the private sector, that will be important in the next generation of risk assessment.

**MORE EFFICIENT AND EFFECTIVE TESTING AND ASSESSMENT PARADIGM FOR CHEMICAL RISK MANAGEMENT.**

**S. Bradbury**, US EPA, Washington, DC.

A major challenge confronting chemical risk management is to address the potential ecological and human health risks for large numbers of chemicals with greater speed and accuracy using fewer resources. From a strategic and tactical viewpoint, an integrated approach that relies on existing knowledge (including mechanistic information of both chemistry and biology from novel computational toxicology) is needed to advance progressive and focused assessment strategies, as well as to advance the utility of risk assessment by providing more relevant information. The use of “Integrative Approaches to Testing and Assessment” is a means to efficiently use existing exposure and toxicity information combined with (Q)SAR predictions and in vitro data in a systematic and coordinated manner for a chemical or a group of similar compounds. Fundamental to using an integrative strategy is an understanding of adverse outcome pathways which provides a biological construct of key events at the different levels of biological organization causally linked to an in vivo endpoint of regulatory interest. By using a battery of models and in vitro assays, the potential of chemicals to initiate molecular interactions can be efficiently evaluated. At each level of biological organization, the potential of chemicals to elicit toxicological effects can be reined as necessary, in the context of likely exposure. This tiered approach leads to a reduction in the amount of testing at each higher level of biological organization. Thus, instead of every chemical being tested for every pos-
credible endpoint in a battery of in vivo studies, only those studies that are rational for the chemicals’ toxicological potential are required. This presentation will describe ongoing efforts to improve and transform approaches for human health and ecological assessment within the Office of Chemical Safety and Pollution Prevention.

**W 832 MABEL: USE OF PRECLINICAL DATA TO SET ACCEPTABLE STANDARDS FOR EXPOSURE.**


Pharmaceutical and personal care product (PPCP) ingredients and other emerging compounds are increasingly detected in water potentially intended for human consumption, and water industry and risk assessment professionals are faced with the need to efficiently establish Acceptable Daily Intakes (ADIs). The growing availability of modern molecular data from chemical exposure studies for agents’ mechanisms of action presents a unique opportunity to explore their use in developing screening levels for these compounds. We investigate the use of the Minimum Anticipated Biological Effect Level (MABEL)—the lowest dose with any measurable effect on biological systems—as a point of departure for deriving ADIs for emerging compounds, particularly PPCPs, in drinking water. Drug industry experience has demonstrated that, for certain types of drugs, “safe” doses identified using traditional methods (e.g., using no observed adverse effect levels, based on toxicological endpoints) may have greater uncertainty than desired; the MABEL is currently being applied in the EU to reduce uncertainties in establishing safe exposure levels for drugs undergoing clinical testing. We characterize data requirements for applying the MABEL methodology to deriving screening levels for PPCPs in drinking water, and evaluate case studies to compare values to those derived using traditional methods (e.g., extrapolating from toxicological endpoints). Based on these findings, we discuss advantages and potential challenges, and propose directions for future research. Results suggest that the MABEL approach offers a transparent means to establish ADIs based on scientific evidence rather than extrapolation modeling, thereby decreasing the uncertainty about the protectiveness of screening levels, and offers a potential means to deriving screening levels for mixtures of compounds with the same or similar modes of action.

**W 835 MIXTURES RISK MANAGEMENT: MOVING BEYOND TEQS AND HAZARD INDICES.**

P.S. Price. Dow Chemical Company, Midland, MI.

The human health and ecological risks posed by cumulative exposures to chemicals, including exposure to mixtures, have traditionally been investigated using additive models. These models include screening models such as the Hazard Indices, systems of TEQs that are based on a common mechanism of action, and Toxicity Units that are based on empirical measurements of acute toxicity endpoints. More recently the IPCS/WHO have proposed a tiered framework of modeling approaches for the evaluation of mixtures. This framework proposes the use of both screening assessments and higher tiered approaches that consider detailed mechanisms of joint action and or use of probabilistic models of toxicity and exposure. The European chemical industry (Cefic) has developed a Decision Tree approach that extends the IPCS/WHO framework to include a determination of the value in performing a cumulative assessment. These new tools provide increased flexibility in the evaluation of risks from mixtures. They allow the use of screening evaluations that can be performed with minimal data and describe how additional data can be used to refine the screening assessments. Finally, the new tools allow the identification of specific groups of chemicals and specific populations where cumulative risk assessments are most needed.

**W 836 USING TRANSCRIPTOMIC DATA IN THE RISK ASSESSMENT PARADIGM.**

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

Current challenges facing chemical risk assessment are the time and resources required to meet the data standards necessary for a published assessment and the incorporation of modern molecular biological information. The integration of transcriptomic data into the risk assessment paradigm may address both challenges by providing an efficient means to quantitatively and comprehensively evaluating the molecular changes resulting from chemical exposure. To assess the value of applying transcriptomics in risk assessment, a series of rodent studies were performed across five chemicals that were positive for lung or liver tumors in a standard cancer bioassay. The target tissues were analyzed for traditional histological and organ weight changes and transcriptional changes using microarrays. The dose-response changes in gene expression were analyzed using standard benchmark dose methods that have been commonly employed in risk assessment. The individual genes were then grouped based on known canonical signaling pathways and the pathway-based responses were used as points-of-departure. A comparison of the transcriptional benchmark dose values with those for the traditional noncancer and cancer apical endpoints showed a high degree of correlation for specific signaling pathways. Many of the correlated pathways have been implicated in noncancer and cancer disease pathways. The results demonstrate that transcriptomic changes in signaling pathways can be used to estimate noncancer and cancer points-of-departure for use in quantitative risk assessments.

**W 837 ADVERSE OUTCOME PATHWAYS AS A UNIFYING CONCEPT IN ENVIRONMENTAL TOXICOLOGY.**

K. Crofton. US EPA, Research Triangle Park, NC.

An adverse outcome pathway (AOP) is a subset of a source to outcome pathway that delineates documented and testable linkages between a molecular initiating event and an adverse outcome at the individual or population levels. AOPs, by definition, span multiple levels of biological organization. The amount of detail and linearity characterizing the AOP between a molecular initiating event and an adverse outcome within an AOP can vary substantially, both as a function of existing knowledge and risk assessment needs. Development of AOPs can provide insight into the uncertainties in linking chemical use, exposure and outcome, thereby focusing research on critical data needs. Well defined AOPs provide utilitarian framework to build quantitative and qualitative models useful in: understanding the degree of perturbation associated with adverse outcomes, target for the development and use of efficient chemical testing methods, and extrapolation between species and lifestages. The development and use of AOPs can provide a framework in which high-throughput and high-content information informs more efficient and predictive risk decisions.

**W 838 BRIDGING THE GREEN CHEMISTRY GAP BETWEEN PRODUCT DISCOVERY AND AVAILABILITY.**

A. A. Li and L. Zeise. 1 Exponent Health Sciences, San Francisco, CA and 2 Cal/EPA OEHHQ, Oakland, CA.

Recent government and industry initiatives seek to identify chemicals of toxicological concern in products to reduce their use or replace them with safer chemicals. At the same time, sustainable approaches to product manufacture and use that account for energy consumption, product life cycle, and societal benefit are being emphasized. This creates the challenge of developing sound scientific approaches for identifying important chemical hazards and evaluating alternative, safer chemicals or products, all done within the context of risk and benefit trade-offs. Two laws recently passed in California (Chapters 559 and 560, Statutes of 2008) direct the state’s implementation of such green chemistry strategies. The US government, several states and cities, and industry are similarly exploring approaches for alternative analyses that can include hazard, risk, environmental, life cycle, and carbon impact assessments. A primary goal is a rapid and streamlined review and assessment to ensure the availability of safer and more effective products for the consumer and general public, at reduced costs and with minimal environmental impacts. Our panel of regulators and scientists from different sectors will explore approaches that can be used to implement green chemistry goals. It begins with a government perspective on challenges of development and implementation of legislation that will be effective, enforceable, and practical. This session features toxicity methods and case studies for hazard and risk-informed screening strategies utilizing high-throughput data, structure activity, and other toxicity information. It also considers how toxicity assessments can be utilized together with evaluations that account for carbon footprint and other impacts of product manufacture, use, and disposal for green chemistry decision making. At the conclusion of this session, two experts will lead a panel discussion and provide their expertise in exposure and risk assessment, regulatory policy decision-making, and occupational clinical medicine.
provide a framework for alternatives analysis to address chemicals of concerns in consumer products. The goal for implementation has been to have approaches in place to guide practical decision-making near term and that address the objectives of the legislation, while maintaining the flexibility to make adjustments as the science and best practices evolve. Exposure to chemicals of concern occurs either through direct product use or environmental pathways. Identification of these chemicals is in part hazard-based, driven for example by classifications by authoritative institutions. The data for “safer” substitutes can be considerably less than for chemicals of concern posing challenges for comparative analyses. Another challenge is weighing sustainability aspects such as life cycle impacts and assessing the carbon footprint associated with product manufacture, use, recycle and disposal. The presentation will share insights gained as Germany has begun to consider alternatives assessment approaches and other aspects of implementing the green chemistry legis-

RI 838 CALIFORNIA’S HAZARD TRAIT FRAMEWORK AND OTHER TOXICOLOGICAL CONSIDERATIONS FOR GREEN CHEMISTRY DECISION-MAKING.


In recent California legislation, green chemistry decision making is initiated by the identification of chemicals of concern. A key consideration for identification is a chemicals’ “hazard traits,” chemical properties that may contribute to adverse effects in exposed humans or the environment. The legislation calls for specifying hazard traits, toxicological and environmental endpoints, and any other relevant data to be included in a Toxics Information Clearinghouse to aid green chemistry evaluations. This talk describes the approach developed to implement this piece of California green chemistry statutes. As a way of organizing hazard information and evaluating evidence, California has developed a hazard trait framework, comprised of four major categories of hazards – toxicological, environmental, exposure potential and physical. Within each of these, a number of hazard traits are identified. There are 19 toxicological hazard traits, those of the major disease “aticies” (e.g., carcinogenicity, cardiovascular toxicity) and traits that can be linked mechanistically with underlying causes (e.g., genotoxicity, reactivity in biological systems). Beneath each toxicological trait are endpoints that are manifestations of that trait, and other relevant data that provide less direct evidence. The framework provides a construct for considering whether evidence is sufficient or suggestive of a particular toxicological hazard trait. Besides hazard information, other types of data can be important in evaluating possible chemicals of concern and alternatives. This includes measures of cancer and non-cancer activities (e.g., cancer and non-cancer benchmark doses, potencies). A challenge for use of the framework in green chemistry evaluations is that the data for possible “safer” substitutes can be considerably less than for chemicals of concern. In vitro and other non-apical data may provide evidence of hazard, and have been used by the industry to develop “greener” products.

RI 839 ALTERNATIVE ASSESSMENT STRATEGIES FOR PRODUCT DEVELOPMENT FOR CHEMICALS REGULATED IN EUROPE: 3 CASE STUDIES.


Toxicological screening strategies are applied to detect potential health hazards or risks early in product development and direct it towards products posing lower risk or hazard to workers, consumers and the environment. The very screening strategy must be tailored to fit the regulatory and scientific basis as well as the intended use of the respective material under investigation as illustrated by three case studies: (i) For pesticides European regulations define cut-offs based on hazards and screening trials to identify critical hazards. A combination of in vitro receptor-binding assays and in vivo metabolomic analysis was successful in identifying potential endocrine disruptors among new candidate compounds. (ii) Cosmetics testing in vitro is limited and shall be banned completely by 2013 in the EU. Complex endpoints - unlike local tolerance - cannot be tested in vitro with adequate accuracy today. Testing strategies based on the in vitro assessment of the uptake of cosmetic ingredients, the exclusion of genotoxic effects and the application of the Threshold of Toxicological Concern concept were, however, able to select cosmetic applications with no or sufficiently low risk. Specifically, sunscreen pigments were brought to market after excluding genotoxicity by in vitro and in vivo studies and demonstrating that they do not penetrate healthy or UV-damaged skin. (iii) For nanomaterials, there is not yet specific regulation under REACH. Classical toxicological methods are generally unable to detect toxic effects of nanomaterials but need some adaptation, especially in test substance preparation and application. Yet, there is also the opportunity to test nanomaterials effectively and efficiently taking their specific material properties into account. Within these testing strategies short-term inhalation studies specifically designed for nanomaterials were a useful tool to select materials with low toxic potency, and to discontinue projects for intended application presenting unclear or unacceptable toxic properties.

RI 840 OPTIMIZING POTENTIAL GREEN REPLACEMENT CHEMICALS—BALANCING FUNCTION AND RISK.

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An important focus of green chemistry is the design of new chemicals that are inherently less toxic than the ones they might replace, but still retain required functional properties. A variety of methods exist to measure or model both functional and toxicity surrogates that could be used in a global green optimization scheme. I present an approach that uses multiple methods including in vitro screening assays, in vitro-based toxicokinetics modeling, QSAR and docking modeling to help rank-order potential replacement chemicals. This ranking needs to account for both differences in inherent toxicity but also differences in properties related to intended use. This approach will be illustrated using data and modeled parameters for a collection of pyrethroid insecticides. These cover a diverse range of chemical structures and differential toxicity (e.g. chemicals in the class hit different ion channel targets and show different rates of detoxifying liver metabolism) and differential pesticidal activity (mainly via different ion channel activities) so provide a good example for testing out “green” optimization approaches. This abstract does not necessarily reflect US EPA policy.

RI 841 WEIGHING MULTIPLE VARIABLES IN IMPROVING THE GREEN PROFILE OF CONSUMER PRODUCTS.

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There are a large number of factors that must be considered in developing greener ingredients and products. These include not only human and environmental safety and environmental fate, but also energy usage during manufacture and product use, disposal considerations, and sustainability of feedstocks, to name a few. Because of the complexity of evaluating such a large number of variables it is important to utilize integrated methods of assessment, such as green chemistry decision making. As a way of weighing the positive or negative impact of changing each variable, current and next generation risk assessment practices can be used to determine human and environmental risk. Life cycle assessment tools are applied to estimate the relative gains/losses each alternative ingredient makes in the manufacture, use and disposal of a product.

RI 842 CROSS-SPECIES EXTRAPOLATION USING PROTEIN SEQUENCE HOMOLOGY TO PREDICT SUSCEPTIBILITY OF NONTARGET ORGANISMS TO CHEMICALS WITH KNOWN MODES OF ACTION.


Cross-species extrapolation has been a prominent challenge in predictive toxicology, both for human health and ecological risk assessment. A common thread that can collectively link species to one another are their genomic and their proteomic similarities or differences. Pharmaceuticals and pesticides are manufactured to act on specific molecular targets to elicit their intended effect. Therefore, through the evaluation of molecular target sequence homology, and the identification of conserved domains within those sequences, it is theoretically feasible to anticipate or predict which non-target organisms may or may not be susceptible to adverse effects from chemicals that act on a given protein target. This presentation will describe an automated algorithm to assess protein sequence homology between species as a means to predict sensitivity to chemicals with known modes of action. Our methodology utilizes data and tools from the National Center for Biotechnology Information database to provide a robust analysis of greater than 240,000 non-target species, with partial or full sequence information, and conduct an in-depth characterization of the sequor each, as well as to have a transparent way of weighing the positive or negative impact of changing each variable. Current and next generation risk assessment practices can be used to determine human and environmental risk. Life cycle assessment tools are applied to estimate the relative gains/losses each alternative ingredient makes in the manufacture, use and disposal of a product.
Work from our laboratory has shown that female killifish (Fundulus heteroclitus) from the chemically impacted Newark Bay, NJ, exhibit signs of reproductive dys-
function, relative to a reference population (Tuckerton, NJ). Our current hypothe-
sis was that the Newark Bay population exhibits a decreased sensitivity of the he-
patic vitellogenin (VTG) pathway to 17β-estradiol (E2), which has resulted in
decreased vitellogenin expression and ultimately, decreased egg production. At the
peak of spawning, Newark Bay females expressed vitellogenin (mRNA and protein)
at lower levels than the Tuckerton reference population. A fecundity study con-
ducted also demonstrated that Newark Bay females produced fewer mature eggs
(11 embryos/female), relative to Tuckerton (140 embryos/female) during spawn-
ing. Average embryo weight and yolk volume in eggs collected from the Newark
Bay population were also found to be decreased by 15% and 25%, respectively, in-
dicating impaired yolk formation. Graded doses of E2 were injected into both
males and females collected from each site and Newark Bay killifish were demon-
strated to have right-shifted dose-response curves for both vitellogenin mRNA and
protein induction by E2. For example, Newark Bay females had significantly lower
levels of VTG protein induction at concentrations of 0.01, 0.1, 1.0 and 100 ng
E2/g (11, 21, 39 and 75% of Tuckerton response, respectively). In addition, cir-
culating 17β-estradiol levels in Newark Bay females (0.26 ng/mL) were measured
to be 8-fold lower than Tuckerton females (2.25 ng/mL). Taken together, we con-
clude that the reduced fecundity in the Newark Bay population is the result of (1)
a refractive VTG pathway (decreased sensitivity) to physiologically relevant of E2,
and (2) a 17β-estradiol deficiency. These studies are the first to report a refractive
VTG pathway, which provides insight into how altered gene-regulation of repro-
ductive genes can manifest population level effects.

**PL 845 CHRONIC TOXIC EFFECTS OF DIETARY TCCD IN ZEBRAFISH.**

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The goal of this project was to investigate the effects of chronic dietary TCCD ex-
posure on global gene expression anchored to histopathologic analysis in zebrafish
by functional genomic, pathologic and analytic chemistry methods. Specifically,
three-month old zebrafish were fed Biodiet starter with TCCD added at 0, 0.1, 1.0
and 10.0% for six weeks, and fish were sampled from each group at 1, 2, 4, and
6 weeks after initiating exposure. Although no significant mortality was found in
TCCD-treated groups, TCCD accumulated in a dose- and time-dependent man-
ner, and the sex ratio between male and female was dramatically changed in 100
ppb TCCD-treated fish. TCCD caused multiple pathologic outcomes in liver, kid-
ney, nose, intestine and ovary of zebrafish, such as depletion of glycogen in liver,
retrobulbar edema, degeneration of neurosensory epithelium, underdevelopment of
intestine, a diminution in the fraction of ovarian follicles containing vitellogenic
ova, etc. Toxicogenomic data analysis showed a number of genes were dysregu-
lated in both TCCD-treated male and female zebrafish that function in lipid me-
tabolism, cholesterol and steroidogenesis, cell death, nervous system development,
 xenobiotic metabolism signaling, endocrine system development. The linkage be-
 tween gene expression profile and pathologies reveal the molecular mechanisms of
sublethal dietary TCCD exposures in zebrafish, and the dysregulated genes can be
potential biomarkers for TCCD exposure in aquatic environments. The results of
the study can be used to assess the potential impacts of environmental TCCD in
the food chain and on health of humans and animals.
Mean plasma sodium levels of surviving fish were the same for control and treatment groups, indicating that osmoregulatory processes were not affected by endotoxin exposure. Results suggest that the threshold for effects of endotoxin (dipotassium salt) exposure on smolts during seawater transition is likely >8 ppm but <12 ppm—far less than the established LC50 of 230-450 ppm for salmonids in freshwater. This finding emphasizes the importance of conducting seawater challenge experiments with species that migrate between freshwater and marine environments before defining chemical toxicity levels.

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The Blesbokspuit wetland system is a former Ramsar site with serious deteriorating conditions. It continues to receive large volumes of effluent water discharge. 1H Nuclear Magnetic Resonance Spectroscopy (1H NMR) is being applied for the first time here to compare toxicant responses in multibiomarkers. The use of Metabolomics is also being linked to and compared with other previous biomarker studies e.g Metallothioneins (MT) and Cytochrome P-450 (CYP). Field surveys were conducted during both the low and high flow seasons of 2008 at 5 sites. Muscle tissues of Resident catfish, Clarias gariepinus and tilapia, Tilapia sparmanni species captured were used for analyses. A transplantation study was conducted in cages at 3 out of the 5 sites during the 2009 high flow with juveniles of laboratory reared tilapia, Oreochromis mossambicus and Chironomids larvae. Whole samples were used. Solvent extraction of metabolites was carried out using methanol, chloroform and water. 1H NMR spectra of each sample was measured at 300MHz on a Varian Unity Inova NMR. The acquired spectra were processed and referenced internally to lactate at 1.3ppm. Metabolites profiling and identification was done using the Chemoxa NMR Suite version 4.6. Principle component analysis of the 1H NMR spectra showed significant differences between the kinetics and Chironomids with more responses found in Chironomids. Also differences in fishes between species and seasons were noted (p<0.05). Responses were higher during the high flow season in both cases. These patterns were also observed during previous biomarker studies. Metabolomics has been used to successfully compare and confirm toxicant response in multibiomarkers. This strategy can be applied within the recommended integrated management framework for this area as well as other catchments.


Polybrominated diphenyl ethers (PBDEs), a class of flame retardants shown to exert neurotoxicity and developmental toxicity, have become an increasing concern over the past two decades as levels in the environment and biota have increased worldwide. Many congeners of PBDEs are classified as bioaccumulative toxins with the ability to biomagnify through the food web. However, little is known about how PBDEs accumulate and are metabolized in lower vertebrates. We exposed northern leopard frog (Rana pipiens) tadpoles to a pentabromodiphenyl ether mixture through their diet (0, 7.1 A, 634 DE-71 ng/g wet mass) for 50 days. At this time, tadpoles in all treatments were fed control food (0 DE-71 ng/g). Elimination of PBDEs was determined during the depuration period by collecting tadpoles on days 8, 14, and 28 days for assessment of body residue tissue. Throughout the 28 day depuration period, tadpoles eliminated over 94% of the ΣPBDEs from their tissues at a rate (tg = 11 days) that cannot be explained simply by growth dilution. To further elucidate the toxicokinetics of PBDEs in frogs, we collected individuals following larval exposure at the beginning and end of metamorphosis, and after ten weeks of growth with no PBDE exposure. During metamorphosis, total body residues per individual did not change, implying little to no elimination. Throughout the ten week depuration period, frogs eliminated 89.7% of the ΣPBDEs from their tissues at a constant rate (tg = 10 days). Ultimately growth dilution. The slope of PBDE elimination in both tadpoles and juvenile frogs was independent of starting concentration and so elimination can be described by first order kinetics. Toxicokinetics of PBDEs in R. pipiens is dependent on developmental life stage as evidenced by the lack of PBDE metabolism during metamorphosis and the difference in half lives of PBDE between tadpoles and frogs. Due to this, developmental life stage, especially for species that undergo metamorphosis, should be considered when determining toxicity of persistent organic pollutants.

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Pesticides accumulate within honey bee hives and concentrate in beecwax, causing bees to experience long term exposures to multiple agricultural chemicals. Standardized toxicity testing methods are often limited to short exposures to foragers, with mortality as an endpoint, neglecting esucial behavior within the hive. The circadian clock coordinates 24 hour rhythms in social behaviors, detoxification, and immune function. Each caste and development stage of bees exhibits unique daily behavioral profiles, and may be uniquely vulnerable to sublethal effects of a given pesticide, potentially impacting the entire superorganism. In particular, the development of circadian rhythms accompanies behavioral maturation of nurse bees to foragers. Neurotoxins such as pesticides may disrupt circadian rhythms, and are likely to desynchronize essential physiological and social systems which are particularly important in coordinating a eusocial behavior. Using a video based Noldus Ethovision behavioral tracking system, we are examining the behavioral responses of queens, drones, and adult bees after chronic exposures to various pesticides singly and in the context of a relevant pesticide mixture. We have developed a method for exposing bees to a range of doses of individual chemicals in wax, in order to calculate behavioral EC50s. Additionally, we are assessing changes in gene expression and enzymatic activity associated with behavioral development, detoxification, and immune responses following chronic exposures. The resulting toxicity values can be used to assess relative risk of each compound to each bee caste, and be used to mitigate the hazards of pesticides to pollinators.

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Bisphenol A (BPA) is now a controversial monomer used in the manufacture of polycarbonate plastics and found in trace quantities in some consumer products and food containers. Unconjugated BPA is a ligand of the estrogen receptor (s). Exposure to BPA has been widely estimated by multiplying the concentration from single urine spot samples by the estimated daily urine volume or creatinine elimination. This approach may be appropriate for chemicals that reach steady state, or when samples sizes are very large, but for BPA, the episodic nature of dietary exposure and rapid absorption and elimination kinetics are not accounted for by the spot sampling approach. In this project, we calibrated a human physiologically based pharmacokinetic model (PBPK) to new BPA blood and urine pharmacokinetic data following dietary exposure obtained in a clinical setting. The resulting model was exercised to estimate the oral exposure associated with single samples from a previously conducted human oral exposure study. The PBPK-based reverse dosimetry approach can be used to calculate oral exposure to BPA with an accuracy of greater than 95%. The approach was then applied to urine biomonitoring data from several large representative studies to estimate distribution of human exposure to BPA, which were consistent with those estimated from 24 hour urine collection studies. Upper bound concentrations of the bioactive form of BPA in blood were orders of magnitude below levels required for activation of estrogen receptors. Combined with adequate data on meal times and urine sampling times, this new approach for estimating human exposure to BPA offers a significant advancement over conventional approaches.

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The objective of this project was to utilize a harmonized physiologically-based pharmacokinetic (PBPK) model for two siloxanes (D4 and D5) to translate expected consumer exposures to internal doses, such as those measured in biomonitoring studies. The 50th and 90th percentile of the daily exposure amounts of siloxanes in personal-care products (hair gel, face lotion, etc) for males and females aged 20-59 years were obtained from an existing probabilistic exposure assessment. These data were the inputs into the human PBPK model of D4 and D5 to simulate...
predicted steady-state blood concentrations of these compounds following dermal, inhalation and aggregate (dermal + inhalation) exposure profiles. Modeling of these exposure data gives us an insight of contributions of the route-specific exposure to the internal dose. The results indicate that about 10% and 2% of the inhaled D4 and D5 dose (respectively) are available for systemic circulation in the inhalation-only scenario. The extensive evaporation of D4 and D5 from the skin surface following dermal application results in less than 1% of the dermally applied dose available for systemic circulation. Our results indicate that although dermal exposure to siloxanes is approximately 50-1000-fold higher than inhalation exposure, the steady-state blood concentrations of D4 or D5 due to dermal exposure is only 2-10 fold higher than that due to inhalation exposure, because dermally applied D4 and D5 evaporate from the skin much more rapidly than they are absorbed. As such, this source-to-biomarker simulation that uses PBPK modeling to characterize route-specific exposures for cyclic siloxanes are critical in interpreting biomonitoring data from consumer populations. This project is supported by Silicones Environmental, Health and Safety Council of North America.

**853 PROBABILISTIC RISK ASSESSMENT OF HUMAN EXPOSURE TO IRON AND STEEL SLAG.**


A probabilistic risk assessment (PRA) was conducted to evaluate the potential for adverse human health effects associated with environmental exposures to iron and steel slags. These materials have a wide range of applications, but are used primarily as construction aggregates. The concentration of 26 metals was characterized in 41 samples of three types of slag, for three size fractions each: 1) processed slag (PS, 0-1 inch), which is the primary commercial product, 2) PS samples screened to <300 μm, and 3) PS samples screened to <75 μm. The bioaccessible fraction for ingestion exposure (Bₐₐ) was also measured for the <300 μm fraction. Chemicals of interest (COIs) were identified through a two-tiered screening process, considering four exposure scenarios: 1) slag on a residential driveway, 2) residence near a slag-covered road, 3) construction worker building road base with slag, and 4) industrial/maintenance worker. Incidental ingestion and inhalation were quantitatively evaluated. Following the screening steps, COIs were limited to CrVI, Mn, and V for residential scenarios only. COIs were found to be concentrated in larger particles, and not readily soluble in gastric conditions. Distributions were developed for soil ingestion rate; inhalation rate; exposure time, frequency and duration; Bₐₐ; BW and PEF. The PRA-calculated risk-based concentrations (RBCs) for each COI and scenario were calculated from distributions of risk/hazard generated using unit concentration endpoints. Only the 95% UCL concentrations of Mn in the largest PS-sized samples exceeded the 90th percentile of the RBC distributions. However, the 95% UCL concentrations of the more biologically relevant size fraction (<300 μm), which is more representative of the fraction that could be incidentally ingested, and the smallest size fraction (<75 μm), which is most representative of the fraction that could be inhaled, were below the Mn RBCs.

**854 A TIERED APPROACH FOR AGGREGATE EXPOSURE ASSESSMENT.**

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The study describes the tiered aggregate exposure assessment methodology followed in the CEFIC-LRI TAGS project, regarding realistic estimation of exposure to substances from multiple sources and routes. The overall TAGS method focuses on the development of guidance and criteria on how an aggregate assessment is to be triggered, the identification of data types/data gaps and quality required, the identification of exposure determinants and modifiers, development of strategies so that the models used are verified and the completion of a range of case studies. As a starting point we performed an extensive review of existing data, models and methodologies, aiming to compile a new overall methodology, adding the necessary elements for an as much as possible realistic exposure assessment. The structured methodology is built upon a computational platform that links existing (e.g. EUSES, SHEDS) and additional in house developed aggregate exposure models, to databases (e.g. Exposac, EXPOLIS), aiming to exposure assessment refinement and data assimilation. Elevation from lower tiers to the higher ones is based on the Risk Characterization Ratio (RCR) derived in each tier; if RCR is higher than one, a refinement of the assessment is required, imposing the use of more refined data (e.g. contamination level distributions instead of worst case estimates), models (e.g. use of more detailed environmental fate models), assimilation of complex data (e.g. biomarkers data and use of toxicokinetic models) as well as Biomonitoring Equivalents for RCR calculation.

The uncertainties embodied in the several stages of the integrated assessment are minimized through Bayesian hierarchical modeling. The applicability of the overall methodology is tested in three distinguished case studies, dealing with classical (benzene) and emerging pollutants (PBDE and BPA).

**855 COMPARATIVE CHEMICAL RISK ASSESSMENT USING BIOMONITORING DATA FROM NHANES: APPLICATION OF BIOMONITORING EQUIVALENTS.**

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Biomonitoring data from the National Health and Nutrition Examination Survey (NHANES) and other large, cross-sectional studies provide powerful data on the levels of chemicals in blood and urine in persons in the general population. These data cannot be interpreted directly in the context of chemical risk assessments that are based on external exposure levels rather than biomarker concentrations. Biomonitoring Equivalents (BEs) are translations of existing risk assessment guidance values such as reference doses or tolerable daily intakes into corresponding steady-state biomarker concentrations, and have now been derived for more than 80 chemicals. BE values were used to examine data from the NHANES program using a hazard quotient (HQ; ratio of biomarker concentration to BE value) approach to provide a risk assessment-based interpretation of the relative levels of detected chemicals in the general population. More than 50 chemicals in the NHANES program with detected blood or urinary concentrations in a proportion of the population can be assessed using available BE values. Median population blood concentrations for chemicals in this group range over nearly four orders of magnitude, while urinary analyte concentrations ranged over nearly three orders of magnitude. However, chemicals with the highest absolute concentrations did not necessarily have the highest HQ values. HQ values did not exceed 1 at the median population biomarker concentration for any analyte. For more than 20 chemicals with derived BE values but non-detectable analyte concentrations in NHANES, the detection limits were nearly always sufficiently sensitive to demonstrate that population exposures remain below current risk assessment-based exposure guidance values. Application of BE values to the screening-level assessment of the NHANES datasets allows comparison of the evaluated chemicals on a risk assessment basis, increasing the utility of the NHANES data for prioritization of research and risk management activities.

**856 AN IN VITRO BIOACCESSIBILITY TEST METHOD FOR THE ESTIMATION OF BIOAVAILABILITY OF ARSENIC FROM SOIL.**

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Accurate assessment of human health risks from incidental ingestion of soil containing arsenic (As) requires knowledge of the relative oral bioavailability (RBA) of As in those soils. RBA can be measured in vivo using animal models, but the cost of in vivo bioavailability tests limits widespread application of this approach for most site investigations. A faster, more economical, yet dependable in vitro method for predicting RBA is highly desirable. One in vitro approach involves measuring the fraction of As that is released from soil into solution under specified extraction conditions. This solubilization fraction is referred to as in vitro bioaccessibility (IVBA). If a strong correlation between RBA and IVBA can be established, then the IVBA method offers a more rapid and less costly alternative to in vivo studies. This study sought to optimize an IVBA method to estimate As RBA in soil for use in human health risk assessments. Phase I we reviewed and summarized the available in vivo RBA data from juvenile swine/monkey studies, and identified 39 candidate test materials for IVBA testing. Phase II investigated the effect of a wide range of experimental variables in the IVBA protocol, and identified three key variables (pH, phosphate, and hydroxyamine hydrochloride concentration). Phase III evaluated various combinations of these key variables in a Latin square design using a selected set of test substrates to identify up to three alternative combinations providing the best IVBA-RBA predictive relationship. Phase IV tested the three most promising extraction conditions on the entire list of test materials. For juvenile swine the best fit model (R²=0.72) is obtained using IVBA measurements at pH 1.5. For monkeys the best fit model (R²=0.75) is obtained using IVBA measurements at pH 7 with phosphate and hydroxyamine. The predictive relationship improves when As mineralogy is included with the IVBA data (R²=0.90 and 0.82 for swine and monkey, respectively).
Polycyclic aromatic hydrocarbons (PAHs) are widely distributed in the environment, and as such, are contaminants of concern when completing human risk assessments. In risk assessment, internal dose of a compound is calculated by multiplying external exposure by an absorption factor. Absorption factors are typically garnered from research done with a single dose. In the case of compounds like PAHs, which cause the induction of enzymes, bioavailability may change after repeated exposure to the compounds. In this study, juvenile swine were orally dosed with a mixture of PAHs in one of four media for 14 days and blood time courses were obtained on day 1 and day 7 of dosing. These time courses were used to calculate area under the curve and from this bioavailability was determined. Different media were incorporated in order to determine if there was different variability observed between media. The media included food, corn oil, artificial soil and historically contaminated soil. Blood samples were also collected on day 14 to determine the formation of micromolecules. A second study was conducted to determine the influence of PAH concentration on bioavailability of PAHs. Bioavailability of compounds in soil are thought to be influenced by soil organic matter content, but this may not be the only factor affecting absorption. In this study, swine were orally dosed with varying concentrations of PAHs spiked into artificial soil. Blood time courses were obtained from these swine and bioavailability was calculated. Preliminary results indicate that bioavailability of PAHs appears to be unaffected by repeat exposure. Also, micronuclei levels do not appear to be changed after repeat exposure. Bioavailability of PAHs does not appear to change as a result of different soil concentrations.

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Arterial baroreflex is one of the body's homeostatic mechanisms that regulate blood pressure (BP) by changing heart rate (HR) and vasomotor tone. Increases in BP reflexively cause HR to decrease, whereas decreases in BP depress the baroreflex and cause HR to rise. As such, baroreflex sensitivity (BRS) is often used as a predictor of impaired autonomic control and cardiovascular disease, and has been found to be
Reduced in various cardiovascular conditions such as chronic heart failure, post-infarction hypertrophy. Given air pollution can alter autonomic nervous system, we hypothesized that exposure to air pollutants would also affect BRS and increase the risk of adverse cardiovascular events. Conscious unrestrained Wistar-Kyoto (WKY) and spontaneously hypertensive (SH) rats implanted with an intraventricular catheter and radiotelemeters were administered increasing doses of phenylephrine (PE-vasoconstrictor) and then sodium nitroprusside (SNP-vasodilator) while HR response, BP, and electrocardiogram (ECG) were continuously measured. This BRS test was done one day before and one day after exposure to either air or 3 ppm acrolein (3hrs). Before exposure, PE caused a dose-dependent increase in BP, which resulted in decreasing HR in WKY rats; SNP caused a dose-dependent decrease in BP and resulting HR increases. SH rats had a similar BRS response however, the reflexive changes in HR due to BP increase/decrease were attenuated at the higher doses. Twenty-four hours after acrolein, BRS was decreased in WKY rats and further blunted in SH rats. SH rats had significantly more arrhythmias than WKY rats during and after exposure to acrolein. Although SH rats had higher baseline BP, there were no other BRS differences between strains. In conclusion, a single exposure to a toxic air pollutant alters the body's ability to reflexively regulate cardiovascular function and predisposes to adverse cardiac events. (This abstract does not reflect EPA policy.)

**862 CHARACTERIZATION OF SOY BIODIESEL EXHAUST AND TOXICOLOGICAL EFFECTS IN MICE.**

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Although biofuel use across the world is increasing, very little is known about possible health effects resulting from biofuel exhaust (BE) from this relatively new source of transportation fuel. The U.S. EPA has instigated an *in vitro* screening approach in rodents to examine whether BE can induce lung and cardiopulmonary responses in healthy and compromised animal models. In our combustion facility, biofuel exhaust is generated by a 0.32 L Yanmar engine driving a 3.8 kW Pramac generator with a constant load of 3 kW. Initial studies tested soy-based biodiesel, either 100% (S100) or a 20% mix with conventional petroleum (S20). Organic solvent extracts of S100 fuel were composed of about 70% methyl esters (e.g. linoleate, oleate) and 15% organic acids (e.g. hexadecanoic). Exhaust from combustion of S100 or S20 was diluted to target concentrations of 0, 50, 150, or 500 μg/m3 as determined by TEOM. Average CO, NO (ppm) at the 500 μg/m3 level were 12.3, 18.7 (S100) and 13.9, 13.2 (S20), respectively, while SO2 and NO2 were not above instrument background (<1 ppm). Female Balb/c mice (8/group) were exposed whole body to these emissions 4 h/ld, for 1, 5, d, or 4 wk (5 d/wk), and necropsied 2 or 24 hr after each of these exposures. Lung inflammation was minimal as neutrophils in BAL fluid were <3% with both S20 and S100 at all concentrations and time points. However, significantly fewer BAL macrophages (62% of neutrophils in BAL fluid were <3% with both S20 and S100 at all concentrations and time points. However, significantly fewer BAL macrophages (62% of BAL macrophages in peripheral blood were higher in mice exposed to summer oropharyngeal aspiration of 50 μg of summer/winter, UF/SFM; and day/night PM in 50 μl Hank’s balanced salt solution (HBSS) or HBSS alone. Results indicate that PM toxicity was significantly dependent on particle size and season and was not influenced by diurnal trends. In general, UF PM elicited significantly more pulmonary inflammation compared to SMF PM regardless of season or time of day. PM size-dependent toxicity demonstrated a more pronounced difference between winter UF and SMF PM compared to summer UF and SMF PM. In contrast, circulating neutrophils in peripheral blood were higher in mice exposed to summer but not winter day UF compared to SMF PM. These findings suggest that particle chemical composition strongly influences PM toxicity, possibly due to seasonal PM sources prevalent in winter compared to summer rather than diurnal impacts. Understanding seasonal impacts on PM-induced adverse respiratory and systemic health effects can lead to more effective targeting of sources for improved control of PM pollution within the SJV and protection of public health.

**863 HYPOTENSIVE AND BRADYCARDIC RESPONSES TO INHALED O3 AND AMBIENT FINE PARTICLES ARE ENHANCED IN RATS ON A HIGH-FRUCTOSE DIET.**

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People with diet-induced cardiometabolic disorders (diabetes, hypertension) may be more susceptible to the adverse cardiovascular effects of air pollution. The present study was designed to determine if a high-fructose diet affects cardiovascular responses to inhaled air pollutants. We used a mobile air research laboratory located in an industrial area of Dearborn, MI, to expose male Sprague Dawley rats, to filtered air (FA), or the combination of ozone (O3; 0.5 ppm) and concentrated ambient fine particles (CAPs; 400 μg/m3). Rats were fed a normal (ND) or high-fructose diet (8% wt/vol fructose) prior to exposure. Exposure periods were 8h/day for 9 days (4-5 days/week). Heart rate (HR), heart rate variability (standard deviation; SDNN), and diastolic, systolic and mean arterial blood pressures were collected by radiotelemetry every 5 minutes during exposures. FA-exposed rats fed an ND had elevated systolic and mean arterial pressures compared to ND rats (10 and 9% greater, respectively). HR was not different between FA-exposed ND and HFD rats. Coexposure to O3/CAPs caused hypotension regardless of diet. O3/CAPs-induced decreases in systolic and mean pressures (decreases of 2 and 3%, respectively) were modest in ND rats compared to HFD rats (6 and 5%). O3/CAPs also induced marked bradycardia in both ND and HFD rats. Decreases in HR were greater in HFD (7%) compared to ND-fed rats (3%). Furthermore, SDNN was increased in HFD, but not in ND rats exposed to O3/CAPs. Observed hypotensive and bradycardic responses to O3/CAPs are consistent with a nasal irritant-induced trigeminal reflex. Mechanisms underlying HFD modification of these cardiovascular changes are yet to be determined. Funded by US EPA RD83479701.
primarily been used for vapor and liquid aerosol atmospheres. Use of this model to assess upper airway irritancy of dry powder aerosols required special technical considerations limiting the use of this model with dry powder aerosols. The RD50 for BA is >1096 mg/m3, and for SB is >1704 mg/m3. Based on the results of this study, BA and SB have low potency as airway sensory irritants. These data support that HDM is a more sensitive allergen for testing PM in acute models of allergic airway inflammation. While the use of P-OVA yielded increased sensitivity in these changes were statistically significant at d31 in DA rats. These results demonstrate that repeated exposure to >150 ppm OA or PD causes airway fibrosis and decreased pulmonary function in rats.

Epidemiological studies have shown increased hospitalizations associated with pulmonary toxicity upon exposure to particulate matter (PM) from wildfire smoke. Macrophages are one of the lung's primary protective cell populations, and it is important to know if wildfire PM induces oxidative stress and toxicity in this cell type. Our research group has shown that intratracheal instillation of coarse wildfire PM (PM10) decreases the number of lavagable macrophages from the mouse lung as compared to control groups. Furthermore, our in vitro studies using the RAW 264.7 macrophage cell line have shown increased cell death from 30 minutes to 24 hours after wildfire PM10 administration as compared to time-matched controls. The studies described here were designed to expand upon these previous findings and test the hypothesis that exposure to wildfire PM10 induces oxidative stress and cell death in the pulmonary macrophage population of the mouse lung. To this end, adult male, BALB/c mice were intratracheally instilled with PM10 suspended in phosphate-buffered saline (PBS) or PBS alone. Bronchoalveolar lavage fluid (BALF) and lung tissue were collected one hour after PM10 instillation. The macrophage population was quantified in BALF and lung tissue sections. Oxidative stress was evaluated using HPLC analysis of the BALF-derived cell pellet for reduced glutathione, and cytoxicity was evaluated using the Trypan blue exclusion method, as well as analysis of TUNEL staining for apoptosis. There was a decrease in macrophages in the BALF of mice instilled with wildfire PM10 as compared to control mice. There was also a decrease in reduced glutathione in macrophages from mice instilled with wildfire PM10. No significant difference in cytoxicity was found between treatments. In conclusion, oxidative stress in macrophages occurs one hour after wildfire PM10 exposure in the mouse lung, and this may give insight to wildfire PM-derived pulmonary toxicity that is seen in people.

Epidemiological data suggests correlations between particulate matter (PM) exposure and increased asthma symptoms and hospitalizations. The San Joaquin Valley (SJV) of California has some of the highest PM pollution in the country and asthma symptom prevalence is among the highest in the state. We sought to evaluate which model of allergic airway inflammation (AAI) will maximize our ability to detect differential particle toxicity collected via a novel source-oriented PM sampling approach in the SJV. BALB/c mice 8-10 weeks old were exposed via intranasal aspiration (IN) on days 1, 3 and 5 to a) saline, b) a select source-oriented particle (SOTox), or c) diffusion flame generated particles (DFP) as a positive control each in combination with ovalbumin (OVA), endotoxin purified OVA (P-OVA) or endotoxin purified D. Fariae house dust mite allergen (HDM). Animals were challenged on day 11, 12, and 13 with allergen alone. On day 14, the animals were sacrificed and tissues collected for analysis. Bronchoalveolar lavage exhibited cellular profiles indicative of an allergic response in all allergen models with elevations in leukocytes characterized by neutrophils, lymphocytes and eosinophils. HDM sensitized animals had significantly stronger inflammatory responses than the P-OVA and OVA models. Mice sensitized with OVA had on average 2-fold greater neutrophils than observed in mice sensitized with P-OVA, with neutrophils still present in all groups. Eosinophils and lymphocytes in HDM sensitized mice were 10- and 2-fold higher respectively than witnessed in OVA/P-OVA animals. These results suggest while purifying OVA reduced the neutrophil component, neutrophil inflammation is not due solely to endotoxin and is present in multiple models of allergic airway inflammation. While the use of P-OVA yielded increased sensitivity for detecting inflammatory differences as compared to OVA, our results indicate that HDM is a more sensitive allergen for testing PM in acute models of allergic airway inflammation.

Addressing the chemical evaluation bottleneck that currently exists can only be achieved through progressive changes to the current testing paradigm. The primary resources for addressing these issues lie in computational toxicology, a field enriched by recent advances in computer science, bio- and chem-informatics, molecular biology, and high-throughput screening (HTS). In vitro testing is resource intensive, particularly for multigenerational reproductive and prenatal developmental assessment. Furthermore, predicting adverse effects of chemicals for reproductive and developmental outcomes has been confounded by the lack of quantitative models that address the complex molecular and physiological factors underlying reproductive decrements and developmental malformations, and the life-stage and gestational windows of sensitivities involved. There is currently a strong focus on identifying endocrine-disrupting chemicals through a battery of in vivo and in vitro screening tests. However, the shared complexities and challenges of modeling reproductive, endocrine, and developmental toxicity, and the parallel need for higher throughput evaluation, creates the need for an integrated application of predictive models for chemical prioritization and targeted testing. Ultimately, predictive models of reproductive, endocrine, and developmental toxicity will provide a critical component in the computational toxicology toolbox that better informs regulatory testing decisions.
associated with reproductive toxicity. The current model is a classification model sufficient for large-scale chemical testing prioritization and begins to infer modes of action, e.g., estrogenicity, anti-androgenicity, or altering steroid metabolism. The model also has transparent inputs and outputs that are biologically translatable improving the chances for the model's acceptance and use in chemical testing prioritization and targeted testing applications. Extension of the model toward risk assessment applications targeted to mechanistic and cellular level information where quantitative and dynamic outputs of dose and time can be placed into a systems modeling framework. This abstract does not necessarily reflect US EPA policy.

871 SPECIES-SPECIFIC PREDICTIVE MODELS OF DEVELOPMENTAL TOXICITY USING THE TOXCAST CHEMICAL LIBRARY.

N. S. Sipes, ORD/NCCCT, US EPA, Research Triangle Park, NC.

EPA's ToxCast™ project is profiling the in vitro bioactivity of chemicals to generate predictive models that correlate with observed in vivo toxicity. In vitro profiling methods are based on ToxCast data, consisting of over 600 high-throughput screening (HTS) and high-content screening (HCS) assays, including embryonic stem cells and zebrafish embryos. The observed in vitro toxicity comes from 30 years of prenatal guideline studies of rodents and rabbits parsed into a publicly available database, ToxReDD. Due to distinct developmental differences, species specific models of developmental toxicity were built from the HTS data on the 309 ToxCast Phase I chemicals with balanced accuracies over 70%. Unique differences between the species specific models emphasized inflammatory signals in the rabbit model, and the retinoic acid receptor (RAR) and G-protein-coupled receptors (GPCRs) in the rodent model. The in vitro HTS profiles for 700 additional ToxCast Phase II chemicals, including failed pharmaceuticals, alternative plasticizers, and food additives are being used to validate and update the initial predictive models of developmental toxicity. These models have the potential to prioritize chemicals for further targeted toxicity testing and risk assessment; generate hypotheses about mechanistic pathways leading to adverse developmental outcomes, and reduce cost and increase throughput of chemical testing. This abstract does not necessarily reflect US EPA policy.

872 TEST STRATEGIES AND ALTERNATIVE APPROACHES FOR DEVELOPMENTAL-REPRODUCTIVE TOXICITY FROM THE EUROPEAN REGULATORY PERSPECTIVE.


Availability of rapid and reliable screening assays is important to the prioritization, selection and optimization of new drugs and chemicals. Recent regulatory initiatives from OECD, US EPA and European REACH legislation will require large amounts of information be generated about toxicity, including reproductive and developmental toxicity, for large numbers of chemicals in a relatively short timeframe. These objectives cannot be met using current testing paradigms, which are time- and resource-intensive, and which require large numbers of animals. New research addresses the need for high-throughput and high-content assays to provide public data on developmental defects, where the toxicity pathways remain largely uncharacterized. This presentation will assess the current status of testing strategies using in vitro and alternative models and will highlight the advantages and limitations of these approaches in the context of reproductive and developmental toxicity, with a special emphasis on classification and labeling.

873 BUILDING BRIDGES BETWEEN HIGH-THROUGHPUT SCREENING DATA AND IN VITRO REGULATORY GUIDELINE TESTS: APPLICATION OF INTERMEDIATE TIER IN VITRO FUNCTIONAL ASSAYS IN THE CHEMICAL INDUSTRY.

E. W. Carney, TERC, The Dow Chemical Company, Midland, MI.

The demand for toxicity data on chemicals used in industry, agriculture, and consumer products has increased tremendously in recent years, largely due to the drive for new, more sustainable products (which will require their own safety evaluation), as well as to increased data requirements for existing chemicals (e.g., European Union's REACH program). This increased demand for toxicity data simply cannot be met by conducting more and more regulatory guideline toxicity tests. This is especially true for developmental toxicity testing, which requires hundreds of animals per compound, is very expensive, and takes long periods of time. In response to these challenges, the US EPA and other government agencies have launched high throughput screening (HTS) and exposure science programs (e.g., ToxCast, ExpoCast) which can be used in a tiered approach to set priorities for further evaluation. In order to bridge the gap between rapid, mechanism-based HTS data and existing whole animal guideline developmental toxicity tests which are mainly descriptive in nature, there is an emerging need for creation of an intermediate tier of testing comprised of functional in vitro developmental toxicity tests, such as cell cultures and the whole embryo culture, zebrafish and C. elegans models. Intermediate tier testing can provide mechanistic read-outs of the initial HTS data. In addition, exposure modeling can be incorporated to ensure that dosing regimens are relevant to human exposures. Examples in which such intermediate tier assays can be used to bridge HTS and in vitro developmental toxicity data will be presented.

874 INTELLIGENT APPROACHES TO DART EVALUATIONS OF THERAPEUTIC PROTEINS.

K. E. Thompson, Reproductive Toxicology, Bristol-Myers Squibb Company, New Brunswick, NJ.

Therapeutic proteins are an increasing presence in the portfolio of pharmaceutical development. Typically, this diverse field of structures is not pharmacologically active in traditional animal models of developmental and reproductive toxicology (DART) evaluations; little is known about the potential placental transfer of these large molecules; and minimal to nothing is known about the ontogeny of the target in development of relevant species. When these drugs are active only in non-human primates (NHP), enhanced pre- and postnatal (ePPND) study designs have been proposed that are large, lengthy, and costly; importantly, however, they are statistically underpowered to detect increases in developmental mortality and dysmorphogenesis, except in cases of extreme toxicity. In face of 21st century initiatives to Reduce, Refine, and Replace, the pharmaceutical DART community is challenged to develop and embrace novel, rigorous approaches for evaluation of therapeutic proteins which permit prediction of target human pregnancy outcomes. These might include assessments of embryofetal exposure to candidate proteins, as well as profiling the ontogeny of therapeutic targets. Customized sets of studies that enhance decision-making, or even replace the ePPND study, will be presented as components of the toolbox available to toxicologists moving into the 21st century.

875 CROSS-SPECIES ANALYSIS OF TOXICOGENOMICS DATA: APPROACHES FOR ASSESSING DIFFERENCES IN SENSITIVITY AND CONSERVATION OF MODE OF ACTION.

R. S. Thomas and C. Rowlands, 1 The Hamner Institutes for Health Sciences, Research Triangle Park, NC and 2 Toxicology and Environmental Research & Consulting, The Dow Chemical Company, Midland, MI.

Two fundamental, but interrelated, challenges in toxicology are to identify a mode of action for a particular chemical and determine whether a particular response will be conserved across species. The determination of whether a response will be conserved usually involves a mechanistic understanding of the molecular events and an assessment of whether the processes and components that comprise those events are present in both the model species and the species of interest. In light of the relationship between gene expression changes and the biological effects, cross-species toxicogenomics studies may be used to identify potential modes of action for a chemical and determine appropriate cross-species adjustment factors for use in a risk assessment. We will evaluate the application of cross-species toxicogenomics analysis of chemical toxicant-induced modes of action across species for use in risk assessment in a series of highly focused presentations. These issues will be of broad interest to investigators and regulators across environmental, industrial, consumer products, and pharmaceutical toxicology who perform and interpret cross-species studies to understand human health risks.

876 CROSS-SPECIES COMPARISONS OF TRANSCRIPTOMIC ALTERATIONS IN HUMAN AND RAT PRIMARY HEPATOCYTES EXPOSED TO DIOXIN-LIKE CHEMICALS.

C. Rowlands, Toxicology and Environmental Research & Consulting, The Dow Chemical Company, Midland, MI.

The relative sensitivities of gene expression changes in human and rat primary hepatocytes exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was assessed using whole genome arrays. Following 24 hours exposure to 11-doses of TCDD, gene expression microarray analysis revealed significantly more genes were affected in rats compared to humans at all concentrations. A total of 479 and 1574 orthologous genes were significantly altered in human and rat hepatocytes, respectively, with 159 orthologous genes affected in both species. When compared on a pathway basis, a total of 49 and 54 pathways were enriched in human and rat hepatocytes,
and better determinations of point of departure for risk assessment can be obtained. From these multi-level analyses, the identification of modes-of-action led to a mechanistic hypothesis on how the peroxisome may be a protective of mitochondrial enzyme induction that explained the increased sensitivity in dog data from rats and dogs treated with a novel PPAR agonist allowed the pinpointing dose (of TCDD) responded very differently based on toxicological endpoints. Microarray data explained temporal and functional outcomes in animals treated with LPS. For example, mice, dogs, and rats administered LPS at doses that produced an equivalent leukon (75% decrease in total white blood cell count four hours post-dose) responded very differently based on toxicological endpoints. Microarray analysis yielded species-specific biomarkers that will allow better monitoring of inflammation-mediated toxicity in preclinical drug testing. Similarly, gene expression data from rats and dogs treated with a novel PPAR agonist allowed the pinpointing of mitochondrial enzyme induction that explained the increased sensitivity in dog and led to a mechanistic hypothesis on how the peroxisome may be a protective organ in rat. From these multi-level analyses, the identification of modes-of-action and better determinations of point of departure for risk assessment can be obtained.

Two species often employed in preclinical drug development are rat and dog, and responses of these species to drugs are sometimes discordant. Gene expression profiling of target organs is a means to produce biologically meaningful endpoints that underlie drug-induced toxicity. We show how probe, gene, and functional level analyses of gene expression data can discern differences in modes of toxicity between two rodent species and suggest species-specific biomarkers that will allow better monitoring of inflammation-mediated toxicity in preclinical drug testing. Similarly, gene expression data from rats and dogs treated with a novel PPAR agonist allowed the pinpointing of mitochondrial enzyme induction that explained the increased sensitivity in dog and led to a mechanistic hypothesis on how the peroxisome may be a protective organ in rat. From these multi-level analyses, the identification of modes-of-action and better determinations of point of departure for risk assessment can be obtained.

β-chloroprene (2-chloro-1,3-butadiene) is a monomer used in the production of neoprene elastomers and is of regulatory interest due to the production of multi-organ tumors in the 2-year rat and mouse rodent bioassays. A significant increase in female mouse lung tumors was observed at the lowest exposure concentration of 12.8 ppm while a small, but not statistically significant, increase was observed in female rats only at the highest exposure concentration of 80 ppm. To assess whether cross-species transcriptomic studies in a sensitive and insensitive species could identify potential key events in the mode-of-action of chloroprene lung tumorigenesis, dose-response analysis was performed and a subset of genes conserved between species were made in the lungs of female mice and female rats at exposure concentrations that spanned those used for the cancer bioassay and provided approximately equal target tissue doses in the lung. Both gene set enrichment and pathway-based benchmark dose analyses were used to identify pathways and the processes involved in oxidative stress or reactive metabolite formation, steroid hormone and amino acid metabolism, inflammation, and apoptosis that are potentially involved in the mode-of-action. The results will be discussed in terms of the broad application of cross-species transcriptomic studies to identify potential modes-of-action.

A remarkable feature of the carcinogenicity of inorganic arsenic is that while chronic human exposures in drinking water at concentrations on the order of 0.1-0.5 mg As/L have repeatedly been associated with increases in skin, lung, and blad-der cancer, inorganic arsenic has typically not produced tumors in standard adult animal bioassays. Whether this difference in the carcinogenicity of inorganic arsenic in animals and humans is primarily limited to studies in which it is co-administered with another carcinogen or when arsenic is administered during the developmental period. There are a number of factors that have been suggested to contribute to the susceptibility of humans to the carcinogenicity of inorganic arsenic: species differences in the expression of the cellular and molecular responses of the species differences in the target tissue dose of the highly potent metabolite, trivalent monomethyl ars-enic, or a mode of action (e.g., co-carcinogenicity, epigenetic programming) that favors tumorigenicity in humans. Genomic analysis indicates a substantially similar response of human and mouse bladder cells to arsenic exposure, both in terms of the tissue concentrations at which effects are elicited and the nature of the pathways affected. In both species, expression changes observed at higher arsenic concentra-tions are largely related to the oxidative stress response, while responses at the lower concentrations are related to inflammation, epithelial-to-mesenchymal transition, survival, and cell cycle control. The evolution of the genomic response over time is consistent with an initial stress response that is suppressed on longer exposure due to down-regulation of Fox. This altered cellular state may, during critical periods of development or on prolonged exposure, lead to cellular transformation.
The regulatory approval standards for biosimilar products are distinct from those for generic chemically-synthesized (small molecule) medicines, which must be identical to the reference listed drug. In contrast to generic medicines, biosimilars are “highly similar” but not identical to the approved biologic product (reference product). The European Union (EU) served as the pioneer for establishing the regulatory framework for the approval of biosimilars following the enactment of legislation in 2003/2004 which empowered the European Medicines Agency (EMA) to approve biosimilar products. Subsequent to the regulatory guidance established by the EMA, guidelines for the approval of biosimilars have been developed in several key regions around the world such as Canada and Japan, as well as the World Health Organization (WHO). This presentation will review the history and development of the regulatory standards for the approval of biosimilars established by various regions and the WHO. The similarities as well as some of the key differences between the regional guidelines will also be reviewed.

**INDUSTRY PERSPECTIVE ON BIOSIMILAR DRUG DEVELOPMENT AND REGISTRATION.**


Prior to the passage of the Biologics Price Competition and Innovation Act (BPCIA), BIO developed a set of key principles that should guide the creation of a pathway for the approval of biosimilars. BIO’s principles state that a pathway should ensure patient safety; recognize scientific differences between drugs and biologics; maintain the physician-patient relationship; preserve incentives for innovation; ensure transparent statutory and regulatory processes; and, continue to prioritize FDA review and approval of new therapies and cures. This presentation will review industry’s perspectives on biosimilarity, interchangeability, and the R&D aspects of and data requirements necessary for a biosimilars pathway.

**US FDA’S OVERVIEW OF THE REGULATORY GUIDANCE FOR THE APPROVAL OF BIOSIMILAR PRODUCTS IN THE UNITED STATES.**

D. Jacobson Kram, CDER, US FDA, Silver Spring, MD.

The Patient Protection and Affordable Care Act (PPACA) was signed into law by President Barack Obama on March 23, 2010. The law contains the Biologics Price Competition and Innovation Act (BPCIA), which creates an abbreviated approval pathway in the United States for biosimilar products that are demonstrated to be highly similar (biosimilar) to a therapeutic protein product previously approved as a Biologics License Application (BLA) under the Public Health and Services Act (PHSAct). The US FDA sought comments from interested parties during a public hearing in November 2010 and submission of comments later that year. The US FDAs scientific standards and data (analytical, nonclinical and clinical) required for the approval of biosimilar products will be reviewed in this presentation. Additionally, factors such as selection of reference product and extrapolation of clinical data, as well as other issues related to biosimilar products will be discussed.

**THE ROLE OF DANGER SIGNALS IN THE DEVELOPMENT OF CHEMICAL SENSITIZATION BY ENVIRONMENTAL AND OCCUPATIONAL AGENTS.**

P. Marc, et al., University of Utrecht, Utrecht, Netherlands.

The adaptive immune response to a foreign antigen needs both the recognition of the specific antigen and the presence of a specific cellular microenvironment at the place of antigen entrance. This specific cellular microenvironment provides signals to antigen-presenting cells allowing the elicitation of the immune response instead of immune tolerance to the foreign antigen. To this extent danger signals (e.g., proinflammatory cytokines, specific molecules from pathogens, necrosis products) play a crucial role in the immune adaptive response to pathogens. Triggering the innate immune system with danger signals, for instance through specific receptors termed PRR (pattern recognition receptors) including TLR (toll-like receptors), is a prerequisite for the immune system to react to pathogens as well as environmental antigens. These danger signals can be considered as adjuvants of immunity and indeed this concept has been extensively used for vaccination by adding chemicals (aluminum hydroxide) or pathogen products in vaccines. By analogy to the immune response to pathogens, the hypothesis that chemical allergens need the presence of danger signals or provide this danger signal to induce chemical hypersensitivity reactions has been developed during the past five to ten years. In this case, the chemical will play the role of an adjuvant. Recent evidence suggests that particulate matters and nanoparticles (SO2, TQ2) can also mimic the presence of danger signals and thus have adjuvant potential on immune responses. Recently, several *in vitro* models have been developed to address the adjuvant effect of chemical sensitizer toxicology programs for specific biosimilar products will be discussed.

**CHALLENGES AND REGULATORY APPROACH FOR THE APPROVAL OF SUBSEQUENT ENTRY BIOLOGICS IN CANADA.**


A “subsequent entry biologic” (SEB) is a term used by Health Canada to describe a biological product that would be considered as similar to an innovator biological product already approved and registered in Canada. As more innovator biological products reach patent expiry in the upcoming years, worldwide interest in developing SEBs will continue to grow. Due to the structural and molecular complexity of biological products, the regulatory standards for the approval of generic medicines are not scientifically appropriate for the development SEBs. In recognizing the distinction between generic medicines and SEBs, regulatory guidance was finalized by Health Canada in March 2010. This presentation will review the origins of the Canadian guidance and regulatory standards for the development of SEBs, as well as present some of the critical challenges in developing SEBs such as manufacturing the SEB, choice of reference product, and determining the extent of the nonclinical and clinical data to demonstrate similarity between the SEB and the reference product. The issue of interchangeability will also be discussed.

**CHEMICALS MODIFY THE CELLULAR MICROENVIRONMENT IN THE SKIN PROVIDING ENDOGENOUS DANGER SIGNALS.**

S. F. Martin, Department of Dermatology, University of Freiburg, Freiburg, Germany. Sponsor: M. Pallardy.

Allergic contact dermatitis (ACD) is an inflammatory skin disease of great and steadily increasing occupational and environmental concern. The disease is induced by xenobiotic organic and inorganic chemicals which penetrate the skin and react with host proteins. This process is accompanied by a strong, allergen-induced inflammatory reaction, which I call xenoinflammation, and leads to the migration of allergen-carrying dendritic cells (DC) from the skin to regional lymph nodes, where they promote generation of allergen-specific T cells. The latter are the ultimate effector cells of the disease. Re-exposure to the causative agent leads to the re-activation of the T effector cells, which then elicit the typical skin inflammatory reaction at the site of allergen contact. A striking finding is the normal contact...
hypersensitivity (CHS) response in germ-free mice. This implies that contact allergens orchestrate the innate immune response by the direct or indirect activation of innate signaling pathways using non-microbial, i.e. endogenous danger signals. In fact, a crucial role for TLR2, TLR4, the NLRP3 inflammasome and reactive oxygen species in ACD and CHS has recently been demonstrated. Thus, contact allergens trigger the same innate pathways that are triggered by microbial pathogens to induce skin inflammation leading to pathogenic T-cell responses. In such cases, ACD can be viewed as an anti-infectious immune response to non-infectious, non-replicating agents.

**889 CHEMICAL SENSITIZERS CAN PLAY THE ROLE OF DANGER SIGNALS.**

P. Marc. INSERM UMR 996, Université Paris-Sud, Chatenay-Malabry, France.

Dendritic cells are the most potent antigen presenting cells, which express a wide variety of receptors on their cell surface, recognizing microbial patterns, damage induced molecules and cytokines that constitute the ‘danger signals’. Dendritic cells become reporters of the microenvironment if exposed to the allergen, subsequently migrating to the draining lymph nodes where they activate naïve T lymphocytes. Dendritic cells could also be indirectly activated by epithelial cells, which express various receptors and secrete a variety of cytokines early after allergen exposure. Recently, it has been shown that chemical sensitizers but not chemical irritants were able to directly activate dendritic cells leading to phenotype modifications, cytokine production and T-cell activation. Specific cellular signalling mimicking ‘danger signals’ has been found to associate with these modifications suggesting a specific recognition of chemical sensitizers by dendritic cells.

**890 PHTHALATES INFLUENCE THE IMMUNE AND ALLERGIC RESPONSES.**

R. Dearman. Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom.

It has been suggested that one possible contributor to the increasing prevalence of atopic (IgE-mediated) allergic diseases and asthma in Europe and the US is exposure to chemicals that may act as adjuvants. Certain commonly used phthalate plasticizers have been implicated in this regard. The evidence for the ability of phthalates to impact on immune and allergic responses will be discussed, encompassing epidemiological investigations and results deriving from studies using experimental animals and from analyses in vitro. There is some evidence from the epidemiological data that exposure to phthalates may be associated with increased risk of development of allergies and asthma, however, the lack of objective exposure information limits the interpretation. It has also been reported that certain phthalates, when delivered at appropriate doses, and via an appropriate route, impact on immune and inflammatory function in rodents, although as yet no consistent pattern has emerged. Effects range from potentiation of immune or inflammatory responses, to the absence of any effect, to inhibitory or immunosuppressive activity. There is clearly a need for more definitive animal studies that will allow development of a more detailed understanding of whether and to what extent phthalates are able to effect meaningful changes in immune function that may in turn impact on human health.

**891 LUNG DENDRITIC CELLS ARE STIMULATED BY ULTRAFINE PARTICLES AND PLAY A KEY ROLE IN THEIR IMMUNOSTIMULATING ACTIVITY.**

R. Pieters. 1, 2, 1Institute for Risk Assessment Science, Research Centre Technology and Innovation, University of Utrecht, Utrecht, Netherlands and 2Research Centre Technology Innovation, Utrecht University of Applied Sciences, Utrecht, Netherlands.

The immunostimulating activity of air pollution particles on allergic airway sensitization is well known, but the cellular mechanisms underlying this so-called adjuvant potential are not yet well defined. Particulate air pollution has been associated with increased induction (sensitization) and exacerbation (challenge) of allergic airway disease. In human subjects diesel exhaust particles (DEP) have been shown to increase sensitization against antigens and various other particles are able to induce allergic sensitization against coadministered ovalbumin (OVA) in mouse models of allergic airway sensitization. Because no sensitization against the air pollution particles per se takes place, the main question is how these airborne particles affect the muco-associated immune system of the respiratory tract so that allergic sensitization against coadministered antigen takes place. Various cellular mechanisms have been suggested, including induction of cellular stress (oxidative stress) and activation of macrophages and epithelial cells. Most of the underlying mechanistic stud-

ies were done in vitro, and mechanistically relevant in vivo data are still limited. Data to support the importance of dendritic cells (DC) and costimulation in particle-induced immunostimulation will be presented in view of the danger hypothesis.

**892 ASSESSING THE BIOAVAILABILITY AND RISK FROM METAL-CONTAMINATED SOILS AND DUSTS.**


Exposure to contaminated soil and dust is an important pathway in human and ecological risk assessment and often is the risk-driver for metal-contaminated soil. Site-specific soil physical and chemical characteristics, as well as biological factors, determine the bioavailability of soil contaminants. Within a single sample, contamination may be from multiple sources of toxic elements that may exist in different forms and species. The bioavailability of soil and dust contaminants has a direct impact on human health risk assessment and risk management practices. Novel research efforts focusing on development and application of in vitro and in vivo methods to measure the bioavailability of metal-contaminated soils have advanced in the past few years. Our panel of experts will provide information on the recent developments in assessing the bioavailability and risk from metal-contaminated soils and dusts. The presentations include the relative bioavailability of arsenic-contaminated soils, metal contamination in urban residences in Canada, and potential children’s exposures to toxic elements in house dust. The information can be found in a community-based study known as the “West Oakland Residential Lead Assessment Study,” which provides details of the bioavailability of soil cadmium, chromium, nickel, and mercury, and human exposures to contaminated Brownfield soils. These presentations cover issues related to human health and bioavailability along with the most recent studies on community participation in assessing metal contamination, studies of children’s exposures to residential contamination, and recent in vitro and in vivo methods development for assessing the bioavailability of metals in soils and dusts. This session seeks to provide a forum for discussing the implications of these latest developments on incorporating bioavailability into the risk assessment and management process.

**893 RELATIVE BIOAVAILABILITY, BIOACCESSIBILITY, AND SPECIATION OF ARSENIC IN CONTAMINATED SOILS.**


Assessment of soil arsenic bioavailability may profoundly affect the extent of remediation required at contaminated sites by improving human exposure estimates. Thus, convenient, rapid, reliable, and inexpensive tools for assessing soil arsenic bioavailability are needed. In this study, in vivo mouse bioavailability and in vitro bioaccessibility of arsenic and physiochemical properties of nine soils from residential sites affected by earlier mining or smelting activity and two NIST standard reference materials were analyzed. In addition, arsenic speciation and select soil physiochemical properties were evaluated as predictors of arsenic bioavailability and bioaccessibility. In the in vivo assay, relative bioavailabilities of soil arsenic ranged from 11-53%. In vitro estimates of soil arsenic bioaccessibility were strongly correlated with soil arsenic bioavailability values (R² = 0.88). Among physiochemical properties, combined concentrations of iron and aluminum accounted for most of the variability in estimates of bioavailability and bioaccessibility, respectively. This multifaceted approach combined in vivo assays, in vitro assays, and physicochemical characterization of soils to yield congruent estimates of arsenic bioavailability and bioaccessibility.

**894 METAL CONTAMINATION IN URBAN RESIDENCES IN CANADA AND POTENTIAL CHILDREN’S EXPOSURES TO TOXIC ELEMENTS IN HOUSE DUST.**

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House dust is both an exposure medium, with ingestion of dust providing a major pathway of Pb exposure for young children, and a transport medium, facilitating the transfer of outdoor contaminants to the indoor environment. Results from the
recently completed Canadian House Dust Study provide a nationally representative sample of typical dust metal concentrations and loadings in urban Canadian homes. Detailed studies employing synchrotron techniques show a strong relationship between metal bioaccessibility and speciation in house dust, and assist in identifying factors for elevated indoor metal concentrations in the national study. The results showed that common Pb carbonate pigments contribute to elevated Pb bioaccessibility of house dust, and demonstrated that Pb bioaccessibility can be predicted from its speciation. This research also investigated variations in potential exposures from one part of the home to another. In humid indoor microenvironments, the dust sink provides a medium for molecular-scale transformations of certain metals (e.g. Pb and Zn) to more bioaccessible forms, which results in a significant (p < 0.05) increase in their overall bioaccessibility in ingested dust. Variations amongst rooms in older homes were also related to the timing of renovation activity, resulting for example in the presence of older paint compounds in bedrooms compared to living rooms. The results underscore the importance of taking appropriate measures to minimize exposures to dust, especially during remodeling and renovation.

W 895  USA EPA WEST OAKLAND RESIDENTIAL LEAD ASSESSMENT STUDY.  S. M. Sera1, S. A. Calanog1, K. D. Bradham2, K. G. Scheckel3 and B. W. Miller4, 1Superfund Division/Region 9, USA EPA, San Francisco, CA. 2NERL/ORD, USA EPA, Research Triangle Park, NC and 3NRMRL/ORD, USA EPA, Cincinnati, OH. Sponsor: M. Hughes

The West Oakland Residential Lead investigation area is comprised of residential blocks adjacent to the AMCO Superfund National Priorities List site. Based on site conditions documented by the 2007 investigation, removal activities, and citizen interest the U.S. EPA conducted an expanded site-wide assessment of lead in residential soils in October 2009. Based on these results and in collaboration with the residents of the South Prescott Neighborhood, EPA is taking an innovative approach to addressing this lead contamination, using phosphates from waste fish bones to reduce the bioavailability of toxic lead species, and covering the treated soil with a green cap of sod and organic material. This would have far less impact on the neighborhood than a traditional dig and haul removal. In addition to the innovative soil treatment, the project will be employing solar power for its electricity needs, electric vehicles for local transportation, and recycled materials, all in order to minimize the environmental and health impacts to the community. The project has three key objectives to protect children’s health, to minimize environmental impact to the community, and to support the community by utilizing local resources. (This abstract does not necessarily represent U.S. EPA policy.)

896 BEYOND LEAD AND ARSENIC: HOW ARE OTHER METALS BEING ADDRESSED?  R. A. Schoof, Environ International Corp, Seattle, WA.

Risk-based clean up levels for metals in soil and dust may be adjusted to correct for differences in the bioavailability of soil metal vs. the metal forms and exposure media from toxicity or epidemiology studies. Research and methods development efforts have focused on evaluation of the relative bioavailability (RBA) of Pb and As in soil; however, extension of these findings to other metals has been a subject of some debate. Factors that could affect applicable methods for a given metal include differences in toxicokinetics and toxic endpoints. Comparison of in vivo study designs used to assess soil As and Pb illustrates some of these differences, with Pb RBA typically being evaluated based on concentrations in blood, liver and kidney after repeated dose studies, whereas, As RBA may be evaluated based on urine or blood concentrations after either single dose or multiple dose studies. Studies of the bioavailability of soil Cd, Cr, Ni and Hg are more limited, but provide evidence that RBA varies with metal species and soil characteristics. Repeated dose feeding studies in rats have been used to measure soil RBA for both Cd and Hg by comparison of concentrations in kidney and other tissues from animals dosed with soil vs. those dosed with reference metal compounds. Evaluation of Cr is complicated by possible co-occurrence of trivalent and hexavalent forms, with reduction of Cr(VI) to Cr(III) in the stomach affecting Cr(VI) absorption. Both Cd and Ni are excreted primarily in the urine and urinary excretion measurements provide a viable approach to estimating RBA. In vitro approaches to estimating RBA may also need to be varied based on both metal characteristics and interactions with soil and dust. Many in vitro methods include an initial low pH “gastric” phase, with an option for a second higher pH “intestinal” phase. Available studies suggest that a single gastric phase approach may be used for both Cr and Ni, although for high Ni concentrations care must be taken to ensure there is sufficient gastric fluid volume so the saturation point is not exceeded. For Cd and Hg, a two phase approach with RBA based on the intestinal phase is recommended.

W 897 CONTAMINANT MIXTURES AND MIXED EXPOSURE EFFECTS: TESTING IN VITRO DIGESTORS TO DISTINGUISH BETWEEN HUMAN EXPOSURE TO BROWNFIELDS AND IDENTIFY ENGINEERING SOLUTIONS THAT REDUCE RISK.  S. D. Siciliano1,2, K. James3, B. Laird4 and D. Peak4, 1Interdisciplinary Toxicology Group, University of Saskatchewan, Saskatoon, SK; Canada and 2Department of Soil Science, University of Saskatchewan, Saskatoon, SK, Canada. Sponsor: M. Hughes

Residents living near or on contaminated sites are typically exposed to a mixture of organic and inorganic contaminants via ingestion and/or inhalation pathways. Estimating cumulative bioavailability across contaminant and pathway mixtures is a daunting task. We conducted a multi-year study on human exposure to soil-bound pollutants from a northern urban brownfield. We determined that residents were exposed to the very fine, mean size 32 μm, fraction of soil enriched in metals and polycyclic aromatic hydrocarbons (PAH) relative to bulk soil. However, the bioaccessibility of these enriched contaminants was reduced compared to bulk soil, thus mitigating the exposure risk. We next studied the chemical basis of this enrichment and were able to predict how mixtures both enrich in this ingested soil fraction and how bioaccessibility varies across mixtures of metals and PAH. Bioaccessibility and enrichment for PAHs was dominated by partitioning. For metals, enrichment was dominated by adsorption processes and bioaccessibility was dominated by metal lability. Metal lability is a chemical property indicating the rate at which water molecules are replaced in the solvation shell of a metal. This term accurately estimated bioaccessibility within a metal mixture and our regression equations predicted bioaccessibility between studies. Combining these ingestion results with air quality monitoring, we found that soil ingestion dominated the risk for As and PAHs, but not for Cr, Cd or Be. During this study, the roads in the study area were paved; exposure estimates were collected before and after paving. Road paving reduced the risk to toddlers and adults by an order of magnitude for Cr, Cd and Be. The premise that inhalation exposure is irrelevant for brownfields is likely incorrect due to vehicular re-suspension. A simple and functional way to reduce risk in northern brownfields is to pave the roads in the area.

W 898 STATE OF THE SCIENCE AND THE FUTURE FOR THE PREDICTIVE POWER OF IN VITRO AND IN VIVO MODELS FOR NANOMATERIALS TOXICITY TESTING.  A. Eldred1 and S. Nadadur2, 1Environmental Medicine, University of Rochester, Rochester, NY and 2NIEHS, Research Triangle Park, NC.

The proliferation of engineered nanomaterials (ENMs) in commerce has led to a growing concern about the consequences of exposure for those who produce and use them. The National Institute of Environmental Health Sciences recognized the importance of gaining a more fundamental understanding of the potential influence of ENMs on human health and, so, initiated a high priority research program (Nanotechnology Environmental Health and Safety, NanoEHS) with the availability of funds through the Americans Recovery and Reinvestment Act. This Nano Grand Opportunity (NanoGO) program was developed to address major issues of inconsistency in results reported for similar engineered nanomaterials investigated at different laboratories, a problem that significantly impedes both hazard and risk assessment for this class of materials. The disparities in results likely arise from poor communication about material characteristics, lack of assay validation, and/or inconsistencies in methodology. The goal of the NanoGO initiative was to support the development of reliable and reproducible methods and models to assess biological response through coordinated research efforts. As a result of this program, the NanoGO Consortium was established with 13 investigators/institutions in November 2009. The Consortium selected a library of ENMs and identified a set of in vitro and in vivo assays to be used in interlaboratory comparisons using standardized protocols. This session will focus on the study design, specific objectives, and consensus findings from interlaboratory in vitro and in vivo toxicity and particle characterization studies that were done with the library of engineered metal oxide and carbonaceous nanoparticles within the NanoGO Consortium, with an emphasis on how the findings can be used in the context of hazard assessment.

W 899 OVERVIEW OF THE NIEHS-SPONSORED NANO GO PROGRAM.  S. Nadadur, NIEHS, Research Triangle Park, NC.

The overarching goals of the Nano EHS program at NIEHS are to address fundamental issues of ENM interactions with biological matrices and to provide leads for defining potential hazard and health effects associated with accidental or incidental exposures. This knowledge could also be utilized to guide the development of safer
ENMs. Towards these goals and in coordination with the priorities identified by the National Nanotechnology Initiative, the NanoGO Program is to address one of the fundamental issues in ENM-biological interactions, i.e. the development of reliable and reproducible assays and methods to predict ENM toxicity using a set of defined materials by a Consortium of investigators across the US. This presentation will detail the origin, structure, composition and contributions of this Consortium. First, a suite of metal oxide (spherical titanium dioxide, zinc oxide; titanium dioxide ‘nanobelts’) and carbonaceous (multiwalled carbon nanotubes, 3 different surface chemistries) ENMs were selected for study based on availability, existence of historical data for comparison, the ability to characterize the material physicochemical properties, and investigator expertise and interest. Data regarding ENM size, morphology, composition, and crystal structure will be discussed for the selected materials. Based on the selected ENMs, a set of short-term in vitro and in vivo toxicological assays was chosen. The time points and outcome measures related to the expected oxidative stress, inflammatory, and profibrotic changes were selected by the Consortium and the rationale for these selections will be described. Through close regular communication amongst Consortium members, problems with reproducibility were identified and corrected through protocol modifications. The results of these collaborative and coordinated efforts by the Consortium investigators highlight the need for integrated efforts in the field of nanotoxicology in addressing the health implications associated with exposure to ENMs.

902 NANOPIRICLE DOSIMETRIC CONSIDERATIONS FOR IN VITRO AND IN VIVO MODEL SYSTEMS: CONSIDERATION OF DISPERSION STATUS, DOSE AND DOSE RATE FOR STUDY DESIGN AND IN VIVO-IN VITRO RESPONSE COMPARISONS.

G. Oberdörster, Environmental Medicine, University of Rochester, Rochester, NY.

This presentation will focus on responses to ENMs in the lower respiratory tract when administered to rodents as a bolus in well dispersed liquid suspensions (intra-tracheal instillation) or as an aerosol (inhalation). The impact of ENM suspension preparation using dispersants or sonication protocols for bolus type deliveries (high dose rate) vs. delivery of pristine nanoparticles by inhalation (low dose rate) will be discussed in terms of the usefulness and relevance of bolus administration to simulate real-life exposures. Implications and consequences for appropriate in vitro exposures of respiratory tract epithelial cells will be highlighted with regard to dosimetry and to particle delivery methodology via media suspension or aerosol (air-liquid interface). Conclusions regarding the influence of changing the dispersion state of ENMs by chemical (dispersants) or physical (sonication) means and of delivery mode on inflammatory responses will be discussed.

903 SUFFICIENT SIMILARITY OF WHOLE REPRESENTATIVE MIXTURES OR A RELATIVE POTENCY FACTOR APPROACH: POLYCYCLIC AROMATIC HYDROCARBONS AS A CASE STUDY.

C. V. Rider, NTP/NIEHS, Research Triangle Park, NC.

Predicting the toxicity of complex and dynamic mixtures represents a difficult challenge for risk assessment, as demonstrated recently by seafood safety concerns following the Deepwater Horizon oil spill. Two established approaches for addressing this challenge are the relative potency factor (RPF) approach and the sufficient similarity (SS) of whole mixture approach. The RPF approach requires knowledge of dose-response relationships of the individual mixture components. These data are then input into a dose additivity model to predict the toxicity of the mixture. Alternatively, with the SS approach, representative whole mixtures are used as a basis for predicting the toxicity of related environmental mixtures. Polyyclic aromatic hydrocarbons (PAHs) offer a useful case study for comparing the advantages and disadvantages of RPF and SS approaches. These ubiquitous contaminants are found in many mixtures to which humans are regularly exposed. PAHs are a large and diverse group of chemicals with complex mechanisms of action. Much work has been dedicated to developing an RPF approach for estimating cancer risk associated with exposure to unsubstituted PAHs. However, there is no clear path forward for expanding risk assessments to include substituted PAHs or noncancer end points, such as immune and reproductive toxicity. Currently, individual PAH dose-response data is incomplete and insufficient for accurately evaluating risk to human health. In order to better characterize toxicity associated with PAH mixtures, more work is needed. Determining which approach is more appropriate is a necessary step in deciding how to focus research resources. In effect, an RPF approach would dictate additional individual chemical toxicity testing; whereas, an SS approach would necessitate deciding on appropriate representative mixtures and testing those mixtures. This session will provide the framework and cover topics including advantages and disadvantages of each approach, consideration of the complex mechanisms of action of PAHs, critical data needs, and novel techniques available for filling data gaps and refining testing.
The Deepwater Horizon blowout in the Gulf of Mexico provided a stark example of the need for risk assessment methods dealing with complex environmental mixtures. The FDA and NOAA protocol implemented for re-opening fisheries used an additive relative potency factor approach to assess risk associated with exposure to polycyclic aromatic hydrocarbons (PAHs) in seafood. An assessment of this protocol based on comparisons with previous oil spills, published testing results, and current knowledge regarding chemicals released will be presented. The data gaps highlighted by this recent event are used to explore risk-benefit scenarios that necessarily incorporate exposure to mixtures, which is particularly important for dietary exposures that may have both positive and negative health implications, such as the case for seafood consumption. Development of pathway-based models that bring together both carcinogenic and non-carcinogenic endpoints may be useful for improving the risk assessment of exposures to mixtures. An approach that relies on information regarding time and spatially dependent activation of evolutionarily conserved transcription factors may provide a useful model construct for mixtures risk assessment of PAHs. Providing evidence for this approach, the examination of extant datasets on transcriptional networks after PAH exposure across several model species suggests downstream perturbation of developmental pathways may help to explain, in part, both carcinogenic and non-carcinogenic endpoints. When data is available, a pathway-based framework can efficiently incorporate evidence of additive, synergistic and antagonist roles in mixtures. In conclusion, the Deepwater Horizon blowout provides a real-world application of PAH risk assessment approaches and highlights the need for prioritization of data and methods development needs in addressing exposure to mixtures in risk assessment.
There is a significant honey bee health crisis in California and globally that may threaten the future of California's agricultural industry. Over half of California crops are dependent upon pollinators, including honey bees, with a potentially staggering economic impact approaching tens of billions of dollars. Some colonies collapse from the rapid loss of adult bees with the queen, a handful of bees, and some brood still present in the hive. In the US, this set of symptoms has been called Colony Collapse Disorder, or CCD. This condition is one component of a more general problem of colony losses, which has reached record highs in the past decade. Colony numbers in the US have been declining since the late 1940s because of the declining number of beekeepers. This trend continues but is now accelerated because of the challenge of keeping bees healthy and productive. Beekeeper surveys indicate annual colony losses of 30% and more. Multiple stresses, including Varroa and tracheal mites, Nosema, foulbrood diseases, numerous viruses, and small hive beetles, may be present in hives and can explain most of the colony losses. Other potential contributing factors are being investigated as well. Our panel brings together scientists, stakeholders, and regulators to discuss different views of the complex web of bees that might be causing the honey bee health crisis. Pesticide testing methodologies and results will also be addressed.

We will provide an overview on the current status of bee and colony health and an introduction into the multifactorial issues that affect bee health. This discussion will be followed by a presentation on the potential health threats posed by infectious agents. Next, a State of California representative will outline the steps being taken to protect bee health while balancing the need for agricultural crop protection tools. Finally, the complex approaches to understanding the relationship between pesticide residues and bee health and the logistical challenges of addressing these in a regulatory context will be discussed.

In the United States, the honey bee (Apis mellifera) is the surrogate for many other non-target insects and insect pollinators. Honey bees are a challenging organism to study. Typically toxicity testing and risk assessment focus on the individual, but in the case of honey bees, the colony is the organism. In a healthy hive, hundreds of foragers die daily and the death of one is of little importance. As a result, U.S. EPA currently has a tiered testing approach. Tier 1 testing includes an acute oral and contact test with young worker bees. Compounds with an LD50 < 2 μg/a.i./bee are considered highly toxic to bees and warrant further testing. Tier 2 testing is currently a foliar residue test. The endpoint of this test is a value indicating how long foliar plant residues are toxic to bees. Tier 3 testing includes either semi-field or field testing, however, standardized protocols for Tier 3 testing have not yet been established. With the introduction of new plant protection products, changes in agricultural practices, and advances in the science of honey bee biology, the ability to characterize potential risks to insect pollinators has become inadequate. In January 2011, a SETAC Pellston Workshop was held to assess and advance the state of the science on pesticide risk assessment for pollinators. The generic problem formulation for pesticide risk to pollinators includes two application strategies, foliar and application of systemic pesticides to seeds or soil. The new risk assessment paradigm is based on exposure estimates and a tiered toxicity testing system consisting of the previous tests plus a larval and 10-day chronic adult test, as well as more structured protocols for semi-field and field testing. California issues include how ecological risk assessment and risk management are blended. California is currently reevaluating neonicotinoid insecticide and has a novel interpretation of a pesticide label based on residual toxicity.

It is common in the pesticide registration and re-registration process to require honey bee effects testing. Effects studies fall into three categories, i.e. Tier 1 and Tier 2 (typically laboratory or semi-field studies), and Tier 3 (field studies). Potential risks identified at Tier 1 and 2, then trigger Tier 3 studies. Field studies are used to determine if effects occur under actual use conditions, but such studies must be designed carefully to ensure scientifically valid conclusions. The purpose and objectives of the study must be clear so that meaningful endpoints can be chosen. The study site must be representative and the surroundings must not compromise the exposure scenario in the study site. The hives must be configured consistently, as sanitary as possible, and bee tight to allow control over the hive entry points. The colonies must be as healthy as possible, which requires considerable lead-in time for bee stock production. Staffing is a challenge since stinging incidents are inevitable which can be a serious safety concern. Keeping qualified staff is essential to consistent observations, continuity of skill sets, and maintenance of healthy colonies. Satisfying GLP requirements while working in a natural environment is subject to endless variables and requires highly trained personnel. In California, almond field studies are difficult because the huge number of hives brought in to pollinate the crop may result in extreme competition among bees for nectar and pollen resources. The difficulty in finding appropriate sites is further exacerbated by the increasing number of bee studies required for regulatory purposes. Tier 3 studies generally provide the best representation of effects under real-world exposure conditions. With the difficulties and expense of conducting field studies, it is essential that they are well designed and targeted to both the concerns raised in lower tier studies and the proposed use patterns of the product. Successful studies ensure that honey bees are adequately protected and that growers have access to the range of tools required for effective integrated pest management.
A large body of research has elucidated various pathways of Acetaminophen (APAP)-induced hepatic cellular injury; however, there are still unknown factors that play a role in the propagation of injury. The role of protein glutathionylation in APAP-induced liver injury was investigated in this study. A single oral gavage dose of 150 or 300 mg/kg APAP in B6C3F1 mice produced increased serum ALT and AST levels and liver necrosis. APAP produced a more oxidizing state within the liver based on a decrease in the ratio of reduced to oxidized glutathione. Despite the increased oxidation state, the level of global protein glutathionylation was decreased at 1 hour and continued to decline through 24 hours. Immunohistochemical localization of glutathionylated proteins showed a complex dynamic change in the lobule zonation of glutathionylated proteins at both doses of APAP. In control animals, the single layer of hepatocytes surrounding the central veins had the highest level of glutathionylation with relatively uniform levels throughout the remainder of the lobule. At 1 hour after APAP exposure, the level of glutathionylation decreased in the single layer of hepatocytes around the central veins but increased mildly in the remaining centrilobular hepatocytes. The areas with increased glutathionylation also had APAP covalently bound to protein based on immunohistochemical detection. Thereafter, the level of glutathionylation decreased dramatically over time in the centrilobular regions with major decreases observed at 6 and 24 hours. Despite the overall decreased glutathionylation, a layer of cells lying between the undamaged periportal region and the damaged centrilobular hepatocytes exhibited high levels of glutathionylation at 3 and 6 hours in all samples and in some 24 hour samples that had milder injury. These zonal and temporal pattern changes in protein glutathionylation exposure indicate that protein glutathionylation may play a role in the pathogenesis of APAP-induced hepatic cellular injury.

Overdose of acetaminophen (APAP) frequently leads to acute liver failure associated with hepatic centrilobular necrosis. An integrated transcriptomic and metabolomics study investigated the mechanism of APAP-induced hepatotoxicity. Groups of rats (n ≥ 5) were orally gavaged with a single dose of 0.5% methycellulose (vehicle), 100 mg APAP/kg body weight (LD), or 1250 mg APAP/kg body weight (HD). Urine and terminal blood were collected at 6 h, 24 h, 72 h and 168 h postdosing. Livers were harvested at sacrifice. UPLC/MS, LC/MS/MS, GC/MS, and NMR, were utilized to examine metabolic changes in plasma/serum and urine. Statistical analysis showed that the HD group was most altered at each time point due to perturbations caused by APAP. A total of 238 compounds were semi-quantitatively detected. Decreases in arginine, potentially from the release of arginase from hepatic necrosis, had statistically significant correlations with alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Besides arginine, further metabolites were identified as potential biomarker candidates of APAP-induced hepatotoxicity. Most importantly, metabolomics and transcriptomics data indicated that oxidative stress, fatty acid metabolism and energy metabolism were significantly disturbed, consistent with previous reports of APAP-induced oxidative stress, which further causes mitochondrial damage to deplete energy production by inhibition of fatty acid beta-oxidation. This integrated approach led to comprehensive insights into the mechanisms of APAP-induced hepatotoxicity, a multisite process involving energy metabolism and oxidative stress perturbations.

Acetaminophen (APAP)-induced liver injury in mice is associated with deposition of hepatic fibrinogen (Fbg). Deficiency in the endogenous inhibitor of fibrinolysis, plasminogen activator inhibitor-1 (PAI-1), has been shown to increase APAP-induced liver injury in mice. However, the role of Fbg and fibrinolytic enzymes in APAP-induced liver injury is not known. We tested the hypothesis that Fbg deposition inhibits APAP-induced liver injury. APAP (300 mg/kg)-induced liver injury in mice was accompanied by thrombin generation, consumption of plasma Fbg, and deposition of hepatic fibrin. Neither Fbg depletion with anadrc or complete Fbg deficiency (Fbg-/-) affected APAP-induced liver injury. PAI-1 deficiency (PAI-1-/-) increased APAP-induced liver injury and hepatic fibrin deposition 6 hours after APAP administration, which was followed by marked hemorrhage at 24 hours. Mirroring PAI-1-/- mice, administration of recombinant human tissue plasminogen activator (tenecteplase, 5 mg/kg) worsened APAP-induced liver injury and hemorrhage in wild type mice. In contrast, treatment of wild type mice with tranexamic acid, an inhibitor of plasminogen activation, significantly reduced APAP-induced liver injury. Of interest, activation of matrix metalloproteinase 9 (MMP-9) in APAP-treated mice was increased in PAI-1-/- and tenecteplase-treated mice, and was inhibited by tranexamic acid. Taken together, the results indicate that Fbg does not contribute to APAP-induced liver injury. Rather, the results suggest that plasminogen activation contributes to APAP-induced liver injury, potentially by enhancing liver MMP activity.

Acetaminophen overdose is currently the most frequent cause of drug-induced liver failure in the U.S. APAP is metabolically activated to the reactive metabolite NAPQI, which forms protein adducts causing mitochondrial dysfunction, oxidant stress, nuclear DNA fragmentation and oncotic necrosis. Recently, it was shown that lysosomal iron translocates to the mitochondria after APAP treatment in hepatocytes, and contributes to opening of the mitochondrial membrane permeability transition pore and collapse of the mitochondrial potential (Kon et al. Tox Sci 2010 Sep;117(1):101-8). Thus, the purpose of this study was to determine if the cytochrome casepse 3/9, another potentially damaging lysosomal constituent, was directly involved in APAP-induced hepatotoxicity. Caspase B activity was measured in subcellular liver fractions of C57BL/6 mice 6 h after 500mg/kg APAP treatment. There was a significant increase in cytosol activity concurrent with a decrease in microsomal activity, indicative of release of lysosomal caspase B associated with cell death. Cell injury was confirmed by a significant increase in serum ALT activity and by histology. To investigate its effect on toxicity, the caspase B inhibitor z-FA-FMK (10µg/kg) was given 2h after 600mg/kg APAP treatment along with either saline, or vehicle control. The caspase inhibitor had no significant effect on the increase in serum ALT activities (4720±370 U/L) compared to vehicle-treated controls (3730±350 U/L) at 12h. Histological evaluation of necrosis (H&E staining) and DNA fragmentation (TUNEL assay) confirmed these findings. However, caspase B activity in the liver was inhibited by 85% compared to controls. Conclusion: APAP overdose causes lysosomal instability and release of caspase B into the cytosol. However, caspase B does not contribute to liver injury under these conditions.

Increased Susceptibility of Natural Killer T-Cell Deficient Mice to Acetaminophen-Induced Liver Injury.

Overdose of acetaminophen (APAP) frequently leads to acute liver failure associated with hepatic centrilobular necrosis. An integrated transcriptomic and metabolomics study investigated the mechanism of APAP-induced hepatotoxicity. Groups of rats (n ≥ 5) were orally gavaged with a single dose of 0.5% methycellulose (vehicle), 100 mg APAP/kg body weight (LD), or 1250 mg APAP/kg body weight (HD). Urine and terminal blood were collected at 6 h, 24 h, 72 h and 168 h postdosing. Livers were harvested at sacrifice. UPLC/MS, LC/MS/MS, GC/MS, and NMR, were utilized to examine metabolic changes in plasma/serum and urine. Statistical analysis showed that the HD group was most altered at each time point due to perturbations caused by APAP. A total of 238 compounds were semi-quantitatively detected. Decreases in arginine, potentially from the release of arginase from hepatic necrosis, had statistically significant correlations with alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Besides arginine, further metabolites were identified as potential biomarker candidates of APAP-induced hepatotoxicity. Most importantly, metabolomics and transcriptomics data indicated that oxidative stress, fatty acid metabolism and energy metabolism were significantly disturbed, consistent with previous reports of APAP-induced oxidative stress, which further causes mitochondrial damage to deplete energy production by inhibition of fatty acid beta-oxidation. This integrated approach led to comprehensive insights into the mechanisms of APAP-induced hepatotoxicity, a multisite process involving energy metabolism and oxidative stress perturbations.
liver homogenates and mitochondrial fractions prepared from APAP-treated NKT cell-deficient mice compared to WT mice, this appears to be due to the up-regulation of CYP2e1 protein expression and activities in NKT cell-deficient mice following starvation. In addition, starvation led to mitochondrial dysfunction and increased ROS formation in the NKT cell-deficient mice compared to WT. It has been shown that elevated ketone bodies up-regulate CYP2e1 expression through protein stabilization. Our data showed that starvation induced a higher increase of ketone bodies in NKT cell-deficient mice compared to WT mice.

Collectively, our data demonstrate that compared with WT mice, NKT cell-deficient mice are more susceptible to starvation-induced increase of ketone bodies, which results in increased CYP2e1 protein expression and thus, elevated liver injury upon APAP-challenge.

919 S-NITROSOGlutathione Protects Against acetaminophen-Induced Liver Toxicity in a Mouse Model.


S-nitrosoglutathione (GSNO) is an endogenous nitrosothiol involved in nitric oxide (NO) signaling and is the primary source of bioavailable NO in the body. Unlike other low molecular weight signaling molecules that bind to and activate target cellular receptors, NO signaling is mediated by covalent conjugation between NO and transition metals or target cellular proteins, often via S-nitrosylation of cysteine residues. A number of recent studies suggest that NO metabolism plays a role in acetaminophen-induced liver toxicity by modulating protein nitrosation and the redo state of glutathione. By increasing the pool of NO, cell signaling could be positively altered. Because NO is a labile gas and endogenous levels are difficult to manipulate, exogenous GSNO could be used as an alternative adjunct therapy with N-acetylcysteine (NAC) in cases of acute liver toxicity due to acetaminophen (ACAP) overdose. We sought in the current studies to assess the safety and efficacy of GSNO with and without NAC in a mouse model of ACAP liver injury. Male CD-1 mice were given a single oral dose of ACAP and two hours later, dosed intravenously with GSNO, NAC, a combination of GSNO/NAC, or a positive control (CO). Mice were observed for signs of clinical toxicity and blood was collected at 6, 24, and 72 hours post-ACAP administration for liver function tests, including AST and ALT. Livers were collected at 72 hours for histopathologic examination. Results demonstrated a significant beneficial effect with GSNO +/- NAC on the clinical and histopathological parameters associated with ACAP-induced liver toxicity. No toxicity was observed in GSNO, NAC or GSH only groups without ACAP administration. The results warrant further examination of GSNO as a potential adjunct therapy for ACAP overdose.

920 Protection Against acetaminophen Hepatotoxicity by Selenocompounds: Role of Thioredoxin Reductase.

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Mammalian thioredoxin reductase (TrxR) is a seleno-containing disulfide reductase which functions in antioxidant defense and cell growth regulation. We previously reported that N-acetyl-p-benzoquinone imine (NAPQI), a reactive acetaminophen metabolite, alkylates the N- and C-terminal redox centers of cytosolic TrxR, a process leading to enzyme inactivation. In the present studies, we determined if selenocompounds can protect against TrxR inhibition by NAPQI. With purified rat liver TrxR, NAPQI caused a concentration-dependent inhibition of enzyme activity. In contrast, selenourea and seleno-L-methionine were ineffective. Bioconjugated iodoacetamide (BIAm) is known to selectively react with free selenol groups on proteins at pH 6.5. NAPQI caused a concentration dependent decrease of BIAm binding to TrxR; this was abolished by co-incubation with esbelen, sodium selenite, aminoethylselenourea, and seleno-DL-cystine, indicating that these compounds can regenerate redox active selenol on proteins. The active selenocompounds also caused a significant recovery of TrxR enzyme activity, which was inhibited by NAPQI pretreatment. Taken together, these data demonstrate that selected selenocompounds can protect against NAPQI-induced TrxR inactivation, and this may be an important mechanism by which selenium protects against acetaminophen hepatotoxicity. Supported by NIH grants GM053410, AR055073, ES004738, CA132624 and ES05022.

921 Inhibition of SPLA2 Rescues Mice from a Lethal Overdose of acetaminophen.

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Earlier studies (Bhave et al., Toxicol. Appl. Pharmacol., 228:225-238, 2008) revealed that secretory phospholipase A2 (sPLA2) is a death protein that mediates progression of liver injury initiated by hepatotoxicants, acetaminophen (APAP), CCl4, and thioacetamide. If progression of initiated liver injury is indeed mediated by sPLA2, inhibiting it even after lapse of time should save the mice. We tested this concept in mice lethally poisoned with APAP (600 mg/kg body weight). Survival/lethality studies, liver histopathology and plasma biomarkers of liver injury were assessed. Male Swiss Webster mice (25-30 g) were injected with a lethal dose of APAP (600 mg/kg, ip, in warm 0.45% NaCl) and then their rescue was attempted with a single injection of sPLA2 inhibitor, 5-(4-benzyloxyphenyl)-4S-(7-phénylheptanoylamino)penicant acid (BPPA, 20 mg/kg, ip) administered at either 2, 4 or 8 h after poisoning. Survival and mortality were recorded four times on day one and twice daily thereafter through 14 days. Plasma alamine aminotransferase (ALT), a marker of hepatotoxicity and sPLA2 activity, a marker of progression of liver injury, were measured on alternate days till day 13. Plasma sPLA2 and ALT activities increased in the mice treated with APAP alone compared to the vehicle controls. Ninety, 70, and 60% of the mice treated with BPPA at 2, 4, or 8 h after the lethal dose of APAP, respectively, survived. The plasma sPLA2 and ALT activities peaked on day 1 and declined thereafter to their normal levels in mice treated with BPPA. Liver toxicity with APAP Elevated plasma sPLA2 and ALT activities were lower in mice intervened with BPPA 2, 4 or 8 h after APAP. We conclude that it is possible to rescue the APAP overdosed mice by intervening with the action of sPLA2 that mediates the progression of tissue injury. The finding may open up a new way of saving lives by therapy that can be administered much later after the injury is initiated.

922 Novel Translational Urinary Biomarkers for Acetaminophen-induced Acute Liver Injury.

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Granted by Dutch Top Institute Pharma (D3-201: Towards novel translational strategies for adverse drug reactions). Introduction: The leading cause of acute liver injury is drug-induced liver injury (DILI). Here, we describe a translational approach using proteomic profiling for identification of urinary proteins related to acute liver injury induced by acetaminophen (APAP). Methods: Male FVB mice were given a single intraperitoneal dose of APAP (0-350 mg/kg bw; n=6-30 per dose), followed by 24 hour urine collection. Subsequently, animals were sacrificed for collection of plasma and kidney and liver tissue. Additionally, two urine samples were collected from a patient with APAP intoxication. Urine samples were profiled using MALDI-TOF MS. Presence of biomarkers were confirmed in mouse and human urine samples using antibodies. Results: APAP treatment in mice resulted in hepatic necrosis and a dose-dependent increase in plasma alamine aminotransferase (ALT) levels (p<0.0001). Proteomic profiling resulted in identification of urinary proteins, such as superoxide dismutase 1 (SOD1), carbonic anhydrase 3 (CA3), and calmodulin (CaM), as novel biomarkers for APAP-induced liver injury. Urinary protein levels of SOD1 and CaM were closely associated with increasing plasma ALT levels (CaM), as novel biomarkers for APAP-induced liver injury. Moreover, hepatic mRNA expression of sod1 and calm2 were significantly decreased with increasing APAP-induced liver injury (p<0.05), probably reflecting a defense mechanism to prevent aggravation of liver injury. Importantly, we showed
923 SOLUBLE COMPONENTS OF ULTRAFINE PARTICULATE MATTER INDUCE PRO-COAGULANT RESPONSES IN ENDOTHELIAL CELLS.

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The mechanisms that underlie the strong association between particulate matter (PM) exposure and adverse cardiovascular (CV) health remain unknown but ultrafine (UF) particles are thought to have a particularly important role. UF particles may not leave the lung to directly mediate adverse CV effects, but their soluble components could enter the circulation and directly interact with vascular cells. We examined the hypothesis that the soluble components of UF particles activate pro-inflammatory and pro-coagulant responses in endothelial cells and thereby lead to a pro-coagulant phenotype. We exposed human coronary artery endothelial cells (HCAEC) to the soluble fraction of UF particles at 10, 50, and 100 μg/ml for 2, 4, 6, and 24 hrs and found significantly increased mRNA expression of pro-inflammatory mediators IL-8, IL-1β, GM-CSF, the adhesion protein E-selectin, and the pro-coagulant protein tissue factor (TF). No significant compensatory changes were observed in mRNA levels of the anti-coagulant proteins thrombomodulin, EPCR, and TFPI following exposure, which suggests an imbalance in the tightly regulated coagulation system. To assess the coagulation balance of the cells, we exposed HCAEC to the soluble components of UF particles and used calibrated automated thrombography to measure their ability to trigger thrombin generation in normal pooled platelet-free plasma. We found that exposed cells triggered earlier thrombin generation as shown by a significant decrease in lag time and time to peak, and that this effect was dose-dependent. These effects were abolished by anti-TF antibody, illustrating that the faster onset of thrombin generation was TF-dependent. These PM-induced pro-inflammatory and pro-coagulant changes in endothelial cells resulted in a pro-coagulant phenotype, which provides mechanistic insight into the enhanced thrombosis and endothelial dysfunction associated with air pollution exposure and increased risk for adverse CV effects.

924 THINKING OUTSIDE THE LUNG: ALTERNATE ROUTES OF NANOPARTICLE EXPOSURE.

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Multi-walled carbon nanotubes (MWCNT) are widely used in many fields to enhance existing material properties; however, more recently their potential as a drug delivery mechanism and other biomedical applications has been investigated. Studies have traditionally focused on the lung, but other exposure routes may be equally important in this regard. Sprague-Dawley rats were exposed to filtered air (control), aerosolized MWCNT with count mode aerodynamic diameter of 420 nm via a single day 5-hour exposure at concentrations of 4.6 μg/m3 which produced calculated respiratory distribution (~170 μg) and segments of the left anterior descending coronary artery were isolated from the left anterior descending artery territory (~170 μm in diameter) based on responses to transmural pressure and to increasing concentrations of phenylephrine (PE), acetylcholine (ACH), A23187, and spermine NONOate (SPR) 24-hours post MWCNT exposure. There were no significant differences observed between groups or microvascular beds in the endothelium-dependent responses to SPR and PE indicating smooth muscle sensitivity to nitric oxide and adrenergic responses were intact in both vascular beds. In the coronary arterioles, vasoactivity of the exposure groups was significantly different than control with respect to the endothelium-dependent reactivity (ACH, A23187, myogenic response); while, in the mesenteric arterioles ACh and A23187 were significantly different. The gavage and inhalation exposure routes were significantly different at the highest concentration of ACh and show an upward shift in myogenic responsiveness. These findings indicate that the endothelium-dependent microvascular impairments that follow MWCNT exposure via the gut, are equal to or greater than those observed in the lung. NIH-R01-E5015022 and RC1-E5018274 (TRN) and NSF-1003907 (VCM)

925 EFFECTS OF MULTIWALLED CARBON NANOTUBE CONCUBINATION ON VASCULAR REACTIVITY AND NITRIC OXIDE (NO) AVAILABILITY.

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Multiwalled carbon nanotubes (MWCNT) are widely used in many fields to enhance existing material properties; however, more recently their potential as a drug delivery mechanism and other biomedical applications has been investigated. Therefore, the inherent toxicity of MWCNT and their direct effects on microvascular tissue function were investigated. These effects were measured using a water jacketed biosensing chamber containing ISO-NOP NO probes connected to a free radical analyzer (WPI) to make direct electrochemical NO measurements. MWCNT in the chamber at concentrations of 100, 50, 25, 10, 5, and 1 μg/ml attenuated the amounts of NO produced by the donor S-Nitroso-N-Acetyl-D-L-Penicillamine (SNAP) by 4% ± 1% to 59% ± 6% inhibition of maximal [NO] sensed by the NO probes. This implies MWCNT have the ability to quench liberated NO. When microsomes isolated from the spinotriapae tearus microsomes are present, co-incubation with MWCNT (100 μg/ml) leads to a 40% inhibition of NO signal after stimulation with a bolus dose of the Ca2+ ionophore A23187, implying a direct effect of MWCNT on the vessels ability to produce NO. Reactivity of sub-epicardial arterioles isolated from the left anterior descending artery territory (~170 μm in diameter) was also impaired with MWCNT co-incubation (100 μg/ml) compared to control maximum dilation based on responses to increasing concentrations of endothelium dependent agonists acetylcholine (38±6%) and A23187 (32%±6%), indicating endothelium-dependent dilation (which relies heavily on NO) is altered. Collectively, these data indicate that MWCNT possess the potential to have a direct effect on liberated NO bioavailability and vascular NO production, leading to an impairment in arteriolar reactivity. NIH-R01-E5015022 and RC1-E5018274 (TRN)

926 PULMONARY EXPOSURE TO MULTIWALLED CARBON NANOTUBES AND C60 FULLERENES ACTIVATE INDOMETHACIN SENSITIVE CORONARY CONSTRICCTOR RESPONSES TO ENDOTHELIN-1.

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The effect of nanomaterials on physiological systems is critical to understand since their widespread use is increasing risks for exposure. Two of the more commonly used nanomaterials include multiwalled carbon nanotubes (MWCNT) and C60 fullerenes (C60). We hypothesized that pulmonary exposure to MWCNT or C60 will induce indomethacin (Indo) sensitive coronary responses to endothelin-1 (ET-1). We exposed male Sprague-Dawley rats to 100 μg of MWCNT or 93.33 μg of C60, or vehicles via intratracheal aspiration. Hearts were excised 24 hr post instillation and segments of the left anterior descending (LAD) coronary artery were isolated and mounted on a wire myograph. After equilibration, LAD segments were subjected to cumulative doses of ET-1 ranging from 0.1 nM - 1 μM with or without 10 μM Indomethacin (Indo). Results demonstrate that Indo produces notable reductions in the magnitude of ET-1 constriction in MWCNT and C60 coronaries (32.96% and 33.14% respectively) compared to untreated segments. Vehicle groups displayed no change in ET-1 response when incubated with 10 μM Indo (0.68% MWCNT vehicle or 1.22% C60 vehicle). We conclude that coronary responsiveness following pulmonary MWCNT or C60 exposure may be changed in a manner that could contribute to vascular dysfunction and susceptibility to cardiac injury. The mechanisms by which Indo sensitivity is induced in our model needs to be further elucidated in order to understand the extent of vascular injury. This work is supported by NIH R01 ES016246 (CJW) and U19 ES019525 (JMB/AL/TF/SS/CJW).

927 CARDIAC ISCHEMIC/REPERFUSION INJURY RESPONSE TO INSTILLED C60 FULLERENE.

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Exposure to small size particulate matter in urban air is regarded as a risk factor for cardiovascular effects, whereas there is little information about the impact on the cardiovascular system by exposure to pure carbonaceous materials in the nano-size.
range. C60 fullerenes are nano-sized particles with potential for widespread use in cosmetics, as industrial intermediates and formulation of medicines. This study was designed to evaluate cardiac ischemia injury following acute pulmonary instillation of C60 fullerene. Male Sprague Dawley rats were instilled with 0.035 mg/kg C60 fullerene suspended in polyvinylpyrrolidone (PVP) and delivered with 200 μl of normal saline. Control animals were instilled with PVP and naive animals did not receive C60 or PVP. Pulmonary instillation and cardiac ischemic/reperfusion injury were assessed at 1 day post-instillation. Cardiac ischemia was induced for 20 mins followed by 2 hours of reperfusion (I/R) in situ. Pulmonary response to C60 instillation assessed by BAL, protein assay revealed a modest elevation in the protein concentration for the C60 group at 24 hours post instillation (4% above PVP group and 13% above the Naive group). BAL cell differential counts indicated a minimal inflammation for C60 exposed rats; showing elevated neutrophils, eosinophils and lymphocytes. The response to I/R injury revealed an expansion of myocardial infarction for the C60 group (46% as compared to the PVP group at 26% and the naive group at 22%). Our study indicates that pulmonary instillation of C60 fullerene results in increased susceptibility to ischemic/reperfusion injury with minimal pulmonary inflammatory response. Even though these effects were observed at relatively high doses they may pose a significant risk to the cardiovascular system under stress. This work is supported by NIH R01 ES016246 (CJW) and U19 ES019525 (JMB/AL/TF/SS/CJW).

928 THE EFFECT OF C60 FULLERENE INSTILLATION ON THE VASCULAR RESPONSES IN PREGNANT SPRAGUE DAWLEY RATS.

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Pregnancy is a physiological state in which the effects of carbon nanoparticles have not been extensively investigated. Fullerenes are reported to have both pro-inflammatory and anti-oxidant properties. Inhaled fullerene particles are translocated through the alveolar capillary membrane to the circulatory system. C60 distributes into maternal and fetal tissues following intravenous exposure. However, the effect of these nanoparticles on the vascular responsiveness has not been investigated. Pregnant Sprague Dawley rats were acutely exposed to C60 at two different gestational periods (14-16 and 17-19 days). C60 particles suspended in polyvinylpyrrolidone (PVP) saline (93.3 μg/kg) or PVP saline alone was administered by intratracheal instillation. The rats were sacrificed 24 hours post-instillation and vascular responses of thoracic aortic rings, first order mesenteric artery segments and uterine artery loop segments were assessed. Cumulative dose-response curves were constructed for phenylephrine, acetylcholine, endothelin 1 and serotonin. The effect of cyclooxygenase inhibition was assessed using indomethacin. The dose-response curves, EC50, and Hill slope values were different for the C60 exposed groups compared with the control groups. Most striking was a 5mN/mm2 increase in the maximum stress to phenylephrine in uterine arteries of C60 instilled animals. The differences were even more pronounced for the 17-19 gestational day group. Pulmonary exposure to C60 during the late stages of pregnancy increases the vasoconstrictor response of the uterine artery. The alteration of the maternal vascular reactivity to support fetal growth may be an important target of the inflammatory response associated with the cyclooxygenase signaling. This work is supported by NIH R01 ES016246 (CJW) and U19 ES019525S5 (JMB/AL/TF/SS/CJW).

929 PULMONARY NANOCERIA EXPOSURE IMPAIRS CORONARY AND MENSETERIC ARTERIOLAR REACTIVITY.

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Ceria oxide (CeO2) is a common fuel catalyst that is present in resultant emissions. However, the health effects of inhaled nano-CeO2 are unknown. We have shown that pulmonary CeO2 exposure reduces endothelin-dependent and -independent arteriolar dilation. However, an effective concentration (EC50) has not been determined. The aim of this study was to determine the EC50 in Sprague-Dawley rats after nano-CeO2 exposure. Rats were intratracheally instilled with CeO2 at 0, 10, 50, 100, 200, and 400 μg/rat. The CeO2’s primary diameter was ~3 nm (determined via transmission electron microscopy). 2 hours post exposure, the rats were anesthetized, the heart and mesentery were removed and bronchoalveolar lavage was performed to assess pulmonary inflammation. Arterioles (<150 μm) were dissected and prepared for isolated vessel experiments. Arteriolar reactivity was assessed by evaluating the responses of thoracic aortic rings, first order mesenteric artery segments and uterine artery loop segments assessed. Cumulative dose-response curves were constructed for phenylephrine, acetylcholine, endothelin 1 and serotonin. The effect of cyclooxygenase inhibition was assessed using indomethacin. The dose-response curves, EC50, and Hill slope values were different for the CeO2 exposed groups compared with the control groups. Most striking was a 5mN/mm2 increase in the maximum stress to phenylephrine in uterine arteries of C60 instilled animals. The differences were even more pronounced for the 17-19 gestational day group. Pulmonary exposure to C60 during the late stages of pregnancy increases the vasoconstrictor response of the uterine artery. The alteration of the maternal vascular reactivity to support fetal growth may be an important target of the inflammatory response associated with the cyclooxygenase signaling. This work is supported by NIH R01 ES016246 (CJW) and U19 ES019525S5 (JMB/AL/TF/SS/CJW).

930 TELEMETRY-BASED MEASUREMENT OF SYMPATHETIC NERVE ACTIVITY DURING PULMONARY NANOPARTICLE EXPOSURE.

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We have reported that pulmonary nanoparticle exposure is associated with systemic microvascular dysfunction. One possible mechanism linking pulmonary nanoparticle exposure to remote microvascular effects is modulation of the influence of autonomic nervous system. Several lines of evidence from heart rate variability and baroreflex studies following particulate matter exposure suggest an altered sympathetic influence on vascular physiology. Our laboratory has recently reported that nanoparticle exposure alters systemic vascular responses to sympathetic nerve stimuli. Based on these data, we developed a model to record sympathetic nerve activity (SNA) during nanoparticle inhalation exposure to determine whether or not SNA is acutely altered. Rats were telemeterized with SNA and blood pressure transmitters to record renal nerve SNA signals and blood pressure from the abdominal aorta. Following 2 weeks of recovery from surgery and a chamber acclimation period, rats were exposed to either sham filtered air or nano-TiO2 aerosols (13.1 mg/m3, 4 day/ 24 h). 24-hours-post-exposure baroreflex sensitivity was determined by i.v. infusion of sodium nitroprusside (SNP; 5-20 μg/kg) or phenylephrine (PE; 1-4 μg/kg). Preliminary results indicate an alteration in heart rate as well as a greater increase in SNA in exposed compared to control animals (max ANSA 0.3 μV control, 0 μV exposed). Furthermore, nano-TiO2 exposure greatly increased the peripheral pressor response to PE (Δmm Hg 6.8±1.6 control vs. 17.5±3.2 exposed) and the magnitude of the decrease in heart rate (Δ BPM -12±1 control vs. -52±3 exposed). Conversely, nano-TiO2 exposure did not significantly alter SNP relaxation responses (Δmm Hg 30.3±9.3 control vs. -44.2±1.8 exposed), nor changes in heart rate (Δ BPM 22.9 control vs. 42.2±14 exposed). Sympathetic activity has not been directly studied in this field and represents a largely ignored pathway in the cardiovascular outcomes of particle exposure. NIH R01-Es015022, RCI-Es018274 (TRN)

931 PROTOTYPE PATHWAY RESEARCH FOR TOXICITY TESTING IN THE 21ST CENTURY (TT21C)—A CASE STUDY USING DNA DAMAGE CHARACTERIZATION.

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The NAS/NRC report on TT21C (Krewski et al., 2010) proposes an in vitro based risk assessment methodology based on toxicity pathway perturbations and human exposure, rather than apical end points measured in experimental animals. Our two organizations are exploring the practical application of the NRC recommendations in relation to safety assessment of ingredients in consumer products. Our first example is for the p53-mdm2 DNA damage/ mutation pathway examining a small number of compounds with diverse manners of interacting with DNA. Core program elements are (a) exposure and consumer use assessment, (b) rapid, high-throughput analysis of test compounds (a total of ~1000 for 2011-2012), (c) dose–response assessments for DNA-damage markers (γH2AX, TP53 and micronucleus), (d) computational models of the dose-dependencies in the citrulline of the p53-mdm2 pathway and (e) pharmacokinetic models supporting in vitro to in vivo extrapolations with specific compounds (e.g. quercetin). The results have been integrated to craft novel risk assessments for the putative ‘genotoxic’ case-study chemicals with the goal of maintaining exposure below levels causing significant pathway perturbations. Here we describe our research strategy and present key results from various HCA and flow cytometry based assays, and empirical modelling...
to assess non-linear dose response characteristics. This prototype toxicity pathway research, if successful, should provide clear support for using T21C principles in risk assessment and foster more rapid development of these much-needed methodologies for other toxicity pathways.

**PL 932 PROFILING DNA DAMAGE PATHWAYS ACTIVATED BY ETOPOSIDE, METHYLTH tanate SULFONATE, AND QUERCETIN.**

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Cells respond to DNA damage by initiating cell cycle arrest, DNA repair and apoptosis, through p53 signaling. How p53 uses different transcriptional and post-translational processes in response to different types of DNA damage is not fully understood. Defining the mechanism of response in relation to dose is an essential step in the application of computational modeling of toxicity pathways for future risk assessments. The current study used immunoblot, flow cytometry, whole genome transcriptomics to profile DNA damage response pathways activated by etoposide (ETP), methylmethane sulfonate (MMS), and quercetin (QUE) in a human cell line (HT1080) expressing wide-type p53. The chemicals induced comparable levels of p-p53 (ser15/ser46), ac-p53 (lys382), p-HAX, p-BRCA1 and total p53 protein. However, the proteins mediated by p53 showed considerable differences among chemicals. ETP activated p53 kinase ATM and Chk2 to a greater extent than MMS or QUE, p53 kinase ATR was not activated with any of the chemicals, and was down-regulated by QUE. While ETP and MMS induced MDM2 expression, QUE (30 µM) reduced MDM2. Finally, at concentrations that produced similar p53 and p-p53 responses, only ETP up-regulated p21 protein. By microarray, all three chemicals increased both MDM2 and p21 mRNA indicating that QUE and MMS down-regulate p21 and/or MDM2 possibly by reducing protein stability. QUE, but not MMS or ETP, down-regulated ATR and ATM, which is in agreement with the reduced protein. Our microarray results also showed that several other p53 responsive pathways were differentially regulated by those chemicals, including cell cycle regulator - cyclin B1, 14-3-3 sigma DNA damage sensor-DBB1, GADD45, DNA repair genes – XPC and PCNA, and the anti-apoptotic Bcl-2 gene. Together with concurrent dose response studies for micronuclei, cell cycle arrest and apoptosis, we have shown that the p53-mediated cellular response differ considerably with these three compounds.

**PL 933 AN ‘OMIC’ BASED METHOD FOR SENSITIVELY MEASURING GENOTOXICITY.**

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DNA damage can occur via endogenous and exogenous genotoxic agents and it can compromise a genome's integrity. This can be caused by the generation of genetic mutations that are often associated with disease. Knowing where damage occurs within a genome is crucial to being able to understand the repair mechanisms that promote genome stability. Using the genotoxin ultraviolet light as a paradigm for the induction of DNA damage, we've developed a novel microarray-based technology that is capable of sensitively measuring the levels and distribution of DNA damage and its repair throughout the entire yeast genome. Following the induction of UV damage, we've affinity captured damaged DNA and separated it from undamaged regions of the genome. By hybridizing the captured damaged DNA to our whole genome DNA microarrays, we are able to sensitively measure the levels DNA damage and their precise location throughout the entire genome. Repeating this process at various times after the induction of DNA damage permits a sensitive and high-resolution estimation of DNA repair capacity throughout the genome. Using chromatin immunoprecipitation on microarrays [ChIP on Chip] we've coupled these DNA damage analyses to an examination of DNA repair protein binding in response to UV damage and correlated these with UV induced epigenetic changes in histone acetylation known to be necessary for efficient DNA repair. In doing so we are developing a system-wide view of how the DNA repair process is organized within the genome in response to the induction of DNA damage and I will describe what we've learned about the intricacies of this process to date. In partnership with Agilent Technologies whose microarray platform we use, we are adapting our technique for use in the human context, with the aim of developing an in vitro alternative to employing animal-based genetic toxicity assays. Our aim is to improve genotoxicity testing in humans, as well as elucidating the underlying mechanisms of genotoxicity.

**PL 934 COMET ASSAY AND MICRONUCLEUS TEST USING CHIMERIC MICE WITH HUMANIZED LIVER (PXB MICE®).**


Genotoxicity studies have been performed as in vitro screening tests to predict carcinogens and genetic disorders in humans. Recently, the comet assay and micronucleus test have been noted for their ability to detect genotoxicity of test compounds and their metabolites in rodents in vivo. However, metabolic activities differ between humans and rodents. We have developed humanized chimeric mice (PXB mice®) whose livers are nearly completely repopulated with human hepatocytes. The PXB mouse liver retains human-type metabolic activities such as cytochrome P450, UDP-glucuronosyltransferase, and transporter activities. In the present study, we performed a comet assay and micronucleus test using PXB mice. Cryopreserved human hepatocytes (from a 2-year-old African–American boy) were transplanted into albinus enhancer/promoter-driven urokinase plasminogen activator transgenic/severe combined immunodeficiency disease (uPA/SCID) mice, and human albumin levels were monitored in the blood to determine the replacement ratio (RI) of human hepatocytes in the mouse liver. Nine-week-old male PXB mice with >7 mg/ml human albumin in the blood (RI >70%) were orally treated with a representative carcinogen, N-ethyl-N-nitrosourea, at 6.25, 12.5, or 25 mg/kg body weight daily for 4 weeks. Then, the liver and femur bone marrow were collected from the tested PXB mice and used for the comet assay and micronucleus test. The number of positive cells increased dose-dependently in the comet assay using hepatocytes and in the micronucleus test using bone marrow. We concluded that PXB mice should be useful for comet assays and micronucleus tests to predict human genotoxicity in vivo.

**PL 935 INTEGRATION OF PIG-4 GENE MUTATION AND MICRONUCLEATED RETICULOCYTE ENDPOINTS INTO 28-DAY REPEAT DOSE RAT STUDIES: EXPERIENCES WITH SEVEN PROTOTYPICAL GENOTOXICANTS.**


Experiments were performed to investigate the feasibility and merits of integrating two blood-based genetic toxicology endpoints into 28-day repeat treatment rat studies. For these studies, Sprague Dawley rats were treated for 28 consecutive days with several doses of 1,3-propane sulstone, melphanal, thiotepa, chlorambucil, cyclophosphamide, 2-acetylaminofluorene, or azathioprine. Low volume blood samples were collected before treatment and at several time points after treatment was initiated. To study induction of gene mutation at the Pig-a locus, flow cytometry was used to quantify circulating mutant reticulocytes (RET CD59-) and erythrocytes. The Pig-a mutation assay was performed using hepatocytes and in the micronucleus test using bone marrow. We concluded that PXB mice should be useful for comet assays and micronucleus tests to predict human genotoxicity in vivo.

**PL 936 DETECTING GENOTOXIC METABOLITES USING HCS-MICRONUCLEUS TEST AND METABOLITE PROFILING.**

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Genotoxicity assays have become an integral part of regulatory requirements, and genetic toxicity has moved towards earlier stages of drug discovery to identify genotoxicity liabilities sooner to avoid late drug attrition. Moreover, if genotoxicity is attributed to the parent compound in the absence of confirmed reactive metabolites, this can prolong discovery cycle.
This study describes the detection and identification of genotoxic metabolites in the same test compound systems using high content screening (HCS) assay and metabolite profiling (MetID). The assay includes conditions for metabolic activation (using S9) using the cytokinesis-block method and fluorescent dyes, enabling visualization of cells, their nuclei (binucleated), and micro-nuclei (MN). The test samples are stored for further analysis using metabolite profiling. The data analysis and image capture are performed using a VT1-HCS imaging platform and an automated data analysis package. Once positive, compound metabolites are further identified using a QTRAP 5500 LC-MS/MS.

The advantages of the HCS-MNT-MetID method makes it ideal for early genotoxicity screening, with small amount of compound required, rapid turnaround times, objective scoring, and metabolite confirmation. Several mutagens were tested showing that this assay can accurately detect micronucleus induction by clastogens (e.g. mitomycin C), aneugens (e.g. griseofulvin) and indirect clastogens (e.g. cyclophosphamide, benzo[a]pyrene, DMBA). Presented data from metabolic activation of benzo[a]pyrene show formation of the toxic metabolite benzo[a]pyrene-diol-epoxide, responsible for intercalation in DNA resulting in genotoxicity, confirmed in the HCS-MNT assay. Such information can distinguish the toxicity associated with the parent compound from the one associated with the metabolites. With this approach, this information will be available to drug discovery programs early, so costly mistakes could be avoided, increasing the odds of developing a drug without genotoxicity liability coming from the parent and/or metabolites.

**THE COMET ASSAY USING A RECONSTRUCTED 3D HUMAN EPIDERMAL SKIN MODEL: EXTENDED DATA SET TO DEMONSTRATE INTRA- AND INTER-LABORATORY REPRODUCIBILITY.**

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The European Cosmetic Association (COLIPA) has initiated a multi-laboratory project to establish and evaluate more predictive in vitro genotoxicity assays using reconstructed 3D human skin tissues. The 3D reconstructed human skin model, EpiDerm™, was combined with the micronucleus (MN) and Comet assays for safety testing because the skin is the first site of contact of many products, including cosmetics. Here, we show the data from the Comet assay using a set of five coded compounds as part of the pre-validation studies. Test compounds were topically exposed to the tissues for 3h following by isolation of basal keratinocytes and assessment of DNA damage. Inter-laboratory reproducibility of the 3D skin comet assay was initially demonstrated for MMS and 4-NQO and results showed good concordance among 4 different labs and with in vivo data. In the current project phase, intra- and inter-laboratory reproducibility was investigated with 5 coded compounds tested at 3 different laboratories. For compounds 2 and 3, all labs found a dose-related increase in DNA damage in every experiment. For compound 4, the overall result from all labs showed a smaller, but significant, dose-re- response. For compound 1, an increase compared to solvent controls was observed only in one lab. However, the response was not dose-related so compound 1 was judged negative overall, as was compound 5, which was the only compound showing clear cytotoxic effects. For compound 5, significant DNA damage generally occurred only at doses that showed substantial cytotoxicity (>30% cell loss), and the overall reproducibility was comparable in all labs despite some differences observed. The results of the collaborative study for the coded compounds were generally reproducible among the laboratories involved and intra-lab reproducibility was also good. These data indicate that the comet assay in reconstructed 3D skin models is a relevant model for the safety assessment of chemicals with dermal exposure.

**EVALUATION OF CHEMICALS REQUIRING METABOLIC ACTIVATION IN A NOVEL 3D HUMAN RECONSTRUCTED SKIN MICRONUCLEUS (RSMN) ASSAY.**

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The in vitro human reconstructed skin micronucleus (RSMN) assay in EpiDerm™ is a promising new assay for evaluating genotoxicity of dermally applied chemicals. A global prevalidation project sponsored by the European cosmetics companies trade association (COLIPA), and ECVAM, the European Center for Validation of Alternative Methods, is underway. Results to date demonstrate international inter-laboratory and inter-experimental reproducibility of the assay for chemicals that do not require metabolism (Aardema et al., Mutat. Res., 701 (2010) 123-131). We have expanded these studies to investigate chemicals that require metabolic activation: 4-nitroquinoline-n-oxide, cyclophosphamide, dimethylnitrosamine, dibenzanthracene and benzo[a]pyrene. In this study, the standard protocol of 2 applications over 48h was compared to an extended protocol involving 3 applications over 72 h. Extending the treatment period to 72 h changed the result significantly only for 4NQO which was negative in the standard 48 h dosing regimen, but positive with the 72h treatment. DMBA, and CP were positive in the standard 48 h assay (CP induced a more reproducible response with 72 h) and BaP gave mixed results: DBA and DMN were negative in both 48 h and 72 h dosing regimens. While further work with chemicals that require metabolism is needed, it appears that the RSMN assay detects some chemicals that require metabolic activation (4/6 chemicals were positive in one or both protocols). At this point in time, for general testing, use of a longer treatment period in situations where the standard 48 h treatment is negative or questionable is recommended.

**INHIBITION OF THE RH/OCK PATHWAY PREVENTS NEURONAL DEGENERATION IN VITRO AND IN VIVO FOLLOWING METHYLMERCURY EXPOSURE.**

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Methylmercury (MeHg) is an environmental neurotoxicant which induces neurotological changes in both the central nervous and peripheral sensory nervous systems. Our recent study demonstrated that down-regulation of Rac-related C3 botulinum toxin substrate 1 (Rac1), which is known to promote neuritic extension, preceded MeHg-induced damage in cultured cortical neurons, suggesting that MeHg-mediated axonal degeneration is due to the disturbance of neuritic extension. Therefore we hypothesized that MeHg-induced axonal degeneration might be caused by neuritic extension/retraction incoordination. This idea brought our attention to the Rac homolog gene (Rho)/Rho-associated coiled-coil-forming protein kinase (ROCK) pathway because it has been known to be associated with the development of axon and synaptic neuronal cell death. Here we show that inhibition of the Rho/ROCK pathway prevents MeHg-intoxication both in vitro and in vivo. A Rho inhibitor, C3 toxin, and 2 ROCK inhibitors, Fasudil and Y-27632, significantly protected against MeHg-induced axonal degeneration and apopotic neuronal cell death in cultured cortical neuronal cells exposed to 100 nM MeHg for 3 days. Furthermore, Fasudil partially prevented the loss of large pale neurons in dorsal root ganglia, axonal degeneration in dorsal spinal root nerves, and vacuolar degeneration in the dorsal columns of the spinal cord in MeHg-intoxicated rat models (20 ppm MeHg in drinking water for 28 days). Hind limb crossing sign, a characteristic MeHg-intoxicated sign, was significantly suppressed in this model. The results of this study showed that inhibition of the Rho/ROCK pathway prevents MeHg-intoxication in both the peripheral nervous system and cerebral cortex. Therefore, we propose that the Rho/ROCK pathway is a potential therapeutic target for MeHg-induced neurological damage.
Manganese (Mn) is a neurotoxin causing Manganism, a Parkinson-like disease. Mn disrupts dopaminergic neurotransmission. The mechanism is not fully resolved and thought to be more related to downstream neuronal pathways than deficits in nigrostriatal function. Lack of effective treatment is an obstacle in clinical management of Manganism. Lateral cilia of gill of Crassostrea virginica are contracted by serotonergic-dopaminergic innervations from their ganglia. Dopamine (DA) causes cilio-inhibition, serotonin (HT) cilio-excitation. We showed post-synaptic DA receptors present in gill lateral cells are D2 type G protein-coupled (Gtido) metabotropic receptors. Gtido inhibits adenylyl cyclase, Gβγ increases K⁺ channel conductance and closes Ca²⁺ channels. We showed Mn blocks cilio-inhibitory effects of DA and Mn exerts its effects by blocking DA post-synaptic receptor. Here we observed membrane potentials of lateral ciliated cells of C. virginica gill with a fluorescent dye while measuring cilia beating rates. Applying HT to gill caused prolonged membrane depolarization and increased cilia beating rates. Applying DA to gill after exciting cilia repolarized the cell membrane and decreased beating rates. Applying Mn prevented the cilio-inhibitory response and corresponding repolarization. Adding ATP 10⁻³M or forskolin (10⁻⁶-10⁻⁴M), an adenylyl cyclase activator, to control or Mn treated gill filaments increased beating without changing the membrane potential. In other experiments applying MDL or SQ, adenylyl cyclase inhibitors, to controls or Mn treated gill, decreased beating without affecting membrane potential. The study shows a correlation between membrane potential of lateral ciliated cells and beating rates of the cilia. It shows the actions initiated by activation of D2 post-synaptic receptors can be differentiated to effects on adenylyl cyclase and on membrane channel conductance, and the neurotoxic effects of Mn can be overcome by application of adenylyl cyclase inhibitors. It helps elucidate the neurotoxic mechanism of action of Mn.

941 ADENYLYL CYCLASE INHIBITORS REVERSE THE NEUROTOXIC EFFECTS OF MANGANESE ON POST-SYNAPTIC DOPAMINE D2 RECEPTORS.


942 TOPICAL PARAOXON TOXICITY REDUCED BY FULLERENES.

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A gadolinium-containing C80 TrimersphereTM fullerene (Gd3N@C80-OH; GdTMS) and C70-tetracrylic acid (C70-TGA) were examined for their ability to protect mice against clinical signs of acetycholinesterase (AChE) inhibition that followed topical administration of the organophosphate (OP) paraoxon. GdTMS or C70-TGA (0.5 – 8 mg/kg) were applied topically or given intraperitoneally (ip) 20 min after topical paraoxon treatment (2 mg/kg in 50% ethanol,10 μL volume). Clinical signs of OP toxicity were recorded every 10 min for 80 min following paraoxon treatment, and mice sacrificed at 80 min for determination of acetycholinesterase (AChE) activities. Clinical effects of paraoxon were seen, but atropine treatment was not required. These signs included changes in posture, activity, gait, mobility, and observation of involuntary movements. Time of onset of these signs was delayed in fullerene-treated mice. Specifically, in paraoxon-treated mice not given fullerene, mobility was altered in 50% of the mice at the 50 min observation time. However, in the GdTMS or C70-TGA treated mice observed at that time, only 17% and 0%, respectively, had altered clinical signs following paraoxon treatment. After 80 min, mobility change was 83%, 50%, and 17% in these groups, respectively. Upon sacrifice 80 min later, improvement in brain AChE activity in mice given 2 mg/kg topical paraoxon + GdTMS or C70-TGA averaged 10% for GdTMS whether given by the topical or ip route. Brain AChE improved about 20% following topical C70-TGA, which was greater than the benefit seen with ip C70-TGA. While further studies are needed to determine optimal routes of fullerene administration, these experiments suggest fullerences may be used to counteract toxicities induced by OP compounds. Supported by NIH 1U01NS063723.

943 THE ROLE OF ENZYME INDUCTION AND INHIBITION ON LAMBDA-CHIHALOTHIRIN HEPATOTOXICITY.

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The wide use of synthetic pyrethroid insecticides makes them an emerging ecotoxicological concern. Lambda -cyhalothrin (LCT) is a new type II pyrethroid used all over the world with extensive public and animal health applications. The present study was planned to investigate the hepatotoxicity of LCT on isolated rat hepatocytes. Rat hepatocytes were isolated using collagenase two-step perfusion technique and were incubated with different concentrations of LCT (100, 200, 400 and 800 ng/ml) at different incubation periods (0, 30, 60 and 120min). LCT induced concentration, time dependent cytotoxicity, and oxidative stress on isolated rat hepatocytes. Cell viability and hepatic enzyme leakages (LDH, ALT and AST) were used as indicators of LCT cytotoxicity. However, GSH content and TBARS accumulation were used as indicators of oxidative stress. The role of cytochrome P450 in the hepatotoxicity of LCT was investigated in fresh hepatocytes isolated either from Phenobarbital pretreated or control rats, and coincubated with SKF525A.

944 STUDY OF BEHAVIORAL AND NEUROPATHOLOGICAL EFFECTS 3 WEEKS AFTER A SINGLE DOSE OF ORGANOPHOSPHATE WITH A SINGLE DOSE OF FULLERENE AS A PROTECTANT.

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The present experiments were done to determine the extent of potential usefulness of fullerences against toxicities induced by organophosphorus (OP) compounds. The OP compound diisopropyl phosphofluoridate (DFP, 2mg/kg ip) has been demonstrated to cause acute clinical detriments in mice, which can be modulated by co-treatment with gadolinium-containing C80 TrimmersphereTM fullerene (Gd3N@C80-OH; GdTMS) (SOT 2011 abstract 2576). Because long-term behavioral sequelae of DFP combined with the fullerene treatment are unknown, the present 3-week study examined effects in mice given single 2 mg/kg ip dose of DFP alone or in combination with 8 mg/kg ip DFP-GdTMS, along with fullerence and vehicle controls (n=9-10/group). In the third week following toxicant/fullerene exposure, the mice were assessed for neurobehavioral deficits using motor activity, rotarod performance, passive and active avoidance, and a modified mouse functional observational battery. At sacrifice, brain samples were obtained for histopathology and determination of acetycholinesterase (AChE) activity. There were no significant differences among the 4 experimental groups using the neurobehavioral tests. No lesions were noted on microscopic examination of multiple brain levels in any group. There were no significant differences in AChE activity among the brains of the four groups, except for a decrease in DFP versus fullerene exposed mice (p<0.0078). As noted above, there were no behavioral effects seen in mice shortly after exposure to this dose of DFP (which are modulated by this fullerene), a 2-3 week post-exposure period eliminates these detriments. Thus potential protection by this fullerene could not be assessed in this longer-term model. Supported by NIH 1U01NS065723.

945 PHARMACOKINETICS OF RESIDUAL FORMALDEHYDE IN VACCINES FOLLOWING INTRAMUSCULAR EXPOSURES IN INFANTS.

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Formaldehyde is a one-carbon, highly water-soluble aldehyde that is a ubiquitous component of the environment and is generated endogenously by all cells of the body as part of normal metabolic processes. Although long-term, inhalation exposure by certain occupational groups is the exposure scenario of greatest concern for
formaldehyde, other sources of exposure to formaldehyde can include tobacco smoke, certain cosmetic products, and food and drinking water. Since formaldehyde can also be a component of certain childhood vaccines in which it functions to inactivate viruses or bacterial toxins, we performed a preliminary safety assessment for formaldehyde that may be found in these vaccines in tiny, residual amounts of no more than 100 μg per 0.5 mL injection. Using the 2011 American Academy of Pediatrics immunization schedule along with pharmacokinetic parameters obtained from the biomedical literature, we determined that a single injection of a vaccine, containing a maximal amount of formaldehyde, inside the quadriceps femoris of infants delivers a median whole-body dose of 29 μg/kg body weight leading to an internal tissue concentration of 53 μM (1.6 ppm) formaldehyde at the site of injection (port of entry). This resulting single whole-body dose is 10-fold lower than the minimal risk level (MRL) established for formaldehyde (300 μg/kg body weight) based on portal-of-entry effects following repeated short-term oral exposure (ATSDR 1999, 2010). In addition, the resulting local tissue concentration is 2-8 times lower than background levels of formaldehyde that have been measured in various mammalian tissues (range: 97-420 μM). Given a background concentration of formaldehyde in blood (2-3 ppm) that is already slightly higher than a single unmetabolized tissue dose to muscle from vaccine, detoxification of formaldehyde by glutathione and formaldehyde dehydrogenase in both muscle and plasma, and the extremely short half-life of formaldehyde in mammalian blood (1-1.5 min), systemic exposure to small amounts of formaldehyde following vaccination is possible but considered unlikely.

946 VITAMIN C MITIGATES SENSORIMOTOR AND COGNITIVE CHANGES INDUCED BY CHRONIC CHLORPYRIFOS IN WISTAR RATS.

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Studies in animal models and humans have shown that chlorpyrifos (CPF), an organophosphate insecticide impairs sensorimotor and cognition. The induction of oxidative stress is one of the mechanisms implicated in CPF neurotoxicity. The present study evaluated the mitigating effect of vitamin C on sensorimotor and cognitive changes induced by chronic CPF exposure in male Wistar rats. Twenty young adult male Wistar rats divided into 4 groups of 5 animals each were used for this study. Group I (Soil) was administered soy oil (2 ml/kg) while group II (VC) was given vitamin C (100 mg/kg) and then supplemented with soy oil (2 ml/kg); group III was dosed with CPF (10.6 mg/kg/1/8th of the LD50); group IV was pretreated with vitamin C (100 mg/kg) and then exposed to CPF (10.6 mg/kg). 30 min later. The regimen were administered orally by gavage once daily for a period of 17 weeks. The animals were evaluated for toxic signs and sensorimotor parameters measuring motor and neuromuscular coordination, learning and short-term memory. The whole brain samples were evaluated for the concentration of malondialdehyde (MDA) and activities of superoxide dismutase (SOD), catalase (CAT), and AChE. The result showed that vitamin C mitigates sensorimotor and cognitive deficits induced by chronic CPF exposure in Wistar rats, partly due to its antioxidative and AChE restoration properties.

947 CHEMOPREVENTION OF GASTRIC CANCER IN HELICOBACTER PYLORI-INFECTED MONGOLIAN GERBILS USING AN NF-kB INHIBITOR (CAFFEIC ACID PHENETHYL ESTER).

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Nuclear factor-kB (NF-kB) plays important roles in host inflammatory responses and carcinogenesis. In this study, we investigated the effect of caffeic acid phenethyl ester (CAPE), a potent NF-kB inhibitor, on Helicobacter pylori (H. pylori)-induced NF-kB activation in cell culture and chronic gastritis and stomach carcinogenesis in Mongolian gerbils. In AGS gastric cancer cells, CAPE significantly inhibited H. pylori-stimulated NF-kB activation and mRNA expression of several inflammatory factors such as TNF-α and IL-8 in a dose-dependent manner, and prevented degradation of IκB-α and phosphorylation of the NF-kB p65 subunit. To examine the effects of CAPE on H. pylori-associated gastric disorders, specific pathogen-free male, 6-week-old Mongolian gerbils were inoculated with H. pylori; administered 10 ppm N-methyl-N-nitrosourea in their drinking water, fed diet containing CAPE (0-0.1%), and sacrificed after 12 or 52 weeks. Infiltration of neutrophils and mononuclear cells, translocation of the NF-kB p50 subunit, and phosphorylation of IκB-α in the gastric mucosa were significantly alleviated by 0.1% CAPE treatment. CAPE also reduced mRNA expression of inflammatory factors including TNF-α in the antrum. Furthermore, 0.1% CAPE significantly decreased the incidence of gastric adenocarcinomas (72/26, 29%) compared with that in the control group (29/54, 53.7%). These results suggest that CAPE has chemopreventive effects on H. pylori-associated chronic gastritis and stomach carcinogenesis in Mongolian gerbils through inhibition of the NF-kB pathway.

948 IDENTIFICATION OF CANCER GENES INVOLVED IN DIFFERENTIAL SUSCEPTIBILITY AND RESPONSE TO RESPIRATORY SYNCTIVAL VIRUS INFECTION.

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Respiratory syncytial virus (RSV) is the primary cause of lower respiratory tract infection during infancy and childhood. Some individuals are highly susceptible to infection and exhibit more severe symptoms which may result in hospitalization and sometimes death. We used human lymphoblastoid cell-lines to evaluate inter-individual variation in response to RSV, test the role of candidate susceptibility genes, and identify susceptibility patterns that express genetic responsivity. Significant inter-individual variation in RSV infectivity across multiple cell lines indicated that genetic background is an important determinant of susceptibility to RSV infection. We then queried databases on human sequence variation and functional annotation for relevant single nucleotide polymorphisms (SNPs) and used to select HapMap cell lines with particular genotypes of interest. Enhanced viral load was found in cell lines with a SNP in MX1, which is involved in suppressing viral replication in infected cells. In a case control study of infants with mild or severe RSV infection, a positive association was found between individuals that were homozygous for the minor allele of the MX1 SNP and the development of severe RSV disease. We also developed a model to predict RSV infection using data from individuals in the HapMap collection. Baseline mRNA expression of HapMap individuals from six different microarray data sets were initially assessed for their correlation with viral load. We have identified cell lines (test set) that have similar expression profiles that will be evaluated for viral loads after RSV infection. Initial studies indicate ~75% accuracy among additional tested cell lines. This novel cell model of RSV disease can thus be used translationally to identify functionally relevant candidate susceptibility genes.
Characterization of Veterans’ Poisonings in the State of Florida

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The Florida Poison Information Centers provide emergency department consultation for suspected poisoning cases presenting to hospital emergency rooms, including Veterans Administration (VA) hospitals. The exposures, case demographics, treatments, and clinical outcomes are recorded for all reported cases. Evidence suggests that veterans may be at increased risk for poisoning. The objective of this study was to characterize patterns associated with poisonings in veterans presenting to VA hospital emergency rooms. A total of 601 poisoning cases from 6 VA hospitals in the state of Florida occurring between the years 2005 and 2009 were evaluated. The population was predominately male (88.1%) with a mean age of 51.5 years (STD 13.7 years). Nearly half (46.2%) of all cases were classified as intentional suspected suicide and 87.6% of poisonings occurred in the patient’s residence. Though death was a rare event (2 cases), 37.7% of cases resulted in clinical outcomes that were considered ‘major’ or ‘moderate’. More than 50% of cases reported a medication as one of the substances implicated in the poisoning. Future research into poisoning interventions among veterans may focus on patient suicide risk, medication monitoring, and medication safety education.

Role of Sensory TRP Channels in Cutaneous Injury by Veniscants and Riot Control Agents

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Transient Receptor Potential (TRP) channels are non-selective cation channels implicated in chemosensation in sensory neurons and other cells. Recently we identified TRP Ankyrin 1 (TRPA1) as the receptor for riot control agents such as CS or CN, oxidants and other reactive irritants, mediating pain and acute irritant responses. Previous studies have shown that agonists of TRPV1 diminished cutaneous veniscant injury elicited by chloroethyl-sulfide (CEES), an analog of sulfur mustard. Here, we hypothesized that TRP channels are involved in cutaneous injury and inflammation by veniscants and riot control agents. Veniscant injury by CEES was characterized in the mouse ear veniscant model in C57BL6 wild-type mice and Trpap1-/- mice. Application of CEES caused a rapid increase in ear thickness, plasma extravasation and production of inflammatory markers such as MMP-9 and IL-1β. Edema, extravasation and marker production, at 24h post-injury, were significantly inhibited by treatment with the TRPA1 antagonists, HC-030031, or A-967079, administered 1h, 8h and 16h post-exposure. Injury parameters showed no rebound, indicating that additional dosing is not required. Trpap1-/- mice showed exaggerated CEES injury, suggesting that TRPA1 has a protective role in the early stages of the injury, but fulfills a pro-inflammatory role at a later time. SB-366791, a TRPV1 antagonist, did not diminish CEES injury parameters. Application of the riot control agent, CS, caused oedema, plasma extravasation and expression of inflammatory cytokines (IL1-β and MIP-2) in the mouse ear. A-967079, administered 30min and 4h post injury, strongly inhibited CS-induced extravasation and marker formation. Trpap1-/- mice were protected from CS-induced plasma extravasation, suggesting TRPA1 is directly involved in neurogenic inflammatory tissue responses.

A Putative Interaction of TSPO with NADPH Oxidase (NOX2) in Primary Microglia


Translocator Protein (18 kDa) (TSPO) is a giall protein that is extensively used as a biomarker of active brain disease in neurodegenerative conditions and to assess neurotoxicity (Chen & Guilarte, Pharm Ther 118: 1-17, 2008). TSPO ligands (TSPO-L) induce cellular functions consistent with microglia activation suggesting an important role in the inflammatory response of the brain to injury (Choi et al., GLIA 59: 219, 2011). Primary microglia exposed to TSPO-L (1-100 nM) induces ROS production that is abrogated by NOX2 inhibitors. To further elucidate the source of ROS production induced by TSPO-L, we measured extracellular and intracellular ROS in the presence of the NOX2 inhibitor apocynin and cyclosporin A, a microchannel permeability transition pore inhibitors. TSPO-L induced ROS production was inhibited by apocynin but not by cyclosporin A, suggesting that the source of ROS production is from NOX2 and not from mitochondria. Co-exposure of TSPO-L with phosphol-12-myristate 13-acetate (PMA), a NOX2 activator, enhanced ROS production and increased the TSPO percent colocalization with NOX2 above PMA alone. Thus, PMA-induced activation of NOX2 may modulate the effects of TSPO-L. Since activated NOX2 is a plasma membrane protein complex, our findings questioned the subcellular localization of TSPO in microglia as TSPO is traditionally viewed as a mitochondrial protein. Triple label immunofluorescent confocal imaging of TSPO with the gp91phox subunit of NOX2, and MitoTracker revealed a high degree of TSPO colocalization with cytosolic and nuclear gp91phox and a lower degree of colocalization in mitochondria. Colocalization of TSPO with NOX2 was also observed in brain tissue from Sandhoff disease mice at an age when the brain is undergoing neurodegeneration. These findings suggest a novel interaction of TSPO with NOX2 in microglia. The results have significant implications for understanding microglia TSPO biology and for the development of potential therapeutic strategies for the treatment of neuroinflammation [Supported by grant ES007962 to TRG].

Neuregulin-1 is Neuroprotective Against Delayed Neuronal Injury Following Acute Organophosphate Intoxication

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Organophosphate (OP) compounds include nerve agents that have been used in military combat and against civilians by terrorists. OP nerve agents are the most toxic and rapidly acting of the known chemical warfare agents. Current post-exposure medical countermeasures against OP nerve agents are useful in reducing mortality, but do not sufficiently protect the brain from delayed neuronal injury and persistent neurological symptoms. In this study, we examined the efficacy of neuregulin-1 (NRG-1) in protecting against acute intoxication with the OP disopropylfluorophosphate (DFP). Pretreatment with NRG-1 did not protect against seizures and mortality in adult male Sprague Dawley rats exposed to DFP (9 mg/kg BW, i.p.) in the absence of standard antidote. Pretreatment with pyridostigmine (0.1 mg/kg BW, i.m.) and atropine methylate (20 mg/kg BW, i.m.) increased survival but did not protect against delayed neuronal injury as determined by extensive Fluorjade-B labeling in multiple brain regions at 24 hours post-DFP injection. However, neuronal injury was significantly reduced in most brain regions by additional pretreatment with NRG-EGF (3.2 μg/kg BW, i.a.) or NRG-GGF2 (48 μg/kg BW, i.a.). NRG-1 also blocked apopoptosis and oxidative stress-mediated protein damage in the brains of DFP intoxicated rats. Administration of NRG-1 at 1 hour after DFP injection also provided significant neuroprotection against delayed neuronal injury. These studies indicate that NRG-1 may represent a novel, potent neuroprotective strategy that has potential therapeutic value in treating individuals after exposure to OP nerve agents. These results further indicate that NRG-1 could be a useful addition to existing antidotal treatment for OP exposure. This research is supported by the CounterAct program, National Institutes of Health Office of the Director, Grant # U01 NS07993.

Mementine and Riluzole do not Reverse the Neuotoxic Effects Resulting from Chronic Exposure to Depleted Uranium

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Continuing concern exists for veterans retaining fragments of depleted uranium (DU) shrapnel from Gulf War I and the resulting CNS effects. Earlier work demonstrated that rats exposed to DU for 15-17 months exhibited increases in evoked extracellular glutamate and NMDA receptor density, and suggested one basis as increased production of oxygen radicals. The goal of this study was to determine if currently prescribed therapeutic drugs can reverse these actions of chronic exposure in vivo, and to assess various markers of oxidative stress. Male Sprague-Dawley rats had 0, 300, or 600 mg of DU pellets implanted intramuscularly at 70 days of age. After 7 months exposure osmotic minipumps were inserted subcutaneously so as to deliver mexitil and riluzole, or the combination for two months during continuing DU exposure. Animals were sacrificed after 9 months exposure and the brains harvested. MK-801 receptor binding in frontal cortex was...
increased 83-96% by DU exposure in agreement with previous observations in hippocampus and parietal cortex; memantine and/or riluzole did not eliminate these changes but caused significant NMDA receptor up-regulation in non-exposed control animals. Cerebellar 8-isoprostane levels exhibited small increases in DU-exposed rats not receiving drugs, while memantine reduced concentrations of this lipid peroxidation marker in all groups, but did not reverse the DU effect. This duration of DU exposure did not produce changes in cortical glutathione peroxidase or cerebellar catalase activities. These findings indicate that the most robust CNS effect of chronic DU is increased access to the NMDA receptor ion channel. While the basis for this observation is unknown, it is possible that increased access to the NMDA receptor ion channel limits the acute behavioural effects of systemic inflammation, thereby limiting the acute behavioural effects of systemic inflammation. In conclusion, our data indicates that chronic administration of ERW resulted in an enhanced activity of Nrf2, regulated at baseline, and its transcriptional activity is further enhanced in mice drinking ERW than in control mice at 2 and 4h, but not at later timepoints. We also assessed the expression of enzymatic antioxidants and found that Nrf2 is up-regulated at baseline, and its transcriptional activity is further enhanced in mice drinking ERW during systemic inflammation. In conclusion, our data indicates that chronic administration of ERW resulted in an enhanced activity of Nrf2, which optimized the neuroinflammatory and antioxidant response in the hippocampus during systemic inflammation, thereby limiting the acute behavioural effects of systemic inflammation. 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Neonatal hypoxia-ischemia (HI) affects 60% of low birth weight infants and up to 40% of preterm births. Severe cases of HI lead to immediate necrosis of neuronal cells, and a secondary apoptosis of surrounding cells due to neuronal inflammation. Our lab has determined that Bax, a cell death signaling protein, is activated after HI and translocates to the nucleus, mitochondria, and ER and thus affects the cell death phenotype (neurotic or apoptotic) observed. Using the well-established Rice-Vannucci in vivo model, as well as an in vitro model of neonatal cerebral HI, we determined whether HI-dependent phosphorylation of Bax determines its multi-organ-ganle localization, and ultimately the cell death phenotype observed. Given that active Bax is known to oligomerize, we characterized Bax oligomerization via western blot analysis of cortical tissue ipsilateral to the injury using an antibody specific to phospho-Bax. We quantified the bands that correspond to dimer, tetramer, and hexameric forms of Bax. Consistent with our previous results, total Bax levels remained constant with no significant increases from 0-4h after HI. We observed a biphasic reaction to HI, in that there was a significant increase in levels of p-BaxThr167 in the nucleus at ½h after HI, and no changes afterward until 6h and 12h, where there was an increase in nuclear and mitochondrial p-BaxThr167 when compared to shams. There was no significant change in p-Bax levels after 12h HI, which is consistent with our previous data that shows that Bax activity is an early event following HI. Additionally, with atomic force microscopy, we were able to visualize the oligomerization of Bax after phosphorylation by JNK. These results indicate that Bax is phosphorylated after HI and that phosphorylation by JNK induces oligomization of the protein. Understanding the mechanisms of Bax translocation will aid in the rational design of specific therapeutic strategies which could potentially involve altering Bax subcellular redistribution to decrease the irreversible trauma resulting from a prolonged inflammatory response.
exposed to either filtered room air or mainstream cigarette smoke for 3 hours, 5 days per week. The dose was adjusted to generate 300mg/m3 total particulate matter, as measured by gravimetric sampling. This dose has been found to generate COHb levels of 20% in mice, and to induce emphysematous lung changes. To our surprise, mice exposed to smoke, while not having major changes in fracture callus composition by micro-CT, histomorphometry, or qPCR, had enhanced vascularization of the fracture callus by vascular CT, associated with increased VEGF-induced angiogenesis that is likely due to nicotine. This is despite the utilization of an exposure paradigm that is well-established in the pulmonary field and that has been shown to induce emphysematous changes. We attribute the failure to establish a model of delayed healing on over-reliance on an established model with insufficient consideration with the toxicokinetics of smoke constituents, and offer this study as a lesson for those undertaking similar studies.

966 EFFECTS OF CACHE VALLEY PARTICULATE AIR POLLUTION ON PULMONARY FUNCTION, AND DISEASE BIOMARKERS IN HUMAN VOLUNTEERS: A PRELIMINARY STUDY.

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Some of the worst particulate air pollution ever measured in the United States regularly occurs during winter inversions in the Cache Valley of Northern Utah. We have shown that Cache Valley PM2.5 (CVPM) induces pro-inflammatory interleukins, C-reactive protein (CRP), and post-translational modifications associated with ER stress, and the unfolded protein response in vitro. In this preliminary study, we sought to determine whether exposure to ambient CVPM would alter pulmonary function, and serum biomarkers of cardiopulmonary inflammation in young, healthy human volunteers (age 18-35 years; n=38) during each of three periods of pre-inversion baseline (PM2.5 < 35 μg/m3), inversion (PM2.5 > 35 μg/m3) and post-inversion periods. There were significant CVPM-associated decreases in both forced vital lung capacity (FVC) and in forced expiratory volume in the first second (FEV1) during unhealthy air days compared to both pre-inversion baseline and post-inversion periods, and a decline, though not statistically significant, in FEV1/FVC ratio between unhealthy air and healthy air recovery days. Serum IL-6, IL-8, and IL-12 all showed significantly elevated levels in subjects self-reporting to be sick during inversion periods compared to pre- and post-inversions; these differences were not observed in healthy subjects. While these results were obtained from a limited sample population, the data provide further evidence that adverse health effects are associated with exposure to CVPM, and provide compelling justification for further study. Supported in part by the Marriner S. Eccles Foundation.

967 ANALYTICAL APPLICATIONS OF FOURIER TRANSFORM-INFRARED (FT-IR) SPECTROSCOPY IN THE CHARACTERIZATION OF CANDIDA.

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Fourier-transform infrared (FT-IR) spectroscopy is known as a high resolution method for the rapid typing of pure cultures of microorganisms, is used as a whole-organism fingerprinting technique since it reflects the bio-chemical composition of intact cells. Through the use of infrared spectroscopy was possible to make a no subjective classification clinically relevant on a series of species of Candida evaluated in the present study, in this way was possible to corroborate some data values obtained in other previously studies. The differentiation capacity of the FT-IR microscopic technique was tested for twenty-eight strains. Candida sp. were cultured on Sabouraud chromolephonic agar at 37°C for 24 h. 4 mg of each sample lyophilized was mixed with 100 mg potassium bromide (KBr). A Shimadzu 8400S FT-IR spectrometer was used, pellets were scanned at 6 cm-1 resolution in the spectral range of 4000-500 cm-1. Cluster and factor analysis methods were used to evaluate the complex spectral data. Excellent discrimination between yeasts and bacteria, and at the same time Gram-negative and Gram-positive bacterial strains was also obtained. Twenty-four selected strains of different species within the genus Candida were repetitively measured and could be grouped into correct species clusters. By exploiting the huge discriminating capacity of this technique, we identified 6 species yeasts (Candida albicans, Candida tropicalis, Candida parapsilosis, Candida krusei, Candida guillermondii and Candida lusitaniae) and from a collection of clinical strains of Candida, isolated from vaginal secretion. Our results clearly show the efficiency and the robustness of FTIR spectroscopy in identifying species with a classification rate of 100% for pure 24-h cultures. FTIR spectroscopy is thus a promising clinical approach, because compared to conventional and molecular techniques, it is time and money saving, has great determination and discriminating potentials, and is amenable to an automated high-throughput routine system.
A family of five and pet dog who rented a water-damaged home and developed multiple health problems are presented. The home was analyzed for species of mold and bacteria. The diagnostics included MRI for chronic sinusitis with ENT evaluation and sinus surgery, and neurological testing for CNS complaints. Bulk samples from the home, tissue samples from the sinuses, urine, nasal secretions, placenta, umbilical cord and breast milk were tested for the presence of macromycete trichothecenes, aflatoxins and ochratoxin A. The family had the following diagnosed: chronic sinusitis, neurological deficits, coughing with wheeze, nose bleeds, fatigue among others. An infant was born with a total body flare and developed multiple Cafe-au-Lait pigmented skin spots. The mycotoxins were detected in bulk samples as well as body fluid (urine, and nasal secretions) as well as in the breast milk, placenta and umbilical cord. Pseudomonas aeruginosa, Acinetobacter, Penicillium and Aspergillus fumigatus were cultured from nasal secretions from the breast milk, placenta and umbilical cord. Pseudomonas aeruginosa, Acinetobacter, Penicillium and Aspergillus fumigatus were cultured from nasal secretions from the father and daughter. RT-PCR revealed A. fumigatus DNA in sinus tissues of the daughter. Finally, the dog developed approximately 72 skin (sebaceous glands and lipomas) from which trichothecenes and ochratoxin A were identified. The identified mold and bacteria as well as the health of the family are discussed in relation to the most recent published literature regarding microbial contamination and toxic by-products present in water-damage.

Formulations of ASA and IS2 derived from human cells have been developed for direct central nervous system (CNS) delivery as enzyme replacement therapies (ERTs) for Hunter syndrome and other lysosomal storage diseases. Previous studies suggest that a direct administration to the CNS via the IT space of the spinal canal may be a relatively safe and effective method for delivery of ERTs. The objective of this study was to evaluate the PK of ASA and IS2 in CSF after IT administration to cynomolgus monkeys. Therefore, a total of twelve male and twelve female monkeys with IT-lumbar (ITL) and IT-cisterna magna (ITCM) catheters were randomly assigned into four treatment groups for IS2 administration (IV = 0.5 mg/kg and IT = 1, 10 and 30 mg) and ASA administration (IV = 1 mg/kg and IT = 1.8, 6 and 10 mg). CSF samples were collected at specified intervals post dosing after the administration of each enzyme treatment for PK analysis. Various compartmental models were constructed using a population approach and their respective ability to fit CSF concentration of ASA and IS2 following IT administration was evaluated using a standard model discrimination process including statistical criteria as well as pertinent graphical representations of goodness-of-fit. Concentrations in the CSF both ASA and IS2 following IT administration were well characterized by a 3-compartment model. Compartmental PK parameters in CSF were derived for ASA (VsCSF = 16.9 mL and CLCSF = 0.973 mL/h) and IS2 (VsCSF = 10.5 mL and CLCSF = 1.22 mL/h). Volume of distribution (Vs) of ASA and IS2 were close to the CSF total volume reported in the literature. Total exposure in CSF predicted with the model across doses levels for ASA (range: 278103 to 16485500 ng.h/mL) and IS2 (range: 278103 to 16485500 ng.h/mL) will be used to perform exposure-response analyses in monkeys and ultimately scale PK and PD in humans.

Formulations of ASA and Idursulfase IS2 derived from human cells have been developed for direct central nervous system (CNS) delivery as enzyme replacement therapies (ERTs) for Hunter syndrome and other lysosomal storage diseases. Previous studies suggest that a direct administration to the CNS via the IT space of the spinal canal may be a relatively safe and effective method for delivery of ERTs. The objective of this study was to evaluate the systemic (SYS) PK of ASA and IS2 in serum of cynomolgus monkeys after intravenous (IV) administration.
female monkeys were randomly assigned into four treatment groups for IS2 administra-

tion (IV = 0.5 mg/kg and IT = 1, 10 and 30 mg). Administration (IV = 1 mg/kg and IT = 1, 10 and 30 mg). Serum samples were collected at specified intervals post dosing after the administration of each enzyme treatment for PK analysis. Various compartmental models were constructed using a population approach and their respective ability to fit serum concentration of ASA and IS2 following IV administration was evaluated using a standard model discrimination process including statistical criteria as well as pertinent graphical representations of goodness-of-fit. Concentrations in serum for both ASA and IS2 following IV administration were well characterized by a 2-compartment model. Compartmental PK parameters were derived for serum ASA (Vss = 1432 mL, CL = 253 mL/h and CLd = 509 mL/h) and serum IS2 (Vss = 409 mL and CL = 149 mL/h). Total volume of distribution of ASA and IS2 in serum is greater than plasma volume (~224 mL) but lower than total body water (~3465 mL/kg) in monkeys, suggesting distribution to peripheral tissues and organs such as the CNS. Total exposure ASA and IS2 in serum were 10505 and 8671 ng/h/mL, respectively. The above model will be used to perform exposure-response analysis in monkeys and ultimately scale the PK of ASA and IS2 to humans.

 formulations of ASA and IS2 derived from human cells have been developed for direct CNS delivery as enzyme replacement therapies (ERTs) for Hunter syndrome and other lysosomal storage diseases. Previous studies suggest that a direct administration to the CNS via the IT space of the spinal canal may be a relatively safe and effective method for delivery of ERTs. The objective this study was to evaluate the systemic (SYS) PK of ASA and IS2 in monkey serum after IT dosing and the CNS PK of ASA and IS2 in cerebro-spinal fluid (CSF) after IV dosing in order to evaluate the intercompartmental relationship between the CSF and SYS spaces. Therefore, a total of 12 male and 12 female monkeys with IT-lumbar (IT-L) and IT-cisterna magna (IT-CM) catheters were randomly assigned into four treatment groups for IV administration (IV = 0.5 mg/kg and IT = 1, 10 and 30 mg) and ASA administration (IV = 1 mg/kg and IT = 1.8, 6 and 10 mg). Serum and CSF samples were collected at specified intervals post dosing after the administration of each enzyme treatment for PK analysis. Available serum (2-compartment) and CSF (3-compartment) models were interlinked to determine the intercompartmental transfer rate constants. The transfer rate constants from serum to CSF (KIN) for ASA and IS2 were 0.258 and 0.00489 h⁻¹, respectively. The transfer rate constants from serum to CSF (KOUT) for ASA and IS2 were 0.258 and 0.00489 h⁻¹, respectively. The transfer rate constants from CSF to serum (KOUT) for ASA and IS2 were 0.779 and 0.374 h⁻¹, respectively. Overall, ASA and IS2 enzymes displayed similar intercompartmental distribution, but presented some differences regarding the rate and extent of distribution between CSF and serum. As expected, transfer from serum to CSF was the rate limiting process, with an equilibration half-life of ~3 hours. The above semi-physiological model will be used to perform exposure-response analysis and ultimately scale the PK of ASA and IS2 in serum and CSF to humans.

A formulation of Idursulfase (IS2) derived from human cells has been developed for direct central nervous system (CNS) delivery as enzyme replacement therapy for Hunter syndrome and other lysosomal storage diseases. Previous studies suggest that a direct administration to the CNS via the IT space of the spinal canal may be a relatively safe and effective method for delivery of enzyme replacement therapies. The objective this analysis was to characterize the relationship between IS2 concentration and its activity measured in CSF after IT and IV administration to cynomolgous monkeys. A total of twelve male and twelve female cynomolgous monkeys with IT-lumbar (IT-L) and IT-cisterna magna (IT-CM) catheters were randomly assigned into four treatment groups for IS2 administration (IV = 0.5 mg/kg and IT = 1, 10 and 30 mg). CSF samples were collected at specified intervals post dosing and were analyzed to determine the PK of IS2 as well as PD activity which was measured using enzymatic activity. The relationship between IS2 concentration and activity was assessed using a linear regression on log-transformed values where the relationship between IS2 concentration and activity measured in CSF was explained with the following power model: Activity IS2 = b x CSF²/m, where ”b” is the intercept (-1.3) and "m" is the slope (-0.92). As a result, the PK/PD relationship of IS2 was deemed linear over the dose range (slope was close to 1), suggesting a very rapid equilibrium between CSF concentration and observed activity. A similar relationship between serum PK and activity was also observed, suggesting that serum can be sampled clinically. CSF or serum concentrations of IS2 can be used as a surrogate marker of activity to demonstrate the feasibility of constructing exposure-response relationships in animals and ultimately extrapolated to humans.

The abuse of cocaine costs the economy billions of dollars and its illicit use remains a major challenge for public health authorities and law enforcement agencies. There is a need to develop improved, simple, portable, low cost aptamer-based sensors for specific, sensitive, rapid and real time detection of cocaine in solution and unprocessed samples. Synthetic oligonucleotide aptamers recognize virtually any class of target molecule with high affinity and specificity. Here we report a 2-amino purine (2-AP)-modified cocaine aptamer with increased affinity for cocaine compared with other reported cocaine aptamers. Atomic molecular simulations of the aptamer-ligand interaction are underway to elucidate the molecular recognition process and to identify possible explanations for the increased cocaine affinity with 2-AP modification. Thermodynamic evaluations by isothermal titration calorimetry and fluorescence quenching were used to evaluate a series of 2-AP-modified cocaine aptamers to identify that with the highest affinity for cocaine. The aptamer with the highest affinity was used to develop a fluorescence-based homogeneous assay to detect cocaine. The limits of aptamer functionality in undiluted and unprocessed biological fluids such as blood serum, saliva and urine will be reported.

A formulation of Idursulfase (IS2) derived from human cells has been developed for direct central nervous system (CNS) delivery as enzyme replacement therapy for Hunter syndrome and other lysosomal storage diseases. Previous studies suggest that a direct administration to the CNS via the IT space of the spinal canal may be a relatively safe and effective method for delivery of enzyme replacement therapies. The objective this analysis was to characterize the relationship between IS2 concentration and its activity measured in CSF after IT and IV administration to
Thus current results suggest that BMAA may act on ER-coupled mechanisms of protein homeostasis. Mis-incorporation of BMAA into the ER leads to misfolded proteins and an accumulation of oxidized proteins, both hallmarks of neurodegenerative diseases, could therefore be involved in the etiology of ALS/PDC linked to chronic BMAA exposure.

PARKIN-DYSFUNCTION AND α-SYNUCLEIN AGGREGATION LEAD TO CELL DEATH IN METHAMPHETAMINE (METH)-TREATED RHEUS MONKEYS AND HUMAN METH USERS.

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Numerous studies have reported that METH induces dopaminergic (DAergic) neurotoxicity and nerve terminal damage via the induction of apoptotic pathways. We have shown that oxidative stress-mediated induction of a tyrosine kinase c-Abl disrupts parkin-dependent ubiquitin-proteasome (UPS) function, leading to DAergic cell death in animal models of Parkinson’s disease (PD) and in human patients. Here, we investigated whether METH-induced oxidative stress leads to parkin-dependent dysfunction of UPS in rhesus monkeys and in human METH users. We also determined if METH-treatment could induce α-synuclein aggregation. METH treatment significantly activated c-Abl leading to an increase in parkin phosphorylation and inactivation in striatum of rhesus monkeys. Inactivation of parkin resulted in significant accumulation of p38/AIMP2, a toxic substrate of parkin known to cause DAergic cell death. Significant increases in a highly active c-Abl, a hyperphosphorylated parkin, and accumulations of p38/AIMP2 were also observed in the striatum of human METH users as compared to their age-matched controls. Significant aggregation and hyperphosphorylation of α-synuclein were also observed in the striatum of both METH-treated rhesus monkeys and human METH users. A significant depletion of dopamine (60%) and its metabolites DOPAC (55%) and HVA (65%) was observed in striatal tissues of METH-treated rhesus monkeys and human METH users. We also observed significant increases in the expression HNE-protein adducts in the striatum of METH-treated monkeys and human METH users as compared to control subjects, suggesting a significant increase in oxidative stress. These results suggest a universal mechanism of dopaminergic neuronal cell death mediated by oxidative stress-induced UPS dysfunction resulting in toxic protein aggregation and by α-synuclein-aggregation due to DAergic dysfunction.

PHARMACOKINETIC STUDY AND EVALUATION OF THERAPEUTIC EFFECTS OF WATER SOLUBLE COQ10 IN A PARAQUAT-INDUCED RAT MODEL OF PARKINSON’S DISEASE.

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Parkinson’s disease (PD) is a progressive neurodegenerative disorder affecting 4 million people worldwide. Environmental toxins leading to oxidative stress and mitochondrial dysfunction causing neuronal death, have been implicated in the development of sporadic form of PD. Exposure to paraquat, a well known herbicide/insecticide, has been correlated to the incidence of PD in human. Currently there is no treatment available that can halt the progression of this disease. We have established a paraquat-induced model of PD in rats. We have evaluated therapeutic potential of a water-soluble formulation of CoQ10 (WS-CoQ10) in this model where WS-CoQ10 was fed after 5 injections of paraquat. We have observed that post-insult feeding of CoQ10 in drinking water blocked the progression of loss of dopamine neurons, decreased oxidative stress in brain tissues and led to better behavioral performances (as indicated by Pole test and vertical climb test) compared to paraquat treated animals fed with un-supplemented water. There was efficient absorption of WS-CoQ10 formulation as the level of CoQ10 doubled in brain and liver tissues of animal fed with supplemented water compared to control. In addition to significant protection of DA neurons in the SNpc, we also observed activated astroglia and increased levels of brain derived neurotrophic factor (BDNF) and glial derived neurotrophic factor (GDNF) in WS-CoQ10 fed rats. We have shown previously the prophylactic effect of WS-CoQ10 treatment, that is WS-CoQ10 prevents the loss of dopamine neurons in the SNpc and ameliorates the symptomatic effects of paraquat-induced PD (Thakur et al., 2010). Therefore the formulation of CoQ10 would be useful to slow or even halt the progression of Parkinson’s disease.

PATHOPHYSIOLOGY OF BAC-TRANSGENIC RATS EXPRESSING PARKINSON’S DISEASE CAUSING MUTATIONS.

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Parkinson’s disease (PD), in most cases is thought to arise from both genetic and environmental influences. Cases arising from purely genetic influences are rare, but provide much insight into the pathogenic features of the disease. Rodent models of genetic PD have been largely unable to replicate the key pathological features of the disease. Here, transgenic rats expressing disease causing mutations in α-synuclein (A53T) or E46K) or leucine-rich repeat kinase 2 (LRRK2, G2019S or R1441G) were created using bacterial artificial chromosomal technology. These rats were assessed at 12 months of age to determine if the key features of the disease were replicated. No overt loss of nigral dopamine cell bodies or striatal dopamine terminals was found by immunohistochemistry. These results indicate that there is not an obvious lesion to the nigrostriatal dopamine system. However, alterations in dopaminergic metabolites, nitrotyrosine levels, and α-synuclein accumulation/aggregation indicate that features of the earliest disease stages may be replicated. Thus, these animals may be ideal for modeling preclinical PD and for potentiating studies with other stressors, such as environmental exposures. Preliminary experiments also indicate that the transgenic animals exhibit heightened sensitivity to rotenone, as evidenced by increased loss of striatal dopamine terminals. Rotenone is a pesticide that has been linked to human PD and replicates the key disease features. In summary, while these animals do not exhibit features of late-stage PD, they are ideal for assessing gene-environment interactions and may be useful in modeling the earliest stages of the disease. Supported by the NIH (NIEHS 1R01ES019879-01, J.R.C.) and the Michael J. Fox Foundation (J.T.G. and C.L.)

Expression of mutant huntingtin impairs striatal manganese (Mn) transport and Mn-dependent enzyme activities by a novel cellular mechanism.


We have previously reported that expression of mutant htt is associated with impaired Mn accumulation in a striatal cell line and mouse models of Huntington’s disease (HD). To explore the consequences of altered Mn homeostasis, we have measured the levels and activity of cortical and striatal arginase, a manganese-containing enzyme. We report that arginase activity exhibits region specific Mn exposure by genotype interactions. To investigate the underlying cause of the Mn transport deficit we used the cellular fura-2 manganese extraction assay (CFMEA) and cellular fura-2 loaded kinetic assays to measure Mn dynamics. We show that cells expressing mutant htt have decreased Mn uptake within minutes of Mn exposure, and decreased total Mn storage capacity yet maintain equivalent concentration dependence. Furthermore, the presence of other di-/trivalent metals, serum proteins, or known co-transporter toxins in the culture media and exposure buffers are not required for this effect. Inhibition of divalent metal transporter 1 (DMT1), as well as a2-APB- and ruthenium red blockable channels, PPAR-gamma, or the mitochondrial transition pore does not affect this Mn uptake deficit. Therefore we are now testing other recently identified Mn transporters for their contribution to this phenotype, including HIP14, PARK9, and Ferroportin 1. Finally, we report that excess divalent metals (Mg2+, Ca2+, and Sr2+) substantially influence Mn uptake and survival in the striatal cells and ameliorate the differences between wild-type and mutant striatal HD cells. We are currently utilizing shRNA knock-down strategies to identify the major transporter(s) responsible for the altered Mn homeostasis phenotype in HD striatal cells and animal models. In conclusion, our data strongly suggest that the HD mutation causes a defect in Mn uptake and storage capacity, which contributes to alterations in the activity of Mn-dependent enzymes in the brain. Supported by NIH/NINDS ES016921, ES016931S1, ES016931S2 and T32 ES007028.
Alzheimer's disease (AD) is the most common age-related neurodegenerative disease affecting millions of people worldwide. Strong evidence supports the role of the amyloid-β peptide (Aβ) induced oxidative stress (OS) and neuroinflammation in the pathophysiology of AD. In brains of AD patients, levels of neuroinflammatory cytokine TNF-α and oxidized proteins, by-products of lipid peroxidation such as acrolein and DNA damage markers are significantly higher in vulnerable brain regions. Bacopa monniera (BM) and Withania somnifera (WS) have a long history of use in memory-enhancing therapy but there is dearth of studies on their neuroprotective effects. The objective of this study was to investigate whether BM and WS extracts can protect against Aβ peptide and hydrogen peroxide -induced toxicity and modulate various redox regulated pathways. We demonstrate that a treatment with BM and WS extracts significantly protected the human neuroblastoma cell line SK-N-SH against Aβ peptide in various cell survival assays. Furthermore, a treatment with BM extract significantly reduced H2O2 and TNF-α induced intra-cellular reactive oxygen species (ROS) generation in SK-N-SH cells. Finally, our results show that BM extract is also a potent inducer of nuclear factor E2-regulated factor2 (Nrf2) and Sirt1 pathways. BM extract also inhibited the TNF-α-induced reactive oxygen species (ROS) generation in SK-N-SH cells. WS extract also inhibited the acetyl cholinesterase (AChE) activity. Thus, our findings indicate that BM and WS extracts may act as an antioxidant and inflammatory modulator and may have beneficial effects in the AD therapy.

Increasing data suggests that concentrated ambient particulate matter (CAPS) adversely impacts brain and could represent a largely underestimated contribution to CNS diseases. Early development may be a period of particular vulnerability of brain to CAPS damage. This study examined the hypothesis that early life postnatal CAPS exposure would sensitize the CNS to subsequent adult challenge with paraquat + maneb (PQ+MB), a well-established pesticide-based model of the Parkinson’s disease phenotype (PDP). Following CAPS exposure from PND 4-8 & 10-13 days, mice were subjected to adult PQ (10 mg/kg) and MB (30 mg/kg) i.p. 2x week for 6 weeks. Mice exposed postnatally to CAPS were significantly more sensitive to the locomotor-reducing effects of PQ+MB than sham controls, or those exposed to CAPS alone or PQ+MB alone. CAPS and PQ+MB significantly altered catecholamine levels in both the nigrostriatal and mesocortical dopamine pathways. CAPS had a particular influence on striatum, increasing NE, DOPAC, and DA turnover (DOPAC/DA) levels, while PQ+MB generally impacted midbrain, decreasing 5HT, DOPAC and dopamine turnover. An interaction between CAPS and PQ+MB on cortical 5-HT levels was evidenced as a significant 5-HT decrease in PQ+MB treated animals compared to both sham controls and CAPS+PQ+MB treated mice. Cortical DA was also increased in PQ+MB treated animals. Collectively, these findings indicate that early life CAPS enhances locomotor reduction in response to PQ+MB challenge and changes striatal catecholamines (DAergic cell terminals), a region critical to PD while PQ+MB, in the same animals produces catecholamine changes in the ventral midbrain (DAergic cell bodies). Collectively this concurrent damage to striatum and midbrain could increase nigral cell death and thereby contribute to a PDP; future analyses will include assessment of TH immunoreactivity and stereological analysis of DAergic cell counts in the SNpc and VTA. Supported by R01-ES019105

Dysfunction of protein handling has been implicated as an important factor in the neurodegeneration that occurs in age-related Parkinson’s disease (PD). Inhibition of the ubiquitin-proteasome system (UPS) has been implicated in the formation of protein aggregates and Lewy bodies in PD. While proteasomal inhibition could trigger an array of downstream protein handling changes including up-regulation of heat shock proteins (HSPs), induction of molecular chaperones, activation of the ER stress/ unfolded protein response (UPR), autophagy and aggresome formation, little is known about the relationship of proteasomal inhibition to the sequence of activation of these diverse protein handling systems. In the current study, we utilized the proteasome inhibitor MG132 (carbobenzoxy-L-leucyl-L-leucyl-L-leucinal) and examined the activity of several major protein handling systems, in the immortalized dopaminergic neuronal N27 cell line. The data from these studies showed that MG132-induced proteasomal inhibition resulted in stimulation of the UPR and autophagic flux in the early phase (up to 6 hours after proteasomal inhibition) as determined by increased levels of phosphorylation of the eukaryotic translation initiation factor 2alpha (eIF2α), C/EBP homologous protein (CHOP) and turnover of autophagosome marker microtubule-associated protein 1 light chain 3 (LC3). Moreover, after prolonged proteasomal inhibition induced by MG132 (> 6 hours) we observed an increased cellular level of polyubiquitinated proteins, HSP70, the formation of vimentin-caged aggresomes and apoptosis. Our data suggest a potential link between proteasome inhibition and activation of other protein handling systems. These data also suggest that the mechanisms of induction of these alternate protein handling systems and their temporal relationship may be important parameters determining the extent of accumulation of misfolded proteins in cells as a result of proteasome inhibition. (Supported by NIH grant R01ES018943)
A combination of risk alleles and environmental stressors are thought to be the major cause of Parkinson's disease (PD): thus, patients sharing a common disease-causing gene can present with significantly different disease progression. We generated induced pluripotent stem cell (iPSC) lines from a control patient and two brothers who share compound heterozygous exonal deletions of the PARK2 gene, which result in complete loss of Parkin function. These cell lines were differentiated to neural progenitors expressing the early neuroectodermal marker Pax6. We assessed cell viability, oxidative stress, mitochondrial morphology, and oxidative stress transcriptional response after exposure to copper and manganese. The LD50 of copper exposure was significantly lower (61 μM) in PARK2 mutant cells than in control cells (130 μM) (n=8, p = 0.019) while the LD50 of manganese exposure was the same in each cell line. Copper exposure resulted in a significantly higher rate of reactive oxygen species production and mitochondrial damage while quantitative RT-PCR analysis indicated impairment in transcriptional activation of the stress response factor heme oxygenase-1 in Parkin mutants. These data suggest that loss of functional Parkin leads to impairment of appropriate mitochondrial stress and oxidative stress transcriptional response pathways, underscoring each as an important mechanism of normal Parkin function. Additionally, the role of copper as a stressor in each of these pathways implies a potential role for copper as a PD risk factor. This work has been supported by the following grants: NIH/NIEHS ES016931, Peterson Foundation for Parkinson's, Vanderbilt Center for Molecular Toxicology Pilot Project, and Subaward RR166-737/4787736 under NIH/NIGMS SPO1 GM08553403.

Inflammatory activation of glia is implicated in the progressive loss of dopaminergic neurons in Parkinson's disease (PD). Suppression of neuroinflammation may prove useful in slowing continuous neuronal degeneration. In the present study we investigate the efficacy of nuclear factor kappa B (NF-kB) inhibition in attenuating progressive dopaminergic neuron loss. We established a model of neurodegeneration employing MPTP in conjunction with probenecid in transgenic NF-kB-EF1α reporter mice in which we observed a progressive reduction of TH positive neurons assessed by stereology. In addition, robust activation of astrocytes and microglia was observed that correlated with increased activation of NF-kB. Using this model, we assessed the efficacy of novel para-substituted diindolylmethane (cDIM) compounds in attenuating continual neuron loss in vivo. cDIM (1,1-bis(3'-indolyl)-1-(p-methoxy)-methylamine), which induces downregulation of prototypic neuroinflammatory gene NOS2 in primary astrocyte cultures, was given via oral gavage (50 mg/kg) once daily to mice following 7 days of MPTP treatment. Stereological assessment revealed a significant attenuation of dopamine neuron loss in animals treated with cDIM, as well as decreased glial activation. This process was repeated using cDIM12 (1,1-bis(3'-indolyl)-1-(p-chlorophenyl)-methylamine), a Nurr1 agonist, that also attenuated dopamine neuron loss. Finally, mice deficient in astrocytic IKKβ were treated using the progressive MPTP and probenecid model and these animals displayed significantly less dopamine neuron loss than their wild type counterparts given the same treatment. These results suggest that NF-kB is an important pathway mediating neuroinflammatory activation of astrocytes leading to loss of dopamine neurons and that interfering this pathway may be a viable therapeutic option for slowing the progression of PD.

The release of pro-inflammatory mediators by activated astrocytes and microglia have been documented in neurodegenerative diseases such as Parkinson's disease and manganese-induced neurotoxicity. Furthermore, research from our laboratory...
has implicated the Nuclear Factor-kappa beta (NF-κB) in release of inflammatory molecules from activated glia; however, the relative impact of these activated glia on neuronal survival and the pathways involved are still poorly understood. In this study, we bred an human Glial Fibrillary Acidic Protein (βGFAP)-Cre mouse line with a floxed IkB kinase beta (IkKβ) mouse line to create a conditional IkB-beta knockout mouse (IkKβ/fl) in which the IkB-beta gene is specifically deleted in astrocytes of the CNS. In vivo comparison of IkB-beta staining in adult knockout mice to wild type (IkKβ/F) littermates revealed maintenance of the IkB-beta in neuronal populations of the basal ganglia, but staining was unsuccessful in glial populations. In vitro staining revealed deletion of IkB-beta in primary neonatal cultured astrocytes, but not in primary cultured microglia. Semi-quantitative analysis via Real Time Polymerase Chain Reaction revealed at least a 50% reduction in IkB-beta activity in primary cultured astrocytes as compared to wild-type cultures. Current data suggests the mouse has been successful in specifically deleting IkB-beta within astrocytes of the CNS and should be a useful tool to determine the role of astrocyte NF-κB in neurodegeneration.

**FUNCTIONAL CHARACTERIZATION OF ABC EFFLUX TRANSPORTERS IN MICROGLIA: IMPLICATIONS FOR THE THERAPY OF PROTEIN FOLDING DISEASES.**

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Efflux transporters are important in limiting cellular and tissue exposure to exogenous chemicals as well as potentially toxic endogenous products. Investigation of efflux transporters, such as Multidrug resistance protein 1 (MDR1), Multidrug resistance-associated proteins (MRP), and breast cancer resistance protein (BCRP), in the pathogenesis of neurodegeneration and neuroinflammation has only recently begun. The purpose of the present study was to characterize ABC efflux transporter function in microglia and examine the effect of microglial activation on the cellular efflux of known MDR1, MRP, and BCRP substrates. A panel of fluorescent transporter substrates and known inhibitors were used to characterize the relative contribution of efflux transporters to the extrusion of these substrates from BV-2 microglia cells using the Nucelom Vision cellometer. Under normal conditions, BV2 cells actively transported rhodamine 123, calcine AM, and Hoechst. Microglial activation also increased cell accumulation of the fluorescent Bodipy-conjugated anxiolytic drug, prazosin, respectively. Activation of microglia with lipopolysaccharide (LPS) significantly reduced efflux transport function in microglia and examine the effect of microglial activation on the cellular efflux of known MDR1, MRP, and BCRP substrates. A panel of fluorescent transporter substrates and known inhibitors were used to characterize the relative contribution of efflux transporters to the extrusion of these substrates from BV-2 microglia cells using the Nucelom Vision cellometer. Under normal conditions, BV2 cells actively transported rhodamine 123, calcine AM, and Hoechst. Microglial activation also increased cell accumulation of the fluorescent Bodipy-conjugated anxiolytic drug, prazosin, respectively. Activation of microglia with lipopolysaccharide (LPS) significantly reduced efflux transporter function, indicated by up to a 90% increase in cellular retention of rhodamine 123, calcine AM, and Hoechst. Microglial activation also increased cell accumulation of the fluorescent Bodipy-conjugated anxiolytic drug, prazosin, by 52% and the pro-apoptotic lipid, NBD-Ceramide, by 119%. Reduced efflux transporter function in activated microglia is consistent with decreased transporter mRNA and protein levels observed previously in these cells. Collectively, these results suggest that microglial activation during neuroinflammation may impair normal regulatory functions of microglia including the efflux of potentially toxic compounds. Supported by NIH ES-0205022, ES-005022, ES-015991, DK-080774, and 2Environmental & Occupational Medicine, University of Medicine and Dentistry of New Jersey, Piscataway, NJ.

**DECREASING THE TOXICITY OF HSP90 INHIBITORS FOR THE THERAPY OF PROTEIN FOLDING DISEASES.**

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The herbicide paraquat (PQ) has increasingly been reported in epidemiological studies to increase the risk of developing Parkinson’s disease (PD). Furthermore, case-control studies report that individuals with genetic variants in the dopamine transporter (DAT, SLC6A3) have higher PD risk when exposed to PQ. However, it remains a topic of debate whether PQ can enter dopamine (DA) neurons through DAT. We report here a novel mechanism by which PQ is transported by DAT: In its native divalent cation state, PQ2+ is not a substrate for DAT; however, when converted to the monovalent cation PQ+ by a reducing agent or NAPDH oxidase on microglia, it becomes a substrate for DAT and is accumulated in DA neurons, where it induces oxidative stress and cytotoxicity. Impaired DAT function in cultured cells significantly attenuated neurotoxicity induced by PQ+. Similar neuroprotection was also observed in mutant mice hypomorphic for DAT injected with this toxicant. In addition to DAT, PQ+ is also a substrate for the organic cation transporter 3 (Oct3, Slc22a13), which is abundantly expressed in non-DA cells in the nigrostriatal regions. In mice with Oct3 deficiency, enhanced striatal damage was detected after PQ treatment. This increased sensitivity likely results from reduced buffering capacity by non-DA cells leading to more PQ+ being available for uptake by DA neurons. This study provides a novel mechanism by which DAT and Oct3 modulate nigrostriatal damage induced by PQ+ redox cycling.
steroidselective neurotoxicity of some new anticonvulsant N,N'-disubstituted chiral spirohydantoins.

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Four new N'-1, N'-3'-disubstituted-2H, 3H, 5'H-spiro-(2-benzofuran-1,4'-imidazolidine)-2,3,5'-triones bearing chiral N-3' and C-4 spiro chiral centers were synthesized and resolved into their four diastereomers. Each was tested for anticonvulsant activity by subcutaneous pentetrazole test and maximal electric shock (MES) assay. Phenobarbital and DMSO were used as positive and negative controls, respectively. A dose of 300 mg/kg of the test compounds was administrated to mice intraperitoneally. Neurotoxicity was determined by the rotorod and righting reflex tests using doses of 30, 100 and 300 mg/kg. The S,5 diastereomer had no anticonvulsant activity. The R.R and S.R diastereomers showed significant anticonvulsant activity in scPTZ test at 300 mg/kg, when compared to DMSO controls. The R,S diastereomer displayed little anticonvulsant activity in the MES test at 300 mg/kg. Compounds showing anti convulsant activity at a high dose (300 mg/kg) were tested for neurotoxicity. Owing to their pharmacokinetics parameters, 30 minutes was allowed before testing for motor coordination and righting reflex. The compound bearing the R configuration in the 1-phenylethyl substituent at the N-3' position significantly impaired the motor activity, (p<0.05) compared to negative control. R.S- and S.R diastereomers did not impair motor activity, but demonstrated anticonvulsant activity. This suggests that compounds with R-spiro carbon configuration selectively act on hippocampal neurons inhibiting the neuronal firing without affecting basal ganglia, whereas S-chiral stereoisomers, act nonselectively on basal ganglia, causing impaired motor coordination. None of the compounds had an affect on the righting reflex.

995 Revised reference concentration for manganese oxide based on recent epidemiological and pharmacokinetic studies.

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In 1993, based on observations of subclinical neurological effects in workers, the United States Environmental Protection Agency (US EPA) published a reference concentration (RfC) of 0.05 μg/m³ for manganese (Mn). Over the last 18 years, numerous epidemiological, toxicological, and pharmacokinetic studies have been conducted that strongly support a revision to the current Mn RfC. We reviewed the most recent data and propose two alternate RfCs for Mn. Of 12 recent occupational studies of eight cohorts with chronic exposure durations, examining subclinical neurobehavioral effects, predominantly on the motor system, three were considered appropriate for development of an RfC, based on use of respirable Mn data, personal air monitoring, and evaluation of an unexposed control population. All three studies yielded no observable adverse effect levels (NOAELs) of approximately 60 μg/m³ respirable Mn. We also reviewed recent physiologically based pharmacokinetic (PBPK) modeling studies that predict similar Mn tissue concentrations (from Mn inhalation exposure concentrations ranging from 1-10 μg/m³) in the target brain region in the human fetus, nursing infant, and child compared to those in the mother and other adults, suggesting that concentrations below 10 μg/m³ Mn in air would not lead to accumulation of Mn in the brains of human fetuses, children, and adults. Based on our review, we determined uncertainty factors (UFs) were not necessary for developmental toxicity or for use of a subchronic or lowest observable adverse effect levels (LOAEL) study. We applied a UF of 10 to account for susceptible subpopulations (abnormal hepatobiological function or sub-optimal iron). Converting the occupational NOAEL (60 μg/m³) to a human equivalent concentration (HEC) of 21 μg/m³ (for continuous exposure) yields a RfC of 2 μg/m³. We derived a similar RfC (7 μg/m³) using a Mn benchmark dose (BMD) of 200 μg/m³ as the point of departure. These RfC’s fall below the recently proposed Mn accumulation threshold of 10 μg/m³, providing additional support for their application in Mn inhalation risk assessments.

996 Comparison of brain metabolite changes in manganese-exposed welders and smelters.

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Excessive manganese (Mn) exposure is known to cause cognitive, psychiatric and motor deficits. Mn overexposure occurs in different occupational settings, where the type and level of exposure may vary. Magnetic resonance imaging (MRI) and spectroscopy (MRS) can be used to evaluate brain Mn accumulation and to measure Mn-induced metabolic changes non-invasively. The aim of this study was to compare metabolite changes among different brain regions of welders and smelters following occupational Mn exposure. Nine Mn-exposed smelters, 14 Mn-exposed welders and 23 male matched controls were recruited from a cohort of workers from two factories in China (mean airborne Mn level: 0.227 and 0.025 mg/m³ for smelters and welders, respectively). Short-echo-time 1H MRS spectra were acquired in each subject from four volumes of interest: the frontal cortex, posterior cingulate cortex, hippocampus, and thalamus. We found that 1) in the frontal cortex, significantly decreased creatine (Cr), glutamate (Glu) and glutathione (GSH) were found in welders, whereas decreased Glu was found in smelters as compared to controls. 2) In the thalamus, reduced myo-inositol was found in both smelters and welders, while Glu and GSH were decreased in welders. These results suggest that Mn-induced brain metabolic changes may be regional in nature and more extensive in welders than in smelters. The frontal cortex seems to show a more profound change than the other brain areas tested among Mn exposed subjects. Further studies are needed to investigate the effects of exposure type and length on the mechanism of Mn neurotoxicity. (Supported by NIH/NIEHS R21 ES-017498, National Science Foundation of China Grant #81072320 and 30760210).

997 Baseline comparison of brain metabolites between rhesus monkeys and humans by rMRS.

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Magnetic Resonance Spectroscopy (MRS) provides a unique possibility to non-invasively detect and quantify brain metabolites in vivo. A variety of studies on manganese neurotoxicity are being performed in monkeys and in humans using MRS. However, little is known about the difference of brain metabolites between monkeys and humans using MRS. Here we compare the baseline difference of brain metabolites, especially of the major inhibitory neurotransmitter gamma-aminobutyric acid (GABA), between the two species using exactly the same imaging sequence on the same type of scanner. Brain metabolites of 7 rhesus monkeys (male, mean age 6.5y, about 20y in human years) and 8 healthy humans (male, mean age 26.5y) were acquired on a 3T Philips Achieva clinical MRSI scanner. GABA data was obtained from a brain volume containing thalamus and adjacent basal ganglia structures by the MEGA-PRESS sequence. Other brain metabolites were obtained and scaled to water in volumes of frontal cortex and thalamus, including glutamate (Glu, the major excitatory neurotransmitter), N-acetyl aspartate (NAA; a neuronal marker), total creatine (tCr) and choline (tCho). We found that monkey thalamus showed higher GABA/Cr than human thalamus, with a difference of 27.6% (p<0.05). Monkeys had lower NAA in both the frontal cortex (p<0.01) and thalamus (p<0.05). Levels of Glu and tCho were higher in human frontal cortex (p<0.05), while tCr was higher in human thalamus (p<0.05). These results provide firsthand information on the baseline differences of various brain metabolites between rhesus monkeys and humans. (Supported by NIH ES017498 and ES010975).

998 Increased cortical GABA levels in manganese-treated rats.

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While manganese (Mn) is important for neuronal function, high exposure levels may cause neurotoxicity leading to Parkinsonism. Both in early idiopathic Parkinson’s disease as well as in Mn intoxication, there is an increase in γ-aminobutyric acid (GABA) in the basal ganglia and thalamus have been reported, suggesting the importance of GABA in Mn-induced neurotoxicity. In this pilot study, we used 1H brain Magnetic Resonance Spectroscopy (MRS) to examine in vivo GABA levels in selected brain areas of rats subchronically exposed to Mn. This longitudinal study included 5 adult rats receiving 6 weeks of ip MnCl₂ injections (10mg/kg, 5 d/wk) and 3 adult rats receiving saline injections as controls. 1H brain MRS was conducted on a 9.4T MRI scanner at baseline, wk 2, 4, and 6. H spectra were acquired from the cortex, thalamus and striatum using an ultra-short-echo-time single voxel...
Excessive occupational and environmental Manganese (Mn) exposure may induce Parkinsonism; yet the mechanism has not been completely elucidated. The regulation of brain Mn depends largely on the blood-brain barrier and blood-cerebrospinal fluid barrier (BCB). The latter is constituted by choroid plexus (CP) epithelial cells, which is specialized for cerebrospinal fluid (CSF) production, has been considered as a primary target in Mn-induced neurotoxicity. This study aims to explore the differential proteome in CSF after severe acute and subchronic Mn exposure in SD rats. Rats (1.5 month) were divided into 6 groups; each received daily ip injections of either MnCl2 (6 mg Mn/kg BW) or saline (as controls) for 30 days. The CSF were collected and analyzed. This Mn-animal model was evaluated and Mn-related proteome in CSF was analyzed by a novel proteomic technique of label-free nanoHPLC-Q-TOF-MS/MS. A total of 123 Mn-related differential proteins in CSF were identified, of which 55 were up-regulated, 68 were down-regulated. Based on the information of Gene Ontology (GO) categories, these differentially expressed proteins were mainly from the nuclei, involving in the function of binding; however, more than half of the proteins have no clear biological function. Biological functions of these CSF proteins generated by PANTHER were produced and excerted by CP, these differential proteins may be valuable for explore the novel potential biomarkers to diagnose and monitor the progression of Mn-induced neurodegenerative disease clinically; meanwhile, the results shed light on the future molecular mechanism study of Mn on choroid plexus epithelial cells. Further verification of these differentially expressed proteins and analysis including protein-protein interaction deserve in-depth investigation.
Environmental exposure to excessive manganese (Mn) is known to cause a neurologically condition termed manganism, resulting from impairment of neurons within basal ganglia. However, the cellular and molecular mechanisms underlying the neurotoxic effects of Mn remain elusive. Because we recently demonstrated that protein kinase Cδ (PKCδ) is an oxidative stress sensitive kinase that plays a causal role in apoptotic cell death in Mn neurotoxicity models, herein we examined the molecular mechanisms underlying PKCδ gene expression in neuronal cells during Mn exposure. As a proof of concept, we first examined the effect of Mn on PKCδ expression in NIE115 cells and primary striatal neurons as well as in animal model of Mn neurotoxicity. Mn exposure (100–300 μM for 24–48h) potently induced PKCδ protein and mRNA levels in primary striatal neurons and NIE115 cells. Importantly, Mn exposure (3 or 10 mg Mn/kg) in C57 black mice via oral route also confirmed the PKCδ upregulation in the basal ganglia. Primary striatal neurons obtained from PKCδ knockout mice showed reduced sensitivity against Mn toxicity, demonstrating that PKCδ plays a critical role in Mn-induced neurotoxicity. We further elucidated the mechanisms underlying the Mn-induced up-regulation of PKCδ in cell culture models. Using both PCK and ChIP assays, we identified that NFKB is essential for both basal and Mn-mediated PKCδ expression. In addition, we investigated the possible involvement of DNA methylation in regulating PKCδ expression. Using bioinformatics method, we found a putative CpG island (+39 to +400 relative to transcription start site) located within mouse PKCδ promoter. By methylation-specific PCR, we identified that the PKCδ promoter is partially methylated in neuronal cells. Collectively, our data suggest that environmental Mn exposure can alter expression of oxidative stress sensitive kinase PKCδ in the basal ganglia and that both NFKB β and promoter methylation play roles in regulation of PKCδ gene expression (supported by NIH grants ES10586 & ES19267).

While much is known about potential cellular mechanisms of manganese (Mn) neurotoxicity at elevated exposure levels, little is understood about cellular responses to lower exposures at the transition between physiologic to toxic levels of Mn. Previously we showed that elevated cellular Mn levels lead to the rapid lysosome-mediated degradation of the cis-Golgi associated protein Golgi phosphoprotein 4 (GPP130) in AF5 cells. In order to determine if GPP130 plays a role in mediating the cytotoxic effects of Mn, we investigated the dynamics of the Mn effect on GPP130 degradation in vitro and in vivo. We determined that GPP130 degradation in AF5 cells is specific to Mn, and not other divalent cations, since cellular GPP130 levels were reduced by ~80% in cells treated with 100 μM Mn for 24 hours, while no effect was observed in cells treated with 100 μM Co, Cu, Zn, Ni, or 300 μM Fe. To determine the sensitivity of the GPP130 degradation response to Mn, AF5 cells were treated with 0.25, 1, 5, 25, or 150 μM Mn for 24 hours. Results show that GPP130 degradation occurs in a dose-response manner starting at the lowest Mn exposure level of 0.25 μM, even though total intracellular Mn levels measurably increased only in the 150 μM treatment. This suggests that GPP130 degradation is sensitive to immeasurably small changes in intracellular Mn levels. To determine the temporal nature of the GPP130 degradation response to Mn, AF5 cells were treated with 5 or 100 μM Mn for 1, 2, 4, or 8 hours; results indicate that GPP130 degradation occurs very rapidly, with significant (~40%) GPP130 degradation by 1 hr at 100 μM Mn and ~25% GPP130 degradation by 2 hours at 5 μM Mn. Collectively, these results suggest an important and novel cellular response to Mn over the transition from physiologic to toxic cellular Mn levels. Studies now underway are exploring the role of GPP130 in mediating the sensitivity of cells to elevated Mn, and the extent that these effects occur in an animal model of early-life Mn exposure.
**PS 1007 MANGANESE DECREASES HUNTINGTIN PROTEIN EXPRESSION AT SERINE 421, INCREASES HUNTINGTIN PROTEIN LEVELS, AND DECREASES BDNF EXPRESSION IN CULTURED NEURONS; IMPLICATIONS FOR STRIATAL MEDIUM SIZE SPINY NEURON DEGENERATION IN MANGANESE NEUROTOXICITY AND IN HUNTINGTON'S DISEASE.**


In Huntington's disease (HD), the polyglutamine expansion in the Huntingtin (Htt) gene is pathogenic and results in medium size spiny neuron (MSN) degeneration in the striatum due to decreased synthesis and disruption of brain-derived neurotrophic factor (BDNF) transport from cortical neurons (Zuccato et al., Brain Path 18: 225, 2008). Phosphorylation of Htt at serine 421 (S421) has been shown to control neurotoxic support and survival of MSN by regulating BDNF synthesis, BDNF vesicular transport (Gauthier et al. Cell 118: 257, 2004) and NMDA receptor excitotoxicity (Metzler et al., J Neurosci 30: 14318, 2010). Similarly, high dose Mn injections have been shown to decrease MSN dendritic length and dendritic spine density in the striatum (Milatovic et al. TAAP 240: 219, 2009). In the present study, we thought to determine if Mn had an effect on Htt protein level and phosphorylation at S421 and BDNF levels. Primary culture of cortical (Ctx) and hippocampal (Hipp) neurons exposed to low levels of Mn (vehicle, 1 or 5 μM) during DIV7-12 decreased dendritic (immuno-cytchemistry) and whole cell (Western blot) proBDNF protein levels in both culture types. Exposure to Mn also significantly reduced pS421Htt levels in cortical and Hipp neurons while increasing total Htt protein at the highest level of Mn exposure. These data indicate that excess levels of Mn can have neurotoxic effects in MSN by decreasing the phosphorylation of Htt at S421, an effect that has been associated with decreased BDNF synthesis and transport. These studies also suggest the possibility that excess exposure to Mn may be able to modify the temporal expression of HD by affecting the same molecular and cellular mechanisms as the polyglutamine expansion of the Htt gene, the principal cause of neurodegeneration in HD. [This work was supported by NIEHS grant ES010975 to TRG]

**PS 1009 HUMAN α-SYNUCLEIN PROTECTS AGAINST MANGANESE NEUROTOXIC INSULT DURING THE EARLY STAGES OF EXPOSURE IN A DOPAMINERGIC CELL MODEL OF PARKINSON'S DISEASE.**

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The role of α-synuclein (α-Syn) aggregation in neurodegeneration is well recognized, but the physiological function of normal α-Syn protein remains unknown. Recently, we demonstrated that α-Syn negatively regulates a proapoptotic kinase Protein Kinase Cδ (PKCδ) and suppresses parkinsonian toxicant MPP+ induced dopaminergic neurodegeneration. In the current study, we further show that α-Syn exhibits a neuroprotective role against acute manganese (Mn) induced neurotoxicity in a dopaminergic cell model of Parkinson's disease (N27 cells). Stable expression of human wild type α-synuclein at physiological levels in N27 cells significantly attenuated Mn (300μM) induced neurotoxicity for up to 24 hr of exposure. To further explore the cellular mechanisms, we studied the mitochondrial dependent apoptotic pathway. Western Blot analysis revealed a time-dependent reduction in the levels of cytosolic cytochrome c release following Mn exposure in the α-Syn expressing cells compared to vector control cells. Further analysis of the caspase cascade suggests that α-Syn significantly attenuated the Mn-induced caspase -3 and -9 activation in a time-dependent manner. Interestingly, the Mn-induced reactive oxygen species (ROS) generation was not affected by stable expression of α-Syn in N27 cells. Stable expression of α-Syn significantly decreased Mn-induced cytochrome c release and cytochrome c translocation for the pro-apoptotic kinase PKCδ. Furthermore, we examined whether α-Syn interferes with Mn transportation into the cells. ICP-MS studies revealed no significant differences in intracellular Mn levels in treated vector and α-Syn cells. Analysis of metal transporter DMT1 expression also showed no differences between α-Syn over expressed cells and vector cells, suggesting α-Syn didn’t interfere with Mn uptake in the cells. Collectively, these results demonstrate that α-Syn exhibits neuroprotective effects against Mn induced neurotoxicity during early acute toxicity in a dopaminergic neuronal model of PD (NIH grants ES19267 ES01586 NS74443).

**PS 1010 EXPRESSION AND AGGREGATION OF A-SYNUCLEIN IN THE BLOOD-CSF BARRIER: INITIAL EVIDENCE FOR THE INFLUENCE OF CELLULAR MANGANESE AND COPPER STATUS.**

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The blood-cerebrospinal fluid barrier (BCB) is a specialized tissue composed of choroid plexus epithelia and functions to produce the cerebrospinal fluid (CSF) and maintain the homeostasis of a wide variety of molecules in the brain. Alpha-synuclein (a-Syn) plays a central role in the pathology of Parkinson's disease (PD) and is detectable in the CSF. Little is known about the BCB's role in the transport and clearance of CSF a-Syn in the early stages of PD. This study was designed to test the hypothesis that choroidal epithelial Z310 cells express a-Syn endogenously, and exposure to toxic metals may increase its expression and/or induce aggregation. Both Western blot and confocal imaging (CI) revealed that a-Syn was expressed naturally in Z310 cells, and that clathrin was incorporated in extracellular a-Syn uptake. When Z310 cells were incubated with 100 μM MnCl2 for 24 and 48 hr, the immunofluorescence data obtained by CI showed that in comparison to the cytosolic, homogenous signal of the control cells, Mn exposure induced a-Syn aggregation characterized by the formation of the small, cytosolic dots emitting a relatively intense signal. Our lab has recently produced an iZCTR1 cell line which, upon adding tetracycline (Tet) to the culture medium, can induce the overexpression of a copper (Cu) transporter CTR1 (see poster by Zheng G & Zheng W). Treatment of iZCTR1 cells with 1 μg/mL Tet for 24 hr followed by incubation with 5 μM CuCl2 for 1 hr resulted in visible a-Syn aggregation in iZCTR1 cells. These results suggest the BCB expresses a-Syn, Mn exposure likely facilitates the cellular aggregation of a-Syn in the BCB. This effect may be due in part to Mn-induced Cu accumulation in the BCB (see poster by Fu et al); the latter in turn induces a-Syn aggregation. These data provide the foundation on which further studies on a-Syn transport at the BCB and its effects of toxic metal exposure are warranted. (Support by NIH/NIEHS ES08146-S2 Minority Supplemental Award.)
Copper (Cu) is an essential trace element that requires tight homeostatic regulation to ensure appropriate supply without any toxic effects as a result of the strong redox potential of the metal ion. This study was designed to elucidate the mechanism by which iron deficiency (FeD) results in excess Cu accumulation at the blood-CSF barrier. To explore this mechanism we examined the effect of FeD on cellular Cu re- tention and transporters Ctr1, DMT1, ATOX1, and ATP7A in choroidal epithelial Z310 cells. Results revealed that deferroxamine treatment (FeD) resulted in signific- antly increased cellular Cu retention (p<0.05). In addition, FeD treatment re- sulted in a significant increase in the mRNA levels of DMT1, ATOX1, and ATP7A. Knock down of Ctr1 or DMT1 resulted in significantly lower Cu uptake by Z310 cells while the knockdown of ATOX1 or ATP7A resulted in substantial in- creases of cellular accumulation of Cu. In addition, DMT1 knockdown followed by FeD treatment in Z310 cells did not result in the significant accumulation of in- tracellular Cu found with FeD treatment alone. mXRF imaging demonstrated that Cu localized in the subventricular zone (SVZ) of the brain ventricles, was not af- fected by systemic Fe status. However, linear regression analysis of the SVZ identi- fied a strong positive correlation between Cu and Fe (r=0.538, p=0.07). Taken to- gether, these results suggest that Ctrl, DMT1, ATOX1, and ATP7A contribute to Cu transport at the BCB and that the accumulation of intracellular Cu found in the Z310 cells during FeD appears to be mediated, at least in part, via the upregulation of DMT1 following FeD treatment.

Zinc has long been touted as a panacea for the common cold. However, there has been some controversy over whether an intranasal (IN) zinc gluconate gel (Zicam), purported to fight colds, causes anosmia, or the loss of the sense, of smell in hu- mans. Prior evidence has shown that IN zinc sulfate solutions can cause anosmia in humans, as well as significant damage to the olfactory epithelium in rodents. However, more recent work has claimed to show that zinc gluconate is less toxic than zinc sulfate. Using an in vitro olfactory neuron model (the rat Odora cell line) to compare the toxicity of zinc sulfate and zinc gluconate on immature and mature rat olfactory sensory neurons, we found that the toxicity of both zinc salts was sim- ilar, with zinc sulfate being slightly more toxic than zinc gluconate. Interestingly, toxicity to Odora cells occurred at significantly lower zinc concentrations (0.1 – 0.4 mM) than that found in Zicam nasal gel (35 mM), which lends credence to the epidemiological link between IN zinc exposure and anosmia. We hypothesized that zinc toxicity was caused by inhibition of the HV CN1 proton channel, leading to acidosis and apoptotic cell death. Olfactory sensory neurons in vivo are able to maintain their intracellular pH through a Na+/H+ exchanger, specifically NHE1, and a Cl/HCO3- exchanger. Zinc sulfate, at non-toxic levels, had no im- pact on intracellular pH via proton transport either after acute exposure or after 24 hours incubation with the cells. In conclusion, zinc toxicity is not mediated through an acidification of intracellular pH in olfactory neurons in vivo.

Accumulation of amyloid beta protein (Aβ) play a major role in the etiology of Alzheimer’s disease (AD). Aβ are generated by the cleavages of amyloid precursor protein (APP) by beta-site APP-cleaving enzyme 1 (BACE1). Decrease of Aβ con- centration in the brain are involved in two major factors that are degradation by peptides such as neprilysin (NEP) and are regulated via two main receptors, the low density lipoprotein receptor related protein 1 (LRP1) and the receptor for advanced glycation end products (RAGE), on blood-brain barrier (BBB). LRP1 is the main receptor that clears Aβ from the brain to body, in contrast RAGE is the main receptor that transports Aβ from the body to brain. Copper (Cu) is well known that AD-related element, especially it’s involved in Aβ aggregation and toxicity. According to a recent study, in addition, it’s reduce Aβ clearance from brain in cho- lesteryl-fed rabbits. However, the critical mechanism is unclear. This study have demonstrated whether Cu would alter accumulation of Aβ in brain, we have used an immortalized line of rat brain endothelial cells (RBE4 and PC12 cells, well-de- fined neurodevelopmental cells, to estimate Cu effect in brain. When PC12 cells were treated with 100 μM Cu for 24 h, 48h and 72h, Aβ accumulation in culture medium was increased by 120.1%, 133.6% and 144.0%, respectively, in compari- son to those of controls. Also PC12 cells were treated with 100 μM Cu for 24 h, mRNA expression of APP was increased by 120.1% in comparison to controls. Moreover, mRNA expression of BACE1 and NPP were 102.5% and 55.1%, re- spectively, in comparison to those of controls. When RBE4 cells were treated with 100 μM Cu for 24 h, mRNA expression of LRP1 and RAGE were 84.2% and 111.4%, respectively, in comparison to those of controls. These results show that Cu have upregulated Aβ production and arrested Aβ degradation, also it has de- creased clearance of Aβ in brain via LRP1 and RAGE on BBB.

Accumulation of amyloid beta protein (Aβ) play a major role in the etiology of Alzheimer’s disease (AD). Aβ are generated by the cleavages of amyloid precursor protein (APP) by beta-site APP-cleaving enzyme 1 (BACE1). Decrease of Aβ con-
we normalized to the endogenous control GAPDH and the untreated animals. Expression of all subunits was upregulated in the treatment group. The α6 subunit was the most affected. At 5ppm the expression of α6, β, and γ subunits is not affected. The relative expression was close to control levels. However, α6 and δ subunits were both downregulated. The α6 subunit which uniquely expressed in granule cells is the most affected of the subunits studied. Its upregulation in the low MeHg treated group may result from a compensatory mechanism, since these are more susceptible at lower concentrations of MeHg than are the purkinje cells. In the high MeHg treated group downregulation of α6 may reflect loss of granule cells. Supported by NIH grants R01ES03299 and R25NS067777.

1016 IDENTIFICATION AND CHARACTERIZATION OF MOLECULAR MODULATORS OF METHYLMERCURY-INDUCED TOXICITY AND DOPAMINE NEURON DEGENERATION IN C. ELEGANS.

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Methylmercury (MeHg) exposure from occupational, environmental, and food sources is a significant threat to public health. MeHg poisonings in adults may result in severe psychological and neurological deficits, and in utero exposures can confer embryonic defects and developmental delays. Recent epidemiological and vertebrate studies suggest that MeHg exposure may also contribute to dopamine (DA) neuron vulnerability and the propensity to develop Parkinson's disease (PD). We have developed a novel Caenorhabditis elegans (C. elegans) model of MeHg toxicity that shows that low, chronic exposure confers embryonic defects, developmental delays, decreases in brood size and animal viability, and DA neuron degeneration. Toxicant exposure results in the robust induction of the glutathione-S-transferases (GSTs) gst-4, gst-5, gst-12, gst-21, and gst-38, and with some GSTs largely dependent on the PD-associated phase II antioxidant transcription factor SKN-1/Nrf2. We also demonstrated that the expression of SKN-1, a protein previously localized to a small subset of chemosensory neurons and intestinal cells in the nematode, is also expressed in the DA neurons, and a reduction in SKN-1 gene expression increases MeHg-induced animal vulnerability and DA neuron degeneration. We will present results from our genome-wide reverse genetic screen to identify mediators and suppressors of MeHg-induced toxicity, as well as our investigations using DA-associated mutants to elucidate the role that the neurotransmitter may play in the toxicity.

1017 METHYLMERCURY (MeHg) DISRUPTS FLUO4 FLUORESCENCE IN CEREBRAL SLICES FROM GABA_A RECEPTOR α6 (-/-) MICE.

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MeHg targets the cerebellum, especially developing cerebellar granule cells (CGCs). The γ-amino butyric acid type A receptor (GABA_A, R) is important in CGC intracellular [Ca^2+] ([(Ca^2+)]_i) control and cell excitability. It is highly sensitive to acute MeHg exposure. GABA_A, R subunit composition may contribute to cell-specific and developmental susceptibilities to MeHg. We examined acute effects of MeHg on cerebellar slices from mice (KO) lacking the α6 GABA_A, R subunit. α6 exists exclusively in mature CGGs and regulates their tonic inhibition. It coexists in CGGs with the abundant α1 subunit, which is not involved in tonic inhibition, nor expressed only in CGGs. Changes in fluo4 fluorescence induced by MeHg were compared in KO and WT mice using fluorescent confocal microscopy of migrating external granule layer (EGL) and post-migratory internal granule layer (IGL) CGCs. MeHg (20μM) increased overall fluorescence in both regions. In KO cerebellar slices, MeHg increased fluorescence significantly at 20 min; peak effect (300±70% over baseline) in IGL occurred at 55 min. In the same slices, MeHg increased fluo4 fluorescence significantly over pretreatment baseline in IGL at 15 min, at 55 min, the effect was 181±20%. Though MeHg treatment appeared to increase fluorescence in WT slices, the effect was not significant within 55 min. In the IGL of KO slices, MeHg increased fluorescence significantly compared to WT from 30 min on. Pulses of the GABA_ R agonist muscimol (100μM) to tissue perfused with MeHg caused a 75±25% reduction compared to MeHg alone in IGL of KO mice. While the α6 subunit is expressed fully in CGGs still is a pronounced effect of MeHg on [Ca^2+]_i in immature CGGs of α6 KO mice. Thus α6 may reflect loss of granule cells. Supported by NIEHS grants R01ES03299 and T32ES007255.

1018 SUSCEPTIBILITY TO METHYLMERCURY-INDUCED (MEHg) CYTOTOXICITY IN HEK-293 CELLS: ROLE OF NEURONAL VOLTAGE-GATED CALCIUM CHANNEL (VGCC) α6 SUBUNITS.

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MeHg, an environmental neurotoxicant, acts on specific cellular targets such as VGCCs to elicit severe pathological effects. Low μM MeHg blocks current through VGCCs and elevates ([Ca^2+]_i) via cytosolic pathways in vitro. onset of MeHg-induced increases in ([Ca^2+]_i) varies with the VGCC phenotype as determined by the pore-forming α6 subunit expressed. Susceptibility of individual VGCC isoforms to MeHg-induced cytotoxicity was examined by transient transfection of HEK-293 cells with a 1:1:1 mixture of α6, α6β, and β2I VGCC subunits. The α6 subunit was varied to produce L-, N-, P/Q- and R-type VGCC isoforms (Cav1.2, 2.2, 2.1 and 2.3, respectively), corresponding to α6, α6β, α6β and α6γ subunits. Cytotoxicity was assayed 1 hr or 24 hrs after 1 hr MeHg exposure (1, 2 and 5 μM). Viability of untransfected HEK-293 cells was concentration-dependent at all [MeHg], though there was no observable time-dependence at 1 or 2 μM MeHg. Expression of VGCCs, regardless of isoform, reduces cell viability at all [MeHg] and time points examined. Cav1.2-transfected cells were most susceptible to MeHg. Viability was reduced to 2.0±0.9% (mean % viability ± SEM) 1 hr after exposure to 5 μM MeHg. In contrast, Cav2.1 were most resistant to 5 μM MeHg (29±8.9%). Cav2.2 (4.5±1.9%) and Cav2.3 (12.7±4.2%) were moderately susceptible at this [MeHg]. The rank order viability at 24 hrs postchallenged that at 1 hr. Cav2.1 was most susceptible (3.4±1.6%) and Cav2.1 was most resistant (26.7±10.3%) to 5 μM MeHg, while Cav2.2 and Cav2.3 were of intermediate susceptibility (6.7±3.0% and 21.7±8.8%, respectively). Viability of transfected cells was only marginally reduced by 1 and 2 μM MeHg. A concentration-dependent effect was observed for all isoforms, as well as a trend towards time-dependent cytotoxicity. Together, these results demonstrate that susceptibility of VGCCs to MeHg-induced Ca^2+-dependent cytotoxicity is isoform-dependent, and the relative expression pattern of VGCCs may affect a neuron’s susceptibility to MeHg.

1019 ACUTE IN VITRO EXPOSURE TO METHYLMERCURY AFFECTS SHORT-TERM SYNAPTIC TRANSMISSION AND PLASTICITY IN CEREBRAL SLICES OF ADULT AND AGING MICE.

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Effects of MeHg on the adult, and particularly the aging brain, have not been well characterized. Changes in synaptic plasticity occur during aging that could be worsened by exposure to an environmental neurotoxicant. In the present study, we examined acute effects of MeHg on synaptic transmission and short-term synaptic plasticity in cerebellar slices prepared from adult (9 mo old) and aging (20 mo old) mouse brains using whole-cell patch clamp recording techniques. Acute bath application of 20 μM MeHg initially stimulated then suppressed spontaneous glutamate-mediated excitatory postsynaptic currents (sEPSCs) recorded from cerebellar Purkinje cells. The onset of the initial increase appears to occur earlier in 20 mo old mice than in 9 mo old mice. Consistent with the increased frequency of sEPSCs, MeHg altered short-term synaptic plasticity in both groups. It promotes paired-pulse facilitation (PPF) by increasing the ratios of the second stimulus-evoked response (EPSC2)/the first stimulus-evoked response (EPSC1) or reversing paired-pulse depression (PPD) to PPF. This suggests that MeHg acts on presynaptic processes to alter the release probability of the transmitter glutamate. MeHg always blocked evoked ESPCs earlier than it did spontaneous ESPCs. This may be due to block by MeHg of volatile, low affinity Cav2.1. The voltage-gated NC channel-mediated outward currents were unaffected by MeHg, suggesting differential sensitivity of different ion channels to MeHg. However, unlike the MeHg-induced transient hyperpolarization of neuronal membranes in young animals, MeHg caused a gradual and persistent hyperpolarization of neuronal membranes in adult and aging mice. Thus, in vitro MeHg exposure affects short term plasticity in both adult and aging mice. However, the effect appears to occur earlier in the aging group, suggesting interaction with age-related senescence of MeHg. Supported by NIEHS grant ES03299.
Disruption of intracellular calcium, [Ca\text{2+}]i, regulation is an early and crucial event in MeHg-induced neurotoxicity. Acute and chronic exposure to MeHg targets the cerebellum, causing loss of the granule cell layer. The striatum is another potential target due to its high level expression in medium spiny neurons (MSNs) of Ca\text{1,3}, an L-type Ca\text{2+} channel that is susceptible to MeHg. Effects of MeHg on aged individuals have not been characterized, however contemporary MeHg exposure occurs over an individual's lifespan. As neuronal [Ca\text{2+}]i homeostasis is significantly altered during aging, aged neurons may be especially susceptible to MeHg. We compared effects of MeHg on [Ca\text{2+}]i dysregulation in cerebellar granule cells and interneurons and in stratal MSNs of 9 or 20.5 mo old mice. Changes in relative fluorescence (RF) of Fura-2, a [Ca\text{2+}]i indicator, were measured in sagittal cerebellar and coronal midbrain slices from male ICR mice using laser scanning confocal microscopy. MeHg (20 μM) was applied for 45 min. No significant difference occurred in RF in cerebellar granule cells or molecular layer interneurons in 9 or 20.5 mo old mice. However, the mean time to maximum RF was reduced in 20.5 mo old mice, suggesting a decreased ability to buffer [Ca\text{2+}]i. In the striatum, the RF increase did not significantly differ, nor did the time to maximum RF in 9 or 20.5 mo old mice. MSNs of 9 mo old mice segregate into two distinct populations: one consisting of a small population of neurons which exhibit a large RF increase and the other exhibiting only a minimal RF increase upon MeHg treatment. There are at least two subsets of MSNs based on expression of dopamine receptors. The observed differential response to MeHg may be due to the neuronal complement of ion channels and neurotransmitter receptors. These results suggest that interaction of the natural aging process with exposure to environmental neurotoxins can alter the cellular response to the toxicant. Supported by NIH grants R01ES03299 and T32ES007255.

Methylmercury (MeHg) causes a time- and concentration-dependent increase of intracellular Ca\text{2+} (Ca\text{2+}[i]) in cerebellar granule cells (CGCs), an effect sufficient to cause their death in vitro. Voltage-gated Ca\text{2+} channels (VGCCs) are important in the onset of this effect. Treatment with VGCC blockers reduces toxicity induced by chronic exposure with MeHg. CGCs with the naturally-occurring Lh1A pore-forming subunit of P/Q-type (Ca\text{V2.1}) VGCCs, and approximately 25% of Lh1β subunits of N-type (Ca\text{V2.2}) VGCCs. To compensate partially for this, increased association of α1 and β1 with β10 and β13 occurs. This could be skew these channels with different properties. Effects of a faster onset of MeHg-induced [Ca\text{2+}]i increase on viability of Lh CGCs were examined using a fluorescent live/dead assay. CGCs were isolated at PND 7 and cultured for 4, 6, 8, and 10 days in vitro (DIV) to account for changes in VGCC gene expression as a result of cell maturation. Cells were exposed to MeHg (0, 0.3, 1, or 3 μM) for 4, 8, and 24 hrs. This was followed immediately by a calcium-AM and ethidium homodimer-1 treatment to assess for live and dead cells, respectively. Lh-CGCS had a higher percentage of cell death at DIV 6 and 8, after 8 hrs of MeHg exposure. There was no difference in cell death percentage between genotypes after 4 hrs of MeHg exposure at any DIV. After 24 hr of exposure, all genotypes had nearly a 100% cell death. For all genotypes, the cytotoxicity was time- and concentration- dependent. These results strengthen the idea of a crucial role of VGCCs in the mechanism of MeHg-induced cytotoxicity. VGCCs, and suggest a gene/environment interaction that may exacerbate the effects of MeHg in the cerebellum. Supported by NIH grants R01ES03299 and R25NS06577.
Hypothyroidism in adulthood has been associated with cognitive decline, and both development and in adulthood. The hippocampus is critical for some types of memory, including trace fear conditioning, amplitudes of excitatory synaptic potentials, and working memory. Adult male rats (PN60-80) were exposed to propylthiouracil (PTU: 0 or 10 ppm), through the onset TH deficiency on hippocampal physiology and learning. Adult male rats were sacrificed by decapitation and their forebrains were dissected and immediately frozen for subsequent western blot analysis probed with a heat-shock protein 90 (HSP90) antibody. Brains were also sectioned and analyzed for astroglial (GFAP) and stress markers (RAGE and HSP90 antibodies). GSSP exposures did not produce any significant changes in HSPP90 levels in either males or females. CDMA IS-95 exposures significantly increased HSP90 at 3, 6, and 9 W/Kg in males. At 9 W/Kg in both sexes, exposure to both GSM and CDMA modulation increase astrocytes and number of RAGE- and HSPP90-positive cells. These preliminary studies indicate that the effects of different types of mobile phone RF are dose- and gender-dependent. Studies are underway to further examine the observed gender differences and to evaluate whether RF exposure might have any potential neuronal damaging effects in specific brain regions. (FDA/NCTR IAG # 224-070-0007) (NIH IAG # YIES1027).

This study was designed to examine the effect of multiple doses of methamphetamine (METH) and MPTP on the retinal dopaminergic system. Six month old C57BL/6 mice were injected i.p. with either a low-dose (LD - 2 doses of 5 mg/kg) or high-dose (HD - 3 doses 10 mg/kg) of METH or MPTP or equivalent amount of saline as a control. Mice were sacrificed 1 day after treatment by cervical dislocation; retinas were removed using the Winkler technique and immediately frozen. Retinas were thawed on ice, homogenized by sonication in 300 μl of 0.2 M perchloric acid and 1 μM DHBAA as internal standard, and centrifuged at 13,000 x g for 10 minutes at 4°C. The supernatant was filtered through a 0.45 μm membrane and a 25 μl aliquot was analyzed using HPLC for dopamine (DA) and its metabolites, 3,4-Dihydroxyphenylacetic acid (DOPAC) and Homovanillic acid (HVA). METH produced no significant changes in DA, or its metabolites in the retina. LD MPTP produced no change in DA level, but significantly decreased DOPAC (R) 19% and 39% respectively. HD MPTP significantly decreased DA, DOPAC, and HVA levels 26%, 28% and 30% respectively. Additionally, LD MPT significantly decreased the DOPAC/DA and HVA/DA ratios 17% and 44% respectively. Mice did not show any significant changes in DA or its metabolites when exposed to multiple doses of METH. Although, METH is known to cause a release of DA, this is likely a transient phenomenon, and levels quickly return to normal after treatment. Where as MPTP, a selective, dopaminergic neurotoxin used to model Parkinson's disease, caused a significant decrease in DA and its metabolites. MPTP also decreased DA utilization as evidenced by significantly decreased DOPAC/DA and HVA/DA ratios. Taken together these results suggest that inhibition of the DA metabolizing enzymes MAO or COMT may take place at lower doses of MPTP treatment; conversely, higher doses of MPTP may cause decreases in DA, DOPAC and HVA through apopotic cell death cell death in the retinal dopaminergic system.
methamphetamine (METH), we documented elevated neuroinflammation, which may serve as a modulator or mediator of astroglial/microglial activation. Activated glia (associated with all types of brain injury) may be neuroprotective or exacerbate neural damage. Our prior genetic and pharmacological interventions have resulted in partial suppression of METH induced neuroinflammation without affecting neurotoxicity/astrogliosis. Here, mice were pretreated with the stress hormone corticosterone (CORT; 400 mg/L drinking water) for 1 week or repeated stress for 5d, consisting of social reorganization and cage shaking. METH administration alone (20mg/kg, s.c.) caused significant increases in proinflammatory cytokine (TNFα, IL6, CCL2, IL1β, IL10, LIF, OSM) mRNA expression in striatum at 12h. By 72h marked astrocytic hypertrophy (GFAP protein/immunohistochemistry(IHC)), microglial activation (isolectin IHC) and dopaminergic nerve terminal damage (TH protein/IHC) was observed in striatum. Chronic CORT pretreatment caused exacerbated inflammation, astrocyte hypertrophy and microglial activation after METH exposure in the striatum, hippocampus and cortex. Of note, chronic CORT pretreatment exacerbated METH-induced decreases in TH protein (to 10% of control) in striatum. However, repeated in vivo stress exposure completely blocked striatal dopaminergic neurotoxicity and reduced the neuroinflammatory response to METH. As the levels of chronic CORT approached or exceeded those associated with high physiological stress, our data suggest that chronic CORT therapy or sustained physiological stress sensitizes the CNS neuroinflammatory and neurotoxic responses to METH. Also, more severe or prolonged in vivo stressor application may be required to produce priming of the CNS similar to exogenous CORT.

**1029 THE MODULATION OF 3, 4-(d)- METHYLENEDIOXYMETHAMPHETAMINE-INDUCED NEUROTOXICITY BY CATECHOL-O-METHYLTANSFERASE.**

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3,4-(d)-Methylenedioxymethamphetamine (MDMA) abuse remains a significant problem worldwide. Systemic administration of MDMA to rodents and non-human primates causes neurotoxicity, and evidence suggests that it is also neurotoxic in humans. Metabolism of MDMA appears necessary for MDMA neurotoxicity. Thus, prior work from our laboratory indicates that cytochrome(s) P450 (specifically, CYP 2D6) inhibition attenuates MDMA-induced neurotoxicity, probably by decreasing the metabolism of MDMA to N-methyl-tetrahydroaminoamphetamine (N-Me-tetrahydromDA). Because N-Me-tetrahydromDA is a substrate for catechol-o-methyltransferase (COMT), we examined the effect of COMT inhibition on MDMA-induced neurotoxicity. COMT converts the N-Me-tetrahydromDA (a catechol) to 4-hydroxy-3-methoxy-methamphetamine (HMMA), thereby limiting the oxidation of N-Me-tetrahydromDA to the reactive ortho-quinone. Pharmacological and genetic models were used to determine the role of COMT in MDMA-induced neurotoxicity. Adult female Sprague-Dawley rats were pretreated with the COMT-inhibitor, Ro 4-0960 (40mg/kg, ip) followed by a neurotoxic dose of MDMA (20mg/kg, sc). In the genetic model, COMT-/- and COMT+/- wildtype mice were dosed with either MDMA (30mg/kg X 3 at 3 hour intervals, sc) or saline. In both models, neurotoxicity was determined one week after dosing via determination of neurotransmitter concentrations. In the pharmacological model of COMT inhibition, MDMA-induced neurotoxicity was potentiated. The data from the genetic model was ambiguous, probably because the COMT activity in the heterozygous animals is sufficient/borderline to process the O-methylation of N-Me-tetrahydromDA. Studies with homozygous COMT-/- mice are ongoing. The findings suggest that enzymes involved in the formation (CYP2D6 et al) and further metabolism of N-Me-tetrahydromDA (COMT) are important contributors to the individual susceptibility to MDMA-mediated neurotoxicity, especially given the polymorphic distribution of these enzymes in the humans. (Supported by NIDA Award DA023525)

**1030 THE EFFECTS OF FLUORIDE ON THE NEURONTRANSMITTERS IN DISCRETE BRAIN REGIONS OF ICR-DERIVED GLOMERULONEPHRITIS MICE BY EXPOSURE VIA THEIR DRINKING WATER.**

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Fluoride (F) has been known as an environmental pollutant. Although homovanillic acid (HVA) in the hypothalamus was altered in the BALB/c mice exposed to F in our previous study, the effects of F on the central nervous system had not been considered a serious health problem. Since a target organ of F is the kidney and F is filtered from the blood by the kidney, the accumulation of F in animals with renal insufficiency may enhance its toxicity. ICR-derived glomerulonephritis (ICGN) mice have been used as a model for idiopathic renal insufficiency. We evaluated whether or not the administration of F via the drinking water induces neurotoxicity in ICGN mice, in which F accumulates, by using neurotransmitters in discrete synapse, primarily of serotonin (5-HT), inducing hyperthermia and hyperactivity (5-HT syndrome). Conversely, an altered neurotransmitter profile as a prolonged depletion in 5-HT, and structural damage to 5-HT nerve terminals. The molecular mechanisms for both the acute and long-term effects remain unclear. The vesicular monoamine transporter 2 (VMAT2) is involved in the transport of monoamine neurotransmitters, in particular dopamine and 5-HT, into intraneuronal storage vesicles. As such, VMAT2 is critical in maintaining neuronal health by preventing neurotransmitter oxidation within the cytosol. We therefore investigated the effects of the pharmacological inhibition of VMAT2, using Ro 4-1284, on MDMA-mediated acute and long-term neurotoxic effects. Sprague-Dawley rats pretreated with the VMAT2 inhibitor (10mg/kg, ip) displayed a significant increase in 5-HT content in the cortex and striatum compared to rats treated with MDMA (20mg/kg, sc) alone. Ro 4-1284 pretreatment delayed and attenuated total horizontal movement distance and mean velocity in animals dosed with MDMA. MDMA-mediated elevation in core body temperature was also significantly reduced in Ro 4-1284/MDMA-treated rats compared to those treated with MDMA alone. Thus, pharmacologic inhibition of VMAT2 appears to attenuate MDMA-mediated 5-HT/5-HIAA depletion, locomotor activity, and hyperthermia in rats. In summary, VMAT2 plays an important role in regulating the acute and long-term neurotoxic effects of MDMA. (Supported by NIDA Award DA023525)

1031 NEUROTOXIC EXPOSURES TO AMPHETAMINE CAN RESULT IN LIPOPOLYSACCHARIDE (LPS) PRESENCE IN CIRCULATING BLOOD, ORGAN DAMAGE, AND IMMUNE ACTIVATION.

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Immunooactivation, organ damage and release of factors such as LPS were observed in rats exposed to environmentally-induced hyperthermia (EIH) or amphetamine (AMPH). Serum levels of substances signaling these events were determined 3hr and 22 hr (1d) after AMPH or saline (EIH and control groups). EIH was induced by a hot (39°C) environment and AMPH neurotoxicity by 4 injections, 2 hr apart, of 5, 7.5, 10 and 10 mg/kg D-AMPH. At 3 hr, creatinine kinase-MB isoenzyme was elevated in 55% (AMPH) and 29% (EIH). At 1d, the enzymes were still 2-fold control in the EIH and 50% of the AMPH animals. Liver enzymes ALT and AST were up 2-fold in 57% of the EIH and 75% of the AMPH animals at 3hr. These enzymes returned to control levels in the EIH group but not in all the AMPH animals at 1d. Creatine and BUN levels were significantly elevated 2-fold by EIH and 3-fold by AMPH at 3hr but returned to control levels by 1d indicating kidney function may be only transiently affected. Detectable LPS was present in only 12% of the control animals but >50% of the EIH and AMPH animals had detectable levels at 3hr and 1d. White blood cell levels were not elevated by either EIH or AMPH at 3hr. However, micro array analysis on whole blood indicated a significant increase in the mRNA expression for cell surface proteins related to macrophages (Cd11b, eosiinophils(Cd52) and T-cells(Cd3, Cd8a). In summary, both EIH and AMPH can result in multiple organ dysfunctions, particularly in muscle, heart, kidney and liver after AMPH exposure. The neurotoxicity induced by AMPH, however, may be dependent on the severity of organ damage. The presence of LPS in the blood raises the possibility that it is involved in the immune response and neurotoxicity produced by AMPH.

**1032 THE EFFECTS OF FLUORIDE ON THE NEURONTRANSMITTERS IN DISCRETE BRAIN REGIONS OF ICR-DERIVED GLOMERULONEPHRITIS MICE BY EXPOSURE VIA THEIR DRINKING WATER.**

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3,4-Methylenedioxymethamphetamine (MDMA, Ecstasy) is a ring substituted amphetamine derivative with potent stimulant properties. MDMA exerts biphasic pharmacological effects on the brain resulting in opposing acute and long-term effects. During the acute effects, MDMA causes major monoamine release into the...
brain regions as indexes. The ICGN mice were administered F via their drinking water at 0, 50, 100, and 150 ppm for 4 weeks, and the viability of each group was observed. Each mouse's brain was dissected into 7 regions (cerebrum, cerebellum, medulla oblongata, midbrain, striatum, hypothalamus, and hippocampus). The concentrations of neurotransmitters (norepinephrine, dopamine (DA), serotonin) and their metabolites (DOPAC [dihydroxyphenylacetic acid], HVA, 5-HIAA [5-hydroxyindoleacetic acid]) were determined by HPLC (high-performance liquid chromatography). All ICGN mice exposed to 100 and 150 ppm F died. The mean values of neurotransmitters and their metabolites were compared between the 0 and 50 ppm-exposed groups. The mean values of body weight in the 100 and 150 ppm groups were significantly lower than those in the 0 and 50 ppm groups. The mean DA in the striatum or hypothalamus of the 50 ppm group was significantly lower than that in the 0 ppm group. The mean HVA in the hypothalamus of the 50 ppm group was significantly lower than that in the 0 ppm group. Although it is not clear whether a direct or indirect effect, F may inhibit DA synthesis in specific regions in the mice with impaired renal function.

1034 MODULATION OF NEUROTRANSMITTER AMINO ACIDS BY PERFLUOROCARBON SULFONATE (PFOS) WITHIN AMYGDALA IN RAT.
M. A. Lafuente1, N. Pereiro1, R. Moyano1 and B. Fernandez1, 1Laboratory of Toxicology, University of Vigo, Orense, Spain and 2Department of Forensic Medicine and Legal Medicine, University of Cordoba, Cordoba, Spain. Sponsor: G. Font.

Perfluorocarbon sulfonate (PFOS) is an emerging environmental contaminant. It has been shown to be neurotoxic and endocrine disruptor. On the other hand, amygdala is a brain region which coordinates behavioral, autonomic, and endocrine responses to perceived threats or danger to an animal. This brain area is involved in the regulation of hypothalamic-pituitary-adrenal axis, which is affected by PFOS exposure. This study was designed to evaluate the possible PFOS-induced alterations of glutamate, aspartate, GABA and tau- rine concentration in amygdala. In this region, morphological changes have been also evaluated. For this purpose, male Sprague-Dawley rats were orally treated for 28 days with PFOS, at the doses of 0.5, 1, 3 and 6 mg/kg/day. Control rats received 0.5% Tween-20 vehicle. Amino acids were separated and analyzed using high-performance liquid chromatography (HPLC), with fluorescence detection after precol umn derivatization with O-phthalaldehyde (OPA), and histological study was performed by using light and electron microscopy. PFOS, at the dose of 0.5 mg/kg/day, increased glutamate concentration but decreased glutamate and GABA content in this region. Animals treated with 1.0 or 3.0 mg of PFOS/Kg/day, showed an increase of glutamate, but no changes in gluta mate, aspartate, GABA or taurine concentration. However, the content of these amino acids was decreased after PFOS exposure with the dose of 6.0 mg/Kg/day. On the other hand, morphological damages induced by exposure to PFOS were observed. The obtained results suggest that PFOS induced several modifications on amino acid concentration as well as morphological alterations, in amygdala, that are dependent of the administered dose. This work was supported by grants from the Ministry of Education and Science, Spain (AGL2009-09061) and from the Xunta de Galicia, Spain (INCITE09 383 314 PR).

1035 PERFLUOROOC TANOC TIC AND PERFLUOROCUC TANESULFONIC ACID EXPOSURES DISRUPT ACETYLECHOLINESTERASE AND GLUTATHIONE S-TRANSFERASE ACTIVITIES IN ZEBRAFISH ADULTS (DANIO SPP).
K. Noell, S. Gunn and S. Chao, Biological Sciences, Fayetteville State University, Fayetteville, NC. Sponsor: H. Tilson.

Perfluorinated compounds such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) are ubiquitous, persistent pollutants that have been detected in wildlife as well as human populations. Due to its wide distribution and possible disruption to various species, concerns have increased regarding its impact on biological organisms and development of potential useful biomarkers of exposure for ecological assessments. Impact of PFOA on nervous system function has been explored; however, few studies have shown similar effects with PFOA, particularly in adults. As a result, acetylcholinesterase (AChE) activity was measured in adults zebrafish (Danio spp) brain and muscle tissue following exposure to PFOS and PFOA. In addition, recent reports show PFOA and PFOA increased gluta thione s-transferase (GST) mRNA expression in the liver temporarily while other reports show inhibition of GST activity in primary cultured hepatocytes of freshwater tilapia. Therefore, our study also examined the possible disruption of GST activity following exposure concentrations of 0.5, 5, 50 mg/L of PFOS compared to PFOA after 22 hours. Preliminary findings support the inhibition of AChE activity by PFOA and PFOA in the zebrafish brain while no observable changes were observed with muscle tissue. In addition, GST inhibition was also observed at lower concentrations in PFOS while no change in GST activity was observed in zebrafish liver following PFOA exposure.

1036 ENANTIOSELECTIVE FORMATION OF HYDROXYLATED METABOLITES OF PCB 95 IN FEMALE C57BL/6 MICE.
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PCB95 (2,2',3,5,6-hexachlorobiphenyl), a developmental neurotoxicant, displays axial chirality and exists as two stable rotational isomers that form non-superimpos able mirror images of each other. These isomers, called atropisomers, can undergo enantiomer enrichment in vivo due to different metabolism rates. The potential toxicological significance of this is underscored by our previous demonstration that chiral PCBs display atropisomer specific neurotoxicity. This study investigates the hypothesis that tissue levels and enantiomer enrichment of PCB95 and its hydroxylated metabolites (OH-PCBs) are dose-dependent in mice. To test this hypothesis, female C57Bl/6 mice (8.5 weeks old; n=16) were fed three different doses of racemic PCB95 (0.1, 1 and 6 mg/kg body weight) or vehicle (peanut oil) in peanut butter daily. The animals were euthanized after 56 days, blood and liver samples were collected, and levels and enantiomer enrichment of PCB95 and its metabolites were determined. Levels of PCB95 and its metabolites increased with increasing dose in liver and blood samples. Livers contained overall higher levels of PCB95 and 4-OH-PCB95 compared to PC95 after 22 hours. Preliminary findings support the inhibition of AChE activity by PFOS and PFOA in the zebrafish brain while no observable changes were observed with muscle tissue. In addition, GST inhibition was also observed at lower concentrations in PFOS while no change in GST activity was observed in zebrafish liver following PFOA exposure. 

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1037 STRUCTURE ACTIVITY OF FIVE RACEMIC POLYCHLORINATED BIPHENYLS TOWARD RyR1 CHANNELS: INFLUENCE OF HYDROXYLATION AT THE META- AND PARA-POSITION.

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Polychlorinated biphenyls (PCBs) are persistent, widespread environmental contaminants banned in 1977. However, chiral PCBs such as PCBs 91, 95, 132, 136, and 149, have persisted at levels of concern to human neurological health. Such non-coplanar PCBs are known to interact with pyrrole receptors (RYRs), ion channels broadly expressed in the central and peripheral nervous system and muscle. RyRs regulate release of Ca2+ from endoplasmic/sarcoplasmic intracellular calcium stores, and thus are pivotal to generating cellular Ca2+ signals. Here we report the structure-activity relationships for 5 chiral non-coplanar PCBs and their 4-OH and 5-OH metabolites toward RyRs. 4-OH and 5-OH derivatives were synthesized and their sensitizing activity toward the type 1 isoform RyR1 was compared to their parent structures using [3H]ryanodine ([3H]Ry) receptor binding analysis and macroscopic Ca2+ flux assay using microsomal membranes. Radioligand binding analysis performed with optimal Ca2+ for [3H]Ry binding indicated a monotonic concentration-effect relationship ranging from 100nM to low μM of parent PCB, 4-OH- and 5-OH-PCB congeners maximally enhancing RyR1 occupancy 1.5- and 7-fold. A 4-C1-substituent reduced activity relative to 5-C1. With few exceptions, 4-OH congeners were less active than the parent PCB, whereas 5-OH congeners were more active. Ca2+ flux measurements indicated that, as predicted by radioligand binding analysis, the rank order of activity was 5-OH-parent->4-OH. Ca2+ release triggered by PCB or metabolites is completely blocked by ruthenium red, a RyR1 channel blocker. These data identify meta-hydroxylated metabolites of chiral PCBs as especially potent sensitizers of RyRs. [Supported by NIEHS grants R01 ES017425, R01 ES04991, P42 ES05695.]

1038 CHLOROACETIC ACID-INDUCED CELL APOPTOSIS THROUGH ROS/ENDOTHELIC RETICULAR STRESS PATHWAY IN NEURONAL CELLS.

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Chloroacetic acid (CA), a toxic chlorinated analog of acetic acid, is widely used as an herbicide and in the synthesis of many organic compounds. Some studies have reported that CA can cause histopathological alterations and functional impairment in brain. However, the toxicological effects and underlying mechanisms of CA-induced neurotoxicity are mostly unclear. Here, we investigated the effects and possible mechanisms of CA in cultured neuron cells (Neuro-2a cells). Treatment of Neuro-2a cells with CA for 24h significantly induced cytotoxicity, reduced cell viability, and oxidative stress damage (membrane LPO production) which accompanied by several features of apoptosis, including the increases in Annexin V-binding cells and caspase-3/7 activity. CA also triggered endoplasmic reticulum (ER) stress as indicated by the enhancement in ER stress-related mRNA and protein molecules induction (such as glucose-regulated protein 78 (GRP78), GRP94, C/EBP homologue protein (CHOP), X-box binding protein 1 (XBPI)), procaspase-12 cleavage, and calpain activation. Transfection of cells with GRP78 or GRP94 siRNA reduced CA-induced caspase-3 expression in Neuro-2a cells. Meanwhile, these CA-induced apoptosis and ER stress-related signals could be effectively reversed by antioxidant N-acetylcysteine (NAC). Therefore, our results suggest that CA caused neuron cell apoptosis via oxidative stress-triggered ER stress signaling pathway.

1039 IN VITRO TOXICITY OF AMPHOTERICIN B AND AMPHOTERICIN B METHYL ESTER EXPOSURE IN OLIGODENDROCYTES.

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Amphotericin B (AMB) is widely used in the treatment of chronic systemic fungal disease; treatment often results in a dose limiting nephrotoxicity. Amphotericin B methyl ester (AME), a polycrane maculide antibiotic with significantly less toxicity and full antifungal activity was introduced in the late sixties. However, its clinical use was terminated following reports of toxicity to CNS myelin. Recent studies in our lab using pure AME suggested that the reported neurotoxicity results from contamination by AMB, leading to oligodendroglial injury. To test this hypothesis, we immortalized human oligodendroglial cell line, MO3.13 were treated with 0.5 - 30 μg/ml concentrations of AMB and AME. A quantitative dose response assessment revealed AMB to be at least 10 times more toxic than AME. The lowest concentration of AMB that induced cell death was 1 μg/ml and 10 μg/ml for AME. AMB induced apoptosis as revealed by activation of caspase 3, cytochrome c release and morphological changes. Activation of caspase 9, but not 8 and 10, at 0.5 μg/ml AMB suggested that there may be a direct effect on the mitochondria. This was confirmed by labeling with MitoView 633 (Biostem, Inc, Hayward, CA, USA), a fluorescent marker of mitochondrial membrane potential, which revealed diminished intensity following AMB treatment of 1 μg/ml, but was unaltered by AME concentrations below 10 μg/ml. These data suggest that AMB kills MO3.13 oligodendrocyte by the mitochondrial apoptotic pathway and that pure AME does not kill myelin-producing oligodendroglia at clinically relevant concentrations. (ES005/022)

1040 VITAMIN A DEFICIENCY INDUCES DYSFUNCTION OF INNER RETINAL LAYER CELLS IN ADDITION TO PHOTORECEPTOR CELLS.

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It is well known that retinoids play important roles in photoreceptor cells and pigment epithelial cells in the retina. Previous studies have demonstrated that vitamin A deficiency (VAD) reduces amplitude of both a- and b-waves in electroretinogram (ERG). Therefore, the study was conducted to determine the level of rhodopsin. While retinoids in the outer retinal layer have been investigated, those in the inner retinal layer including amacrine cells and ganglion cells still remain largely unknown. Since retinoids are known to be associated with development, differentiation, and function of neurons, the function of retinoids in the inner layer cells of the retina was investigated using a VAD rat model in the present study. VAD was induced by feeding a VA deficient diet to Brown Norway (BN) rats (18 males, 22 days old) for 10 weeks. The retinoid concentrations were measured in the plasma and retina between 6 and 10 weeks after initiation of VA deficient diet to confirm VAD. The effects of VAD on the retina were evaluated functionally by ERG and morphologically by routine ophthalmologic and histologic examination. VAD decreased the amplitude of a-, b-, and op-waves in ERG without histological changes at 10 weeks after initiation of feeding VA deficient diet. The degree of reduction in op-wave amplitude was more prominent than that of a- and b-waves, and coincided with the degree of reduction of retinal retinoids. The a-wave is generated from amacrine cells and ganglion cells in the inner layer of retina. Therefore, it is considered that retinoids have important roles also in the inner retinal layer cells. In this study, we also discuss a possible mechanism of action of retinoids in the control of op-wave in the inner retinal layer cells.

1041 NEUROACTIVITY OF THE β-CARBOLINES, HARMANE AND NORHARMANE, FOUND IN TOBACCO SMOKE: AN IN VITRO STUDY.

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Nicotine is one of the most frequently used drugs of abuse, and its use is associated with millions of deaths annually. It has long been speculated that tobacco, in addition to nicotine, contains other compounds that are addictive or act in concert with nicotine. One of the promising candidates are the β-carbolines (βCs) harmane and norharmane, which are condensation products of the major smoke constituent acetaldehyde. As harmane and norharmane are monoamine oxidase (MAO) inhibitors with potent central nervous system (CNS) activity, the toxic effects of exposure to these βCs warrants investigation. In the current study, we investigated the neuroactive profile of harmane and norharmane (1-750 μM) alone and after 24 hr exposure in MN9D cells. The effects of these βCs (1-10μM) alone and in combination with nicotine (5-50μM) were also assessed. Cell viability and mitochondrial function were evaluated using LDH and XTT assays, respectively. HPLC was used to...
Polycyclic diphenyl ethers (PBDEs) are a major class of brominated flame retardants. Their chemical structure and toxic effects are largely comparable to those of polychlorinated biphenyls (PCBs). Previous research identified the nervous system as being among the most sensitive target organ for flame retardants, with modulations of inhibitory GABAergic neurotransmitter receptors and excitatory nicotinic acetylcholine (nACh) receptors as a potential mode of action for halogenated flame retardants.

Therefore, the present study aimed at comparing effects of the abundant congeners PBDE-209, PBDE-47, PCB-47 and a hydroxylated metabolite 6-OH-PBDE-47 on the function of human α5, β2, GABA B, and αβ nACh receptors. The human neurotransmitter receptors were expressed in Xenopus oocytes and their function was investigated using the two-electrode voltage-clamp technique. The results demonstrate that inhibitory GABA B-mediated signaling was concentration-dependently activated or potentiated by PBDE-47 and 6-OH-PBDE-47, while PBDE-209 inhibited the activation of the receptor. Contradictory, small but concentration-dependent inhibition of the excitatory ACh-mediated signaling was induced by PBDE-47, 6 OH PBDE-47 and PBDE-209. Notable, no effects were observed for PBDE-47 on the receptors. The data indicate that oxidative metabolism increases the neurotoxic potential as PBDE-47 was without effect whereas the hydroxylated metabolite affects both receptors. Moreover, since both the GABA B and nACh receptor are critically involved in brain development, the effects described in this study may (partly) underlie previously observed neurobehavioral and neurodevelopmental effects of PBDEs and PCBs. [This work is part of the European Union-funded project ENFIRO (grant agreement FP7 ENV-2008-1-226563)].

Microelectrode arrays (MEA) have been proposed as a tool for detecting functional changes in electrically active cells, including neurons, exposed to drugs, chemicals, or particles. However, conventional single well MEA systems lack the throughput necessary for screening large numbers of chemicals. The current experiments used primary cortical neurons grown on Multielectrode Array (MEA) plates to screen a training set of 30 chemicals. Each MEA plate contained 12 wells with 64 microelectrodes/well, for a total of 768 channels. Of the 30 chemicals evaluated, 23 were known to alter neuronal function (“positives”), including 6 GABAergic and 3 glutamatergic compounds, 4 pyrethroids, 3 metals, 2 cholinesterase inhibitors, 2 nicotinic acetylcholine receptor agonists, valproic acid, verapamil, and fluoxetine. Seven compounds (glyphosate, acetaminophen, dimethyl phthalate, paraglutamic acid, saccharin, d-sorbitol, amoxicillin), expected to have no effect on neuronal function, were tested as “negatives.” Chemical effects (50μM or highest soluble concentration) were assessed for 30 min between 14 and 22 DIV, after collection of 30 min baseline activity. Twenty of the positives altered either the mean network spike rate, number of active electrodes or both by more than the DMSO control. The 3 positives without effect were valproic acid, nicotine and imidacloprid. None of the negative compounds changed activity more than by DMSO. A Bayesian approach was then used via a multinomial distribution to compare the electrode activity levels on a chemical by chemical (rather than well) basis. This analysis displays chemical responses in a more detailed manner and has greater sensitivity to detect responses that differ from control. Based on these results, the MW MEA assay has both high sensitivity and specificity, indicating that cortical neurons grown on MW MEAs are a useful approach to screen chemicals for neurotoxic effects mediated by a broad variety of mechanisms. (This abstract does not reflect Agency Policy).

Microelectrode arrays (MEA) detect and chemical-induced changes in action potential “spikes” in neuronal networks and can be used for neurotoxicity screening. Analytical “fingerprinting,” using Principal Components Analysis (PCA) on spike trains may improve the utility of MEAs to classify unknown chemicals by mode of action. The current study developed such approaches using MEA data from well understood chemicals (bicuculline, lidocaine, RDX, picotin, muscimol, verapamil, fluoxetine, chlorpyrifos oxon, domoic acid, and deltamerthrin) and “negative” controls (dimethyl phthalate, acetaminophen, and glyphosate). Bursting parameters (rate, duration, interspike intervals, /spike/burst/) were computed and averaged to yield parameter values as a function of concentration, then standardized to control. These data were compared with spike rate and synchrony data and PCA was performed across all concentrations. The first 5 principal components accounted for 50, 23, and 8% of the data variability. To determine how well PCA separated the high dose groups, confidence ellipsoids were drawn around data from concentrations ± each chemical’s IC/EC50 for spike rate. Chemicals in the same class grouped together and there was noticeable separation of GABA B antagonists from other chemicals. As expected, the negative controls grouped with baseline activity in the absence of chemical. Chlorpyrifos oxon grouped closely with fluoxetine, muscimol and verapamil, and MEA- and PCA-simulated data. Both groups were dispersed, while deltamerthrin separated from the other chemicals. The separation of chemicals within chemical classes and the separation of different chemical classes indicates that the sets may be distinguishable. This demonstrates that burst analysis with PCA offers a promising approach for identifying neurotoxicological modes of action for unknown chemicals. This abstract does not reflect Agency Policy.

Microelectrode arrays (MEA) have been proposed as a tool for detecting functional changes in electrically active cells, including neurons, exposed to drugs, chemicals, or particles. However, conventional single well MEA systems lack the throughput necessary for screening large numbers of chemicals. The current experiments used primary cortical neurons grown on Multielectrode Array (MEA) plates to screen a training set of 30 chemicals. Each MEA plate contained 12 wells with 64 microelectrodes/well, for a total of 768 channels. Of the 30 chemicals evaluated, 23 were known to alter neuronal function (“positives”), including 6 GABAergic and 3 glutamatergic compounds, 4 pyrethroids, 3 metals, 2 cholinesterase inhibitors, 2 nicotinic acetylcholine receptor agonists, valproic acid, verapamil, and fluoxetine. Seven compounds (glyphosate, acetaminophen, dimethyl phthalate, paraglutamic acid, saccharin, d-sorbitol, amoxicillin), expected to have no effect on neuronal function, were tested as “negatives.” Chemical effects (50μM or highest soluble concentration) were assessed for 30 min between 14 and 22 DIV, after collection of 30 min baseline activity. Twenty of the positives altered either the mean network spike rate, number of active electrodes or both by more than the DMSO control. The 3 positives without effect were valproic acid, nicotine and imidacloprid. None of the negative compounds changed activity more than by DMSO. A Bayesian approach was then used via a multinomial distribution to compare the electrode activity levels on a chemical by chemical (rather than well) basis. This analysis displays chemical responses in a more detailed manner and has greater sensitivity to detect responses that differ from control. Based on these results, the MW MEA assay has both high sensitivity and specificity, indicating that cortical neurons grown on MW MEAs are a useful approach to screen chemicals for neurotoxic effects mediated by a broad variety of mechanisms. (This abstract does not reflect Agency Policy).

Microelectrode arrays (MEA) separate chemicals by class.
as trauma, stroke, and drug-induced toxicity. A widely used and reliable method for labeling degenerating neurons in ex vivo brain tissue involves the use of Fluoro-Jade (F-J) stains. To date, however, all published labeling results utilizing the F-J dyes have been based on fixed tissue samples. Here, we report a method for labeling degenerating neurons in unfixed brain tissue using F-J dyes, showing results from a rat model of neurodegeneration induced by the i.p. injection of the excitotoxin, kainic acid. The labeling procedure is simple and fast (it can be finished in minutes). Since the brain tissue is unfixed, the identified degenerating neurons can be subsequently harvested using a laser capture microdissection system and mRNAs or proteins can be extracted for further molecular analyses. This new method of F-J labeling does not involve basic alcohol, potassium permanganate, or acids as does conventional F-J labeling, thus, this approach avoids the potential interactions between these molecules and extreme pH conditions and mRNAs or proteins. In addition, the labeling procedure works at 4°C, thus, further reducing or minimizing potential degradation and/or modifications of mRNAs and proteins. We also dissolved F-J in acetic acid, saline, or neutral phosphate buffer and tested it on formalin-fixed brain tissue sections from rats treated with kainic acid. Only F-J in acetic acid showed good staining, while F-J in saline or phosphate buffer did not label any cells in fixed-tissue sections, suggesting that an acidic environment is required for F-J labeling in fixed brain tissue. We anticipate that this one-step labeling method will be especially useful for studies that elucidate mechanisms of neurodegeneration at the genomic and proteomic levels.

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1047 FUNCTIONAL OBSERVATION BATTERY IN NONHUMAN PRIMATES WITH CONTINUOUS INTRACEREBRAL INFUSION.

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A functional observation battery (FOB) is often conducted as part of the ICH S7A safety pharmacology core battery while this evaluation is normally conducted in rodents, it can also be performed in large animals such as nonhuman primates when justified. Intracerebral infusion is used in patients for delivery of therapeutic agents for treatment of serious brain diseases. Nonclinical biological studies may not often require the use of the clinical route of administration. The impact of continuous intracerebral infusion of saline in Cynomolgus monkeys on clinical signs (CS), body weight (BW), FOB and histopathology were evaluated. Four males (5.6 to 6.3 kg) were instrumented with a left parietal catheter (Medtronic, Ref. 41207) and standard jacket and tether system. The animals were continuously infused using a clinical grade syringe pump for 28 or 42 days. Catheters remained patent except for 1 animal which presented a transient occlusion after 38 days of infusion. Intracerebral infusion did not result in any abnormality at CS and FOB. No macroscopic observation related to the procedure was noted except for 1 animal at 42 days post-surgery which presented dark parenchymal cerebrovascular atrophy. After 28 days, histopathology revealed minimal multifocal necrosis with gliosis and white matter vacuolation at the infusion site and cerebral parenchyma. At 42 days, mild to moderate necrosis and inflammation with minimal vacuolation were noted at the infusion site. Mild necrosis and hemorrhage were also noted in the cerebral parenchyma. In conclusion, continuous intracerebral cerebral infusion did not have any effect on CS, BW and FOB in freely moving Cynomolgus monkeys under the conditions of this study. Minimal to moderate local changes to the parenchymal tissue observed histopathologically were considered related to the presence of a foreign material.

1048 FUNCTIONAL OBSERVATIONAL BATTERY IN PERSPECTIVE.

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Hypothesis: The Functional Observational Battery (FOB) is a valuable tool used for assessing compounds for on target or off target effects (i.e. posture, gait, grooming, grip strength, etc). FOB panels have been highly cited and were originally designed to measure the efficacy of psycho-active drugs (i.e Irwin 1968), plus to re-interpret FOB data since some signs can indicate effects in both the CNS and peripheral tissues. Detection of FOB alterations alone does not fully characterize the true hazard of the compound. Confirmatory studies are needed to determine the more precise cause of the FOB alterations, consequences and localize the pathology to a specific tissue (i.e. specific tissue histology or enzyme assays, etc). The specific tissue of action is important to the hazard characterization of drugs, since the CNS has a very limited ability for regeneration, while some peripheral tissues can have a robust ability for reversibility, repair and regeneration (i.e. muscles). Disclaimer: The views expressed are those of the authors alone and do not necessarily reflect those of the US EPA.

1049 COMPARISON OF MOTOR AND COGNITIVE FUNCTION IN TWO SUBSTRAINS OF C57BL/6 MICE.

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The most common strain of mice used in animal research is C57BL/6 or B6. This control mouse is used as a standard for many different knockout mice in labs across the world. Although this strain of mouse is inbred, random mutations and separations of B6 mice in geographically distinct facilities resulted in an accumulation of genetic differences. Recently, single nucleotide polymorphisms (SNPs) have been identified in multiple substrains of the B6 line. To determine if these differences could affect results in common behavioral tests, we compared motor and cognitive function in two of the most commonly used substrains: C57BL/6, originating from Jackson laboratory, and C57BL/6N, originating from the National Institutes of Health. Motor function was analyzed with rotarod. We used Morris water maze to assess spatial learning and memory and novel object recognition to assess nonspatial learning and memory. We found no significant differences in rotarod performance, although there was a significant difference in the learning curve from days 3 compared with days 5. In the water maze, C57BL/6N mice outperformed the C57BL/6 mice in all three phases of hidden platform testing even after correcting for a statistically significant difference in swim speed. Our findings suggest the choice of substrain as a control or background strain might influence the interpretation of results from classic neurobehavioral tests.

1050 ASSESSMENT OF THE RESPIRATORY SENSITIZATION POTENTIAL OF PROTEINS USING AN ENHANCED MOUSE INTRANASAL TEST (MINT).


There remains a need for a simple and predictive animal model to identify compounds with the potential to induce respiratory sensitization and allergy. The mouse intranasal test (MINT) has been applied for the assessment of the relative allergenic potential of detergent enzymes; however its assessment was limited, evaluating only total and enzyme-specific antibody levels as endpoints. The present study was designed to evaluate an enhanced MINT which included multiple endpoints (serum antibody levels, airway inflammation and airway hyperresponsiveness (AHR)) to determine their value in improving the specificity and predictive accuracy of the MINT. BDF1 mice were intranasally instilled on days 1, 3, 10, 17 & 24 with Subtilisin (SUB), ovalbumin (OVA), beta-lactoglobulin (BLG), mouse serum albumin (MSA), or Keyhole Limpet Hemocyanin (KLH). On test day 29, wholebody plethysmography was used to measure AHR following methacholine or specific protein challenge followed by evaluation of bronchoalveolar lavage (BAL) fluid, pulmonary histopathology, and serum immunoglobulin responses. Evaluation of the results indicated that the assessment of AHR measured via wholebody plethysmography did not provide data that allowed for the evaluation protein allergicity. Reaginic antibody responses and IgG1 and IgG2a isotype characterization proved to be the most sensitive and reliable indicators of the protein allergenic potential, with SUB and OVA producing the most robust responses. BAL responses after protein-specific challenge were also indicators of allergen potential, with SUB, OVA, and KLH showing dose dependent induction of eosinophils in the BAL. Both BLG and MSA resulted in minimal to no responses for all endpoints. These data highlight that the evaluation of the respiratory sensitization potential of proteins can be best informed when multiple parameters are evaluated, and further improvements and refinements of the assay are necessary.
1051 ACUTE INHALATION TOXICITY IN RATS EXPOSED TO GASEOUS ATMOSPHERES OF HYDROGEN FLUORIDE AND CARBONYL FLUORIDE.

The acute Fire Extinguishing System (AFES) in Army vehicles commonly employs the “clean” agents Halon 1301 or FM-200. As a consequence of their fire extinguishing mechanism, toxic pyrolys products are formed as an essential chemi-cal step. These toxic gas pyrolysis products studies in rats conducted with hydrofluoric acid (HF) and carbonyl fluoride (COF2) which Army personnel may be exposed to. Groups of 10 rats each were exposed nose-only to a single, 5- or 10-minute exposure to gaseous atmospheres of either HF or COF2. Chamber concentrations were measured with a Fourier Transform Infrared Spectrometer (FTIR). Respiratory rates were measured during exposures and rats were monitored for mortality, clinical signs, and body weights. The 10-minute acute lethality concentration (LC50) for HF was 49,000 ppm and 1,100 ppm for COF2. Evaluation of the toxicity issues related to the potential exposure to these gases is an integral part of armored vehicle development and live fire testing.

1052 IN SEARCH OF PREDICTIVE PULMONARY HAZARD ASSAYS: LUNG TOXICITY AND IN SILICO STUDIES TO ASSESS TITANATE PARTICULATE MATERIALS.

The development of accurate lung toxicity studies are a necessary component of risk assessments. The aims of these studies are to develop less expensive/animal-intensive alternatives. Terraces compounds are a variety of metal titanium oxide (TO) particulate products, which include potassium TO varieties, such as sodium, magnesium or lithium TO or titanate particles of different shapes and particle sizes. Ninety day inhalation toxicity studies in rats conducted with the magnesium and potassium TO varieties demonstrated low toxicity (NOAELs = 50 and 10 mg/m3, respectively). In addition, pulmonary bioassay studies with Terraces JS (another type of potassium TO particle) indicated benign effects in rats compared to positive (i.e., quartz) controls. Previously we reported that the Vitamin C assay could serve as an effective screening tool for predicting in vivo lung effects of titanium dioxide (TiO2) particulates as an in silico indicator of particle reactiv-ity for fine and ultrafine (ul) TiO2 particulates; and results correlated with in vivo pulmonary toxicity effects in rats as reported in an earlier study (Warheit et al., 2007). Accordingly, we postulated that the Vitamin C assay might likewise serve as an animal alternative, predictive screening tool for other titanium oxide-containing compounds. To test the hypothesis, two different forms of potassium TO, magnes-ium potassium TO, and lithium potassium TO particulates were tested in the Vitamin C assay, using positive (P25) and negative (TiO2 control) particles. Using Vitamin C yellowing assay in silico methodology, the results were mixed – moderate surface reactivity for two potassium titanate compounds and low reactivity for lithium or magnesium potassium titanate particles. Thus, the data did not always correlate with low in vivo lung toxicity results on the same previously tested compounds. Based upon these results, the Vitamin C yellowing assay may be an effective screening tool for TiO2 particulates but not titanate (TO) particle-types.

1053 ACUTE TOXICOLOGICAL RESPONSES OF FISHER RATS TO NATURALLY OCCURRING ASBESTOS FROM THE UNITED STATES AND CANADA.
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This study was designed to provide understanding of the toxicity of naturally occurring asbestos (NOA) including Libby amosite (LA), Sumas Mountain chrysotile (SM), El Dorado Hills tremolite (ED) and Ontario actinolite/tremocto- nolite cleavage fragments (ON). Rat-respirable fractions (aerodynamic diameter ≤ 2.5 μm) were prepared by water elutriation and delivered via a single intratracheal (IT) instillation at doses of 0.5 and 1.5 mg/rat. Bronchoalveolar lavage fluid (BALF), lung histopathology and baseline pulmonary function were analyzed 1 and 3 μg pos-IT. One day post-IT, low-dose NOA exposure resulted in a 3-4 fold in-crease in BALF cellularity compared to dispersion media (DM) controls, whereas high-dose exposure had a more severe effect on lung inflammation which varied by source. Although inducing less acute inflammation than ON and ED, exposure to either LA or SM resulted in increased eosinophilia, as well as a greater degree of acute lung injury. Additionally, an increase in Penh (correlated with airway resist-ance) was only noted in rats exposed to high-dose LA or SM. Three months post-IT, most BALF parameters had returned to DM control levels. Despite a relative re-turn to baseline of lung inflammatory and injury markers, the development of fibrosis, as determined histologically, was greatest in SM-exposed rats (SM>LA>ON>ED). Consistent with this, elevated Penh was only noted in high-dose SM-exposed rats. These data demonstrate that, in the rat, SM results in more significant fibrosis and impairment of baseline lung function than exposure to LA. This study suggests that there may be cause for concern for people at risk of being exposed to NOA from the Sumas Mountain landslide, and highlights the need for further study of sites where NOA is present. (This abstract does not represent US EPA policy).

1054 DETECTION OF NAPHTHALENE (NA)-DNA ADDUCTS IN MOUSE OLFARY AND AIRWAY EPITHELIAL TISSUES BY ACCELERATOR MASS SPECTROMETRY.
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Humans are exposed to the ubiquitous PAH, NA, through a variety of industrial and environmental sources. NA is a byproduct of fossil fuel manufacturing, is re-leased following the combustion of wood, gasoline, and tobacco and is a major component of jet fuel. NA metabolites are consistently detected in human urine. Concern over the potential health effects of NA focuses in part on a role in the de-velopment of lung cancer, which is the number one cancer killer of men and women. Chronic rodent exposure studies showed that NA causes tumors in mice and rats. However, the majority of standard in vitro short-term mutagenicity assays for NA are negative. Formation of both depurinated and stable DNA adducts in vitro and in skin painting studies has been reported. Skin is not a known target tissue for NA, however. NA-derived DNA adducts have not been demonstrated in the respiratory system of any species. The goal of this research is to determine if stable NA-DNA adducts form in target tissues. Dissected airway and nasal olfactory epithelial tissues were incubated with 50 μM 14C-NA for 1 hr. DNA was isolated and its purity veri-fied by A260/A280 ratios. DNA samples were analyzed for 14C by accelerator mass spectrometry. NA-DNA adduct levels were slightly increased in treated airways compared to control, indicating DNA adduct formation occurs at low levels. The nasal olfactory epithelial samples yielded too little DNA to generate conclusive data and tissues will be pooled in the future. This preliminary ex vivo work demonstrates the feasibility of AMS to measure these low concentrations. More ex vivo studies to eliminate the chance of protein contamination will be needed as will in vivo studies. These studies provide the necessary data in target tissues to establish whether stable naphthalene-DNA adducts form and potentially clarify the mechanism of action of naphthalene tumorigenicity in animal models. Supported by NIHES 04699 and NCRR 13461.

1055 PULMONARY RESPONSES AFTER INHALATION OF RESISTANCE SPOT WELDING FUME USING AN ADHESIVE.

Resistance spot welding (RSW) is commonly used in the automotive and aircraft industries where high speed, repetitive welding is needed and relatively thin metal sections are welded. Epoxy adhesives are applied as sealers to the seams of the met-als that are joined. RSW produces complex aerosols composed of both metals and volatile compounds which have caused respiratory symptoms, including bronchitis and asthma, in exposed workers. The goals of the study were to develop a RSW generation and inhalation system, characterize the generated aerosols, and expose laboratory animals to determine the potential mechanisms that may be involved with the development of lung toxicity. RSW was configured so that two aerosols were generated and compared: (1) high metal (HM) fume and (2) low metal (LM) fume (mostly volatiles). Male Sprague-Dawley rats were exposed to 20-50 mg/m3 of the aerosols for 4 h/d x 3 d. Control animals were exposed to filtered air. Bronchoalveolar lavage (BAL) was performed at 1 and 7 d after exposure, and lung injury and inflammation parameters were measured in the recovered lavage fluid (BALF). Mass median aerodynamic diameter was determined by a Micro-Orifice Uniform Deposit Impactor and found to be 0.77 and 0.93 μm for the HM and mostly volatile LM fume, respectively. The HM fume was composed of greater than 90 % Fe, whereas little to no metal was present in the LM fume. Volatiles present in
the LM fume included benzene, toluene, 2-propan-1-ol, acetone, and others. An increase in lung injury parameters (albumin and lactate dehydrogenase) in the BALF was observed at 1 d after exposure to the HM fume that had resolved by 7 d, whereas exposure to the LM fume had no effect on lung injury and inflammation. Initial findings of this pilot study indicate that the metal, and not the volatile, component of RSW aerosol may influence the development of lung injury. Additional inhalation studies are ongoing to determine if either of these components alters lung function.

1056 CARDIOPULMONARY HEALTH EFFECTS OF TRAFFIC-RELATED AIR POLLUTANTS.

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There is emerging evidence that the inhalation of certain components of ambient air PM is associated with adverse health effects. These studies, combined with others that looked at the sources of toxicants, implicate traffic markers. Our study is designed to integrate the associations between health effects and exposures to traffic-related PM. In this case-control study, 11 healthy, non-smoking participants were recruited to measure pulmonary function (FEV1, FVC), exhaled NO, and blood pressure prior to, immediately after, and 24 hours after they walked along 3 diverse roadway types for 1.5 hours between July and September 2011. Prior to participation, volunteers went through a consenting process as determined by protocols approved by NYU’s IRB. The 3 roadway locations differ by traffic type: the George Washington Bridge (GWB) carries truck and car traffic, the Garden State Parkway (GSP) carries car traffic, and Sterling Forest, NY (SF) acts as a control location with little traffic influence. Levels of PM2.5, PM10, and BC were found to be 0.034 μg/m3, 0.043 μg/m3, and 8321 ng/m3 at the GWB; 0.017 μg/m3, 0.027 μg/m3, and 2452 ng/m3 at the GSP; and 0.017 μg/m3, 0.020 μg/m3, and 1496 ng/m3 at SF, respectively. Using a two-tailed paired t-test, p-values were generated between the GWB or GSP and SF results.

Despite the limited number of subjects, a trend of increasing eNO between the GWB and SF was observed (p=0.11 and 0.23) and a decreasing trend of systolic blood pressure both directly and 24 hours after the exposure between the GWB and SF (p=0.07 and 0.12) were observed. While no statistically significant differences in health effects were observed, a trend of increased eNO and decreased blood pressure at the GWB, in comparison to SF, suggests that PM10, and BC were found to be 0.034 μg/m3, 0.043 μg/m3, and 8321 ng/m3 at the GWB; 0.017 μg/m3, 0.027 μg/m3, and 2452 ng/m3 at the GSP; and 0.017 μg/m3, 0.020 μg/m3, and 1496 ng/m3 at SF, respectively. Using a two-tailed paired t-test, p-values were generated between the GWB or GSP and SF results.

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According to the US Energy Information Administration, coal remains to be the number one source of electricity, it fueled about 48.5% of the world’s electricity production in 2008. This has many implications on the health of coal workers, such as increased risk of developing coal workers’ pneumoconiosis (CWP). Epidemiological studies have determined that the prevalence of CWP is much higher in the Eastern US than the Western US. This indicates that the chemical compositions of coal may have a great affect on the development and prevalence of CWP. Acidity and the amount of Bioavailable Iron (BAI) have been demonstrated to be two important contributing factors to the toxicity of coal. BAI is the result of the oxidation of pyrite (FeS2) and its stability is pH-dependent. Coals from the western US have a high levels of calcite (CaCO3), and subsequently high pH, and low BAI. Based on these findings, we hypothesize that by adding calcite into a coal suspension, the amount of BAI will decrease thereby decreasing toxicity of the coal. We determined dose-response effects of coal and calcite samples from PA and UT in three models: cell-free, cell culture (Beas 2B cells) and mouse (129/SvEsvTac).

The BAI in PA coal originally at 400 ppm, was significantly reduced to 3-5 ppm within 20 minutes of adding a 10% calcite solution suspended in water. Also, RT-PCR results show that calcite helps reduce EMT markers such as fibroblast-specific protein-1 (FSP-1) in the murine model. We also speculate that inflammatory markers such as IL-6 and IL-1α would be down-regulated in the calcite and PA coal solutions as compared to the PA coal solution. These results demonstrated that introduction of calcite into coals of eastern regions of US could help reduce the incidence of CWP. Future studies should be aimed to corroborate findings in humans, as well as investigate the potential preventive effects of calcite in pulmonary fibrosis upon coal exposure.

1059 CALCITE AS A PREVENTIVE AGENT FOR COAL WORKERS’ PNEUMOCONIOSIS.

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Exposure to airborne particulate matter (PM) pollution has been correlated with the development and exacerbation of asthma, especially in susceptible populations such as young children. Inhalation of polymeric aromatic hydrocarbon (PAH) rich particles exacerbates respiratory disease and has been shown to cause oxidative stress. Polymorphisms in various antioxidant enzymes have correlated asthma with exposure to traffic generated PM. PM is a dynamic mixture with source and temporal variations that confound systematic studies of acute effects. In response, we have developed a laboratory-based PAH rich ultratine particulate (PPF) atmosphere that is devoid of metals and allergens and can be replicated for use in animal models. We exposed 7-day old neonatal and adult rats to a single 6 hour dose of 22.4
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1061 TEMPORAL PATTERN OF INFLAMMATION IN NEONATAL AND ADULT RATS FOLLOWING INHALATION OF PARTICULATE MATTER.


Over 23 million children under age 18 live in counties with unhealthy levels of short term particulate air pollution. This can predispose to respiratory disease. In this study, 7 day old and 18 week old rats were exposed for 6 hrs to laboratory synthesized PM (170 μg/m3, mean diameter 190nm) or to filtered air (FA). Peripheral blood and bronchoalveolar lavage fluid (BAL) was collected at 2 and 48 hours post exposure to monitor local and circulating markers of inflammation. Serum was collected for a multiplex cytokine array exposing neonates and adults; interleukin (IL)-4 gene expression was significantly increased only in neonate airways at 48 hr. Within the lung tissue, gene expression of colony stimulating factor (CSF)-1 was significantly increased after 48 hr of exposure to PM in the airways of both neonates and adults. Interleukin (IL)-4 gene expression was significantly increased only in neonate airways at 48 hr. Baseline expression of IL-1alpha, IL-1beta, IL-4, IL-6, IL-10, granulocyte macrophage colony-stimulating factor (GM-CSF), IFN-gamma, IL-6 was significantly greater in adult airways and parenchyma, as was interferon (IFN)-gamma. White blood cell counts in the circulation were not significantly altered by exposure at either age. In adult animals exposed to PM, serum cytokine levels were significantly increased at 48 hr compared to FA for IL-1alpha, IL-1beta, IL-4, IL-6, IL-10, granulocyte macrophage colony-stimulating factor (GM-CSF), IFN-gamma, and tumor necrosis factor (TNF)-alpha. PM exposed neonatal rats had significant decreases of IL-2, IL-4, IL-6, IL-10 and TNF-alpha at 48 hr in serum. We conclude that: 1) local inflammatory responses, particularly PMNs, are more robust to the same concentration of PM in neonates than in adults and 2) circulating cytokines are more likely to be increased in adults than in neonates following an inhalation exposure to fine/ultrafine PM, even in the absence of lung cytotoxicity or elevation of BAL cells. Supported by USEPA RD-83241401, ES06700 and ES05707.

1062 PULMONARY TOXICITY OF AMINES USED FOR CARBON CAPTURE AND STORAGE.

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Carbon dioxide (CO2) absorption with aqueous amine solvents is among the leading candidates for use in carbon capture and sequestration (CCS) techniques aimed at reducing greenhouse gas emissions from flue gases (coal-fired power plants, refineries, etc.). The environmental consequences this technology have been poorly characterized. A concern is that amines or degradation products that may form in the capture system or via interaction with flue gases may be emitted to the atmosphere and create unintended human exposures. The potential for these emissions to occur, the potential for the emissions to result in exposures, and the resulting health risk of these exposures are unknown. This study evaluated the potential health risks of inhalation exposure to amines and amine degradation products. Three model amines (monoethanolamine (MEA), methylidithiolanethanolamine (MDEA), piperazine (PIP) and their laboratory generated degradation products were used to create inhalation exposure atmospheres for exposures in mice. C57bl/6N mice were exposed for 7 days of inhalation to 25 ppm amines or the equivalent containing or degraded amines. Inflammatory response in lungs was assessed by counting inflammatory cells in lavage fluid and measuring cytokine expression in lung tissue. Inhalation exposure to degraded MEA resulted in significant increases in total cells, neutrophils, and lymphocytes compared to control mice. Significant increases in inflammatory cytokine expression were seen in mice exposed to the degraded MDEA atmosphere. These exacerbated inflammatory responses in mice exposed to degraded amines suggest that oxidative products of amines may produce increased toxicity. Work supported by the Electric Power Research Institute.

1063 COMPARISON OF HEALTH EFFECTS AND COMPOSITION OF SECONDARY ORGANIC AEROSOLS.

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We present findings from the Secondary Particulate Health Effects Research (SPHERES) program, which was created to define the composition and resulting health hazard of secondary organic aerosol (SOA) synthesized under varying reaction conditions. Reaction conditions were varied to evaluate SOA derived from a biogenic (α-pinene) or anthropogenic (toluene) hydrocarbon precursor in the presence of NOx/sunlight. The reactions were conducted with/without the addition of SO2 that would add additional acidity, and then a subsequent exposure where ammonia was added to titrate the acidity. Target SOA exposures were conducted at 300 μg/m3 of particulate material. Direct comparisons of composition and toxicity were conducted with normalization of atmospheres to either total particulate matter or total organic particulate matter. Exposures were conducted for 7 days (6 hr/day) to ApoE mice (male, 8-10 weeks) down-stream of honeycomb de-nuders employed to remove ozone. Measurements of pulmonary inflammation (lavage white blood cells) and indicators of vascular injury and remodeling were assessed. The presence of SO2 led to differences in the observed SOA composition for both α-pinene and toluene. SO2 participated directly with α-pinene to form organosulfate compounds, and had an apparent modification of toluene chemistry that led to enriched amounts of organonitrates compounds. The vascular responses showed increased expression of metalloproteinase activity and collagen expression. In our model, high ozone flux plays a role in the site-specificity of this injury. To test this hypothesis, we determined that cyclic ozone exposure causes epithelial necrosis, squamous metaplasia, and neutrophilic rhinitis in the nasal airways of infant monkeys, a model for the developing nasal airways of children. These lesions are not uniformly distributed across the nasal airways and vary in location and extent, increasing injury at the nasal septum. The site-specificity is likely due to a complex relationship between tissue susceptibility and local ozone dose. The purpose of this study was to test the hypothesis that ozone flux plays a role in the site-specificity of this injury. To test this hypothesis, we developed a three-dimensional (3D) computational fluid dynamics (CFD) model of the left nasal cavity of a 6-month-old rhesus monkey. The computer model was used to simulate inspiratory airflow and ozone uptake. Simultaneously, 6-month-old rhesus monkeys were exposed to 1 cycle of ozone (5 days of filtered air + 9 days of 0.5 ppm ozone, 8h/day), 11 cycles of ozone, or filtered air. The left nasal passage was processed for light microscopy. The presence or absence of epithelial necrosis was plotted on the corresponding regions delineated on the CFD model. Model predictions of ozone flux were compared to lesion incidence along the perimeter of two transverse cross-sections by correlation analysis. In our model, high ozone flux was not limited to the regions of ozone-induced injury, but was also found in other regions of the nasal airways. As a result, ozone flux was not significantly correlated with ozone-induced epithelial necrosis. These findings suggest high dose alone is not sufficient for ozone-induced nasal airway injury, and that additional biological factors may render some sites more susceptible to injury after ozone exposure.

1064 CORRELATION BETWEEN COMPUTER-DERIVED OZONE FLUX AND OZONE-INDUCED INJURY IN THE NASAL AIRWAYS OF INFANT RHESUS MONKEYS.

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The mechanisms by which ozone, the principal oxidant pollutant in photochemical smog, causes nasal epithelial injury are incompletely understood. We have demonstrated that cyclic ozone exposure causes epithelial necrosis, squamous metaplasia, and neutrophilic rhinitis in the nasal airways of infant monkeys, a model for the developing nasal airways of children. These lesions are not uniformly distributed across the nasal airways and vary in location and extent, increasing injury at the nasal septum. The site-specificity is likely due to a complex relationship between tissue susceptibility and local ozone dose. The purpose of this study was to test the hypothesis that ozone flux plays a role in the site-specificity of this injury. To test this hypothesis, we developed a three-dimensional (3D) computational fluid dynamics (CFD) model of the left nasal cavity of a 6-month-old rhesus monkey. The computer model was used to simulate inspiratory airflow and ozone uptake. Simultaneously, 6-month-old rhesus monkeys were exposed to 1 cycle of ozone (5 days of filtered air + 9 days of 0.5 ppm ozone, 8h/day), 11 cycles of ozone, or filtered air. The left nasal passage was processed for light microscopy. The presence or absence of epithelial necrosis was plotted on the corresponding regions delineated on the CFD model. Model predictions of ozone flux were compared to lesion incidence along the perimeter of two transverse cross-sections by correlation analysis. In our model, high ozone flux was not limited to the regions of ozone-induced injury, but was also found in other regions of the nasal airways. As a result, ozone flux was not significantly correlated with ozone-induced epithelial necrosis. These findings suggest high dose alone is not sufficient for ozone-induced nasal airway injury, and that additional biological factors may render some sites more susceptible to injury after ozone exposure.
PM composition as a result of the partition of semivolatile organic compounds (SVOCs) to the vapor phase once released into the atmosphere. Therefore, the aim of this study was to assess the difference in cardiovascular toxicity between semivolatile and refractory nonvolatile constituents of ultrafine PM. Apoprotein E knockout (ApoE-/-) mice, which are prone to develop atherosclerosis, were exposed to ultrafine concentrated ambient particles (CAPs), or to either its semivolatile components or to nonvolatile CAPs. Animals were exposed for 5 hours a day, 4 days a week, over an 8 week period, 100m downwind of a major freeway. A subset of animals was implanted with a telemetry device to record electrocardiograph (ECG) readings. Heart rate variability (HRV), a measure of autonomic cardiovascular function, was calculated from ECG readings during each experiment and compared to baseline values. The progression of atherosclerosis was determined by analyzing aorta tissue sections for plaque build-up and lipid accumulation. Serum levels of total cholesterol and protein carbonyls were also measured. Greater HRV changes from baseline were observed in mice exposed to unmodified CAPs and SVOC components. Removal of SVOCs present in ultrafine CAPs resulted in a reduction of both plaque formation and lipid accumulation in the aorta. No differences among groups were observed for total cholesterol and protein carbonyls. The results indicate that SVOCs are important contributors to the cardiovascular toxicity attributed to ultrafine PM. Further analyses will measure additional serum biomarkers (i.e. lipid peroxidation and CRP) and will attempt to correlate the physiological and biological endpoints with specific PM components (i.e. PAHs).

**PARTICULATE MATTER EXPOSURE MODULATES OXIDATIVE AND INFLAMMATORY PARAMETERS IN SPECIFIC REGIONS OF THE APOE-/- MOUSE BRAIN.**

L. Mendez, A. Campbell, A. Keebaugh and M. T. Kleinman.

Exposure to particulate matter (PM), present in urban environments, has been shown to induce pro-inflammatory central nervous system (CNS) effects in apoprotein E knockout (ApoE-/-) and Balb/c mice. In this study pro-inflammatory and oxidative stress responses in different regions of the ApoE-/- mouse brains were evaluated after a chronic exposure to fine (2.5-10μm) concentrated ambient particles (CAPs). ApoE-/- mice were exposed to either CAPs or particle-free air for 5 hours a day, 5 days per week, for a period of 6 months. Subset of animals were euthanized at 2, 4 and 6 months to evaluate the progression and persistence of pro-inflammatory and oxidative stress responses in the brain. To elucidate whether specific regions of the brain are more susceptible to the adverse effects of CAPs, brains were dissected into 3 regions (rostral, mid, and caudal) and each region was analyzed separately. Baseline levels of the pro-inflammatory cytokines IL-6 and TNFα and the transcription factors NF-κB and AP-1 were different based on the region analyzed and the exposure length. CAPs treatment did not significantly alter these baseline levels. Higher levels of IL-6 were observed in the rostral region after 2 and 6 months of exposure. Increases in TNFα were only seen after 6 months of exposure. Baseline levels of the anti-inflammatory cytokine IL-10 were higher in the caudal region and there was less evidence of inflammation in that region. Baseline levels of protein carbonyls were higher in the rostral region with an increase of protein oxidation after two months of exposure. The results indicate that different brain regions may respond uniquely to exposure to ambient air pollutants such as PM. To what extend these changes contribute to the pathology associated with different neurodegenerative diseases is the focus of our current research endeavors.

**A FOUR-WEEK INHALATION TOXICITY STUDY OF CUPROUS OXIDE IN RATS.**


The objective of this study, in which albino rats were exposed (whole-body) to cuprous oxide (Cu2O) aerosols for 4 weeks (5 days/week), was to evaluate inhalation toxicity with an emphasis on respiratory tract pathology. Exposure concentrations were 0, 0.2, 0.4, 0.8 and 2.0 mg/m3. Groups exposed at 0 and 2.0 mg/m3 were also evaluated following a 13-week recovery period. Pathological assessments consisted of hematologic, bronchoalveolar lavage fluid (BALF) evaluations, gross necropsy and organ weight evaluations and microscopic examination of selected tissues. Test article-related effects on quantitative endpoints included higher blood neutrophil counts and BALF total protein, lactate dehydrogenase and percent neutrophils at 0.2 mg/m3 and higher BALF total cell counts, lung weights (wet and dry) and lung histopathology in animals exposed to 0.4 mg/m3. Microscopic effects in the lungs consisted of alveolar histiocytosis (higher numbers of large, foamy macrophages) and acute inflammation at ≤0.2 mg/m3 and mononuclear cell infiltrates at ≥0.4 mg/m3. The lungs of animals exposed at 0 and 2 mg/m3 were also examined using Masson’s Trichrome stain, revealing slightly greater collagen staining relative to control in alveolar septa and alveolar ducts. Lymphoid hyperplasia of the lung draining lymph nodes was seen at ≥0.2 mg/m3. Microscopic effects in the nasal cavity were rare and limited to the 0.8 and 2.0 mg/m3 groups. At the 13-week recovery evaluation, the only effects were slightly higher lung weight and slightly greater collagen staining relative to control in alveolar septa. Results of these investigations indicate that Cu2O-related effects were found at all exposure levels. However, the microscopic findings in the lungs and lymph nodes represented a typical response in the rat to an inhaled insoluble dust, and there were no microscopic indications of alveolar epithelial injury or repair or of the presence of edema. Nearly complete resolution of findings was observed following a 13-week recovery period.

**EVALUATION OF INHALATION EXPOSURE TO AEROSOLIZED D-LIMONENE IN SPRAGUE DAWLEY RATS.**


D-limonene is a commonly used fragrance material that has been associated with dermal allergies. The study objective was to evaluate the potential toxic effects, including pulmonary inflammation, of aerosolized d-limonene when administered to Sprague-Dawley (SD) rats by nose-only inhalation at 10, 100, or 1000 ppm for 2 weeks (6 hours/day, 5 days/week). Standard endpoints included clinical observation, body and organ weights, hematology and serum chemistry evaluation, macroscopic/microscopic examination, and bronchoalveolar lavage fluid (BALF) analysis to determine pulmonary inflammation. Cytokines in BALF and serum were measured for immune-mediated mechanisms of action. For comparison to test compounds and to validate the utility of cytokine measures for evaluation of pulmonary inflammation, a nose-only inhalation pilot was conducted with 25 mg/m3 amorphous silica (0, 1, 2, 5 or 10 exposures). Consistent with published data, SD rats had higher lung weights and a time-dependent increase in pulmonary irritation, evidenced by increase in BALF and histopathologic parameters. For d-limonene, respiratory tract changes in the 1000 ppm group animals were minor. BALF parameters were unaffected compared to controls and to silica-treated animals. No inflammation was indicated by cytokine profiles in BALF and serum. Low thymus weights in the 1000 ppm group correlated with lymphoid necrosis/depletion in females, while low spleen weights in this group had no microscopic correlate. High-dose males had mild/moderate hyaline droplet accumulation in the kidney proximal tubules, common post oral ingestion. Exposure to 1000 ppm is higher than any occupational or consumer exposure scenario representing a threshold of response that inhalation exposure to d-limonene in the general population poses little to no safety concern.

**EVALUATION OF INHALATION EXPOSURE TO AEROSOLIZED 2, 3-PENTANEDIONE IN SPRAGUE DAWLEY RATS.**


2,3-Pentanedione is used in butter flavoring for consumer products. A related material, diacetyl, has been suggested to cause pulmonary effects associated with the development of bronchiolitis obliterans. The study objective was to evaluate the potential toxic effects, including adverse pulmonary effects, of aerosolized 2,3-pentanedione when administered to Sprague Dawley (SD) rats by nose-only inhalation at 8.8, 17.5, and 35 ppm for 2 weeks (6 hours/day, 5 days/week). Standard endpoints included clinical observation, body and organ weights, hematology and serum chemistry evaluation, macroscopic/microscopic examination, and bronchoalveolar lavage fluid (BALF) analysis to determine pulmonary inflammation. Cytokines in BALF and serum were measured for immune-mediated mechanisms of action. For comparison to test compounds and to validate the utility of cytokine measures for evaluation of pulmonary inflammation, a nose-only inhalation pilot was conducted with 25 mg/m3 amorphous silica (0, 1, 2, 5 or 10 exposures). Consistent with published data, SD rats had higher lung weights and a time-dependent increase in pulmonary irritation, evidenced by increase in BALF and histopathologic parameters. For 2,3-pentanedione, all animals survived and no pulmonary effects were observed (no microscopic, BALF or cytokine effects) Nasal
transitional epithelial hyperplasia, subacute inflammation and a dose-dependent in-
crease in hemorrhage, necrosis, and edema, as well as laryngeal sub-acute inflam-
mation, were noted at 17.5 ppm. It was previously reported that inhalation expo-
sure to >200 ppm diacetyl (6 hr/for 5 days) caused acute necrotizing rhinitis, er-
ose/necrotizing laryngitis and death. This study presents a threshold of exposure at
which the lowest dose tested, 8.8 ppm (>31 ppb STEL), failed to induce acute
necrotizing rhinitis/laryngitis.

1070 THE ROLE OF CLAUDIN 4 IN EPITHELIAL REPAIR FOLLOWING LUNG INJURY.
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San Francisco, CA.
Repair of epithelium in the lung is fundamentally important to recovery and sur-
vival in patients with acute lung injury and ARDS. Regulation of fluid balance in
the lung via paracellular pathways is managed by the Claudin family of tight junc-
tion proteins. While the involvement of Claudins in regulating ion and fluid trans-
port is apparent, other potential functions of these tight junction proteins is un-
clear. Previous studies have observed a marked increase in Claudin 4 (cldn4) mRNA
expression in mice following ventilation induced lung injury. Similarly cldn4 levels
have been shown to be induced in a variety of epithelial injury models, not specific
only to the lung. In our current study we utilized both in vitro and in vivo models to
study the role of cldn4 in response to epithelial wounding and to delineate spe-
cific pathways critical to the unique regulation of this protein and its involvement
in epithelial repair. Naphthalene (NA), a ubiquitous environmental pollutant,
causes selective toxicity of Clara cells lining the conducting airway of mice. At 24
hrs following NA treatment, we found that mice exhibited an increase in lung per-
meability coupled by a 4 fold induction of cldn4 protein. Cldn4 levels returned to
control levels as lung permeability was restored following epithelial repair. The in-
duction of cldn4 was inhibited by pretreatment of animals with an EGFR inhibitor,
indicating cldn4 acts downstream of EGFR in wound repair and likely is implicated
in cellular proliferation. In our cell culture model of epithelial injury, 16HBE cells
were induced for cldn4 mRNA and protein levels along the migrating edge of cells
following scratch wounding. Early events following scratch wounding included in-
creased tyrosine phosphorylation of cldn4. siRNA knock down of cldn4 was suffi-
cient to inhibit wound closure rates. We conclude from these studies that cldn4 is
induced with epithelial injury and is necessary for timely epithelial repair and resto-
arion of barrier function in pulmonary epithelium.

1071 AN INHALATION TOXICITY TIME-COURSE EVALUATION OF CUPROUS OXIDE IN RATS.
J. T. Weinberg 1, B. Danaisien 2, C. Mackie 3, D. T. Kirkpatrick 1 and J. B. Nold 1, 
1, 2, 3

For this study phase, albino rats were exposed to cuprous oxide (Cu2O) aerosols at
0 and 2.90 mg/m3 by whole-body inhalation for 1, 2, 3 or 4 weeks (5 days/week).
The objective was to evaluate possible temporal relationships between duration of
exposure and test article-related effects and whether a plateau was observed for ef-
ficacy differences related to NO exposure. These results suggest that in a healthy animal
model exposure to CO or NO alone, at levels reflecting concentrations found in
primary components of diesel emissions, on vascular function in systemic arteries.
We hypothesized that exposure to these monoxide gases, at concentrations similar to
those of diesel emissions, would reproduce the negative vascular effects seen pre-
viously. Whole body rodent exposures were conducted for both NO and CO or
room air for one or four hours. CO exposure was 0, 50 and 100 ppm; NO exposure
levels were 0 and 10 ppm. Vascular rings, two per animal, were isolated immedi-
ately after exposure, mounted on a force-transduction myograph and then chal-
lenged with potassium, phenylepherine (PE) and acetylcholine (ACh). The NO ex-
posures were conducted for one hour, preceded by N-acetylcysteine (NAC) or
vehicle supplementation (intraperitoneal injection; 200 μg/kg). Additionally, both
young (4-6 weeks) and old (4-6 months) mice underwent this one hour NO/NAC
exposure regimen. Vascular response to lower concentrations of ACh was enhanced
after CO exposures. NO inhalation did not alter the vascular responsiveness to ei-
ther PE or ACh in mice or rats, although rat plasma nitrate/nitrite levels were sig-
ificantly elevated after NO exposure. NAC supplementation did not produce the
enhanced plasma NOX levels previously seen with diesel emissions exposures. Old
mice had a noticeable decrease in vascular responsiveness overall, but showed no
differences related to NO exposure. These results suggest that in a healthy animal
model exposure to CO or NO alone, at levels reflecting concentrations found in
whole diesel emissions, do not have deleterious effects on systemic vascular reactiv-
ity.

1072 VASCULAR FUNCTION EFFECTS OF ACUTE INHALATION OF CARBON MONOXIDE AND NITRIC
OXIDE.
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Cardiovascular disease (CVD) remains a major cause of morbidity and mortality in
the developed and developing world. Exposure to emissions from diesel and gaso-
line engines is widely recognized as an important risk factor for CVD. In an effort
to determine the drivers of toxicity in the complex diesel exhaust mixture, we have
examined the effects of nitric oxide (NO) and carbon monoxide (CO), two of the
primary components of diesel emissions, on vascular function in systemic arteries.
We hypothesized that exposure to these monoxide gases, at concentrations similar to
those of diesel emissions, would reproduce the negative vascular effects seen pre-
viously. Whole body rodent exposures were conducted for both NO and CO or
room air for one or four hours. CO exposure was 0, 50 and 100 ppm; NO exposure
levels were 0 and 10 ppm. Vascular rings, two per animal, were isolated immedi-
ately after exposure, mounted on a force-transduction myograph and then chal-
lenged with potassium, phenylepherine (PE) and acetylcholine (ACh). The NO ex-
posures were conducted for one hour, preceded by N-acetylcysteine (NAC) or
vehicle supplementation (intraperitoneal injection; 200 μg/kg). Additionally, both
young (4-6 weeks) and old (4-6 months) mice underwent this one hour NO/NAC
exposure regimen. Vascular response to lower concentrations of ACh was enhanced
after CO exposures. NO inhalation did not alter the vascular responsiveness to ei-
ther PE or ACh in mice or rats, although rat plasma nitrate/nitrite levels were sig-
ificantly elevated after NO exposure. NAC supplementation did not produce the
enhanced plasma NOX levels previously seen with diesel emissions exposures. Old
mice had a noticeable decrease in vascular responsiveness overall, but showed no
differences related to NO exposure. These results suggest that in a healthy animal
model exposure to CO or NO alone, at levels reflecting concentrations found in
whole diesel emissions, do not have deleterious effects on systemic vascular reactiv-
ity.

1073 ALTERATIONS IN NEURAL TELOMERE SYSTEM FOLLOWING CHRONIC INHALATION EXPOSURE TO
COAL COMBUSTION.
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Air pollution exposure is associated with premature mortality due to heart and lung
disease, an observation that has been well characterized epidemiologically. Recent
studies indicate a reduction in life expectancy related to particulate matter (PM) levels,
suggesting that the US population loses more than 7 months of life span for
every 10 μg/m3 of PM2.5. Additionally, telomere length in peripheral blood cells
has been found to be shorter in association with high pollution regions. Together
these observations suggest that air pollution may contribute to premature cellular
aging. In this study, we hypothesized that chronic exposure to a Simulated Downwind
Coal Combustion Atmosphere (SDCCA) would result in decreased telomere length and increased apoptosis in brain cortex. Brain tissue from rats ex-
posed daily for 6 months to low (100 μg PM/m3), medium (300 μg PM/m3), high
(1000 μg PM/m3), PM-filtered high and control (room air) coal combustion at-
mospheres were assayed for alterations to the telomere system. mRNA expression of
TERT was reduced by 70% (p<0.05) in the cortex of female rats exposed to high
levels of SDCCA compared to both control animals and male rats. This reduction
was partially ameliorated in female animals exposed to PM-filtered high SDCCA,
which had a 29% reduction in TERT mRNA expression compared to control ani-
mals. Males showed a more modest reduction in TERT (45% decrease) that was
also restored with the filtration of PM. TRF2 mRNA, but not TRF1, also showed
differences related to NO exposure. These results suggest that in a healthy animal
model exposure to CO or NO alone, at levels reflecting concentrations found in
whole diesel emissions, do not have deleterious effects on systemic vascular reactiv-
ity.

1074 THE EFFECTS OF COPPER PYRITHIONE ON DEVELOPING ZEBRA FISH EMBRYOS.
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Queens, NY.
Copper pyrithione, an active component in antifouling biocides predominantly
found in marine paints, has been shown to cause anomalies during early develop-
ment of mummichogs (Fundulus heteroclitus). Since a strict regulatory use of organ-
ants as antifouling agents has been imposed, a frequent substitute within the Japanese market has been the use of metal pyrithiones, principally zinc and copper. Zebrasfish (Danio rerio) embryos were exposed within the first hour after fertilization to increasing concentrations of copper pyrithione (2, 4, 8, 12, 16, 32 and 64 μg/L) for 24 hours. Morphological abnormalities at 30, 60, and 120 hours post fertilization were recorded. Hatchability was measured every 12 hours for 5 days. Following the 24 hour exposure, results showed morphological abnormalities, particularly of the notochord at concentrations of 12 μg/L and higher when compared to controls. Notochords were severely twisted as concentrations increased. Distortions of the notochord began in the caudal region and proceeded rostrally. Edema was observed in the cardiac and yolk sac regions at the 12 and 16 μg/L concentrations. Hatching rate also decreased in a dose dependent manner. At 120 hours post fertilization, 47 percent of embryos hatched, while all of the controls hatched by 96 hours post fertilization. Copper accumulated in whole embryo tissues and was significantly different in 32 and 64 μg/L CuPT treatment groups as compared to controls. Lipid peroxidation was also significantly increased in animals exposed to 32 and 64 μg/L of copper pyrithione. These results demonstrate that oxidative stress plays a partial role in the observed toxicity. Copper pyrithione has shown to be toxic to zebrasfish at concentrations of 12 μg/L or greater. The abnormalities and deformities observed in fish larvae would significantly decrease survival in non-controlled environments.

**1075 DIFFERENCES IN UPTAKE, METABOLISM, AND CLEARANCE OF ATRAZINE AND TAMOXIFEN IN A FISH AND A RAT SPECIES.**


Atrazine and tamoxifen are known endocrine-disrupting chemicals (EDCs) that have metabolites exhibiting biological activities that are equally or more potent than the parent compound. To evaluate if uptake, metabolism and clearance of such EDCs is a concern in interspecies extrapolation, plasma concentrations of these chemicals and major metabolites were compared over time in fish and rats treated with a single oral dose of atrazine (50 mg/kg) or tamoxifen (25 mg/kg). For atrazine, plasma samples were collected 0.25, 0.5, 1, 3, 24, 72, and 120 hours after gavage. Atrazine and metabolites were quantified using GC/MS. Atrazine was detected in rat and fish plasma within minutes, indicating rapid uptake in both species. Rats metabolized atrazine more slowly at 3 hours, than fish, demonstrating dissimilar concentrations than atrazine at all time points and concentration of the metabolite diaminochlorotriazine (DACT) in rat plasma was 30 times that in fish at 3 hours. In fish, atrazine was metabolized more slowly at 3 hours, atrazine predominated in fish plasma at concentrations over 100 times that in rat plasma. By 120 hours, only DACT was still detectable in both rats and fish. For tamoxifen, animals were sampled 1, 4, 8, 16, 20, 24, 48 and 72 hours post-dosing. Tamoxifen and metabolites in plasma were quantified using HPLC. Peak plasma concentrations of tamoxifen (about 600 ng/ml) were observed at 1 hour in fish and at 4 hours in rats, indicating a slightly faster uptake in fish. From 4 hours forward, tamoxifen and metabolite 4-hydroxytamoxifen were detected in fish plasma, but not rats, at each time point. In rat plasma, from 16 hours forward, tamoxifen and metabolite N-desmethyltamoxifen were prevalent, again at similar concentrations. These results indicate uptake and metabolism of environmental EDCs by different species can vary substantially and could be a significant issue when extrapolating effects across species.

**1076 TOXICITY OF LEGACY AND REPLACEMENT FLAME-RETARDANT CHEMICALS ON DAPHNIA MAGNA**


Chemical flame-retardants (FRs) have been added to consumer products and used extensively in California since the 1970s. Since the FRs are not covalently bonded to materials in which they are added, they leach into the environment and end up in animals such as humans and marine mammals. However, very little data exists about the toxicity of flame retardants in environmentally relevant concentrations that occupy the lower ranks of the food web, such as Daphnia magna. In this work, we determined the acute 48-hour toxicity of flame retardants to neonate D. magna. Flame retardants tested include banned legacy chemical pentabromodiphenyl ether (penta-BDE), related octa- and deca-BDE, and penta-P derivative’s formulation Firemaster®550 (FM550). We also tested FM550 components tetra-bromo phenyl (TBB), triphenyl phosphate (TPP), and Firemaster®7Z-54, which is a mixture of TBPB and tetra-bromo benzene (TBB). We found that FM550 has a higher acute LC50 to D. magna than its predecessor, penta-BDE. We also performed microarray gene-expression studies on adult daphnids at 1/10L C, and found that exposure to each chemical elicited a unique genomic response in the daphnids. It is therefore possible that these chemicals with very low water solubility could be acting via unique modes of toxicity instead of by a purely narcotic toxic effect.

**1077 ANXIOLYTIC EFFECTS OF FLUOXETINE ON THE STARTLE RESPONSE AND EXPLORATORY BEHAVIOR OF THE ZEBRAFISH.**

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Pharmaceuticals, including the antidepressant fluoxetine (Prozac) are released in waste-water effluent and are present in measurable quantities in effluent-impacted streams. Environmental exposure to pharmaceuticals may produce subtle aberrant behaviors that could impact the survival of affected individuals and populations. Fluoxetine, a selective serotonin reuptake inhibitor, is an anxiolytic and has been linked to decreases in social aggression, reproductive success and C-start (startle) behaviors in several fish species. Startle responses in fish vary, but often include changes in swimming velocity as the fish freeze or flee and decreased exploratory behavior. This study examines the effects of fluoxetine on the startle response and exploratory behavior of individual zebrafish following an acoustic/vibrational stimulus. Fish were exposed in tank water at an environmentally relevant low dose 0.1 μg/L or a high dose of 1.0 μg/L for 1 week or were injected with a pharmacological dose via IP microinjection at 10 μg/g body weight. The movement of individual fish in a 10 L tank digitally divided into 4 zones was tracked by computer (Ehrovision ver. 3.0) for 2 min. prior to and 2 min. following a startle stimulus (ball pendulum). Swimming velocities were used to assess baseline-swimming behavior and startle responses, while frequency of crossings between and total duration of time spent in each zone were used to assess exploratory behavior. Under the influence of fluoxetine, zebrafish showed a decrease in acute startle response intensity, decreased baseline-swimming velocity, more rapid recovery from startle behaviors and increased exploratory behavior. Such anxiolytic effects in the wild might increase the likelihood of a fish encountering a predator and decrease its likelihood of surviving an attack. Environmental and injection protocols produced disparate results, suggesting that acute treatments by injection might not be an accurate way to model environmental effects of pharmaceuticals on fish in the wild.

**1078 FISH TISSUE LEVELS OF FIVE METALS ASSOCIATED WITH CRUDE OIL FROM THE DEEP HORIZON WELL.**

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Scad mackerel, TRACHURUS LATHAMI, were collected from the northern Gulf of Mexico as part of the 2010 NOAA Pelagic Trawl Survey. Fish tissues were analyzed by ICP for five metals associated with crude oil from the BP Deep Horizon Well. On samples analyzed to date, nickel concentrations ranged from 1.67 μg/g to 8.35 μg/g Vanadium levels ranged from 0.86 to 49 μg/g. Chromium, lead and thallium, the high values were 0.40, 0.63 and 1.30 μg/g respectively. Low values for each of these metals were not detected. Metal concentrations in these fish varied with sampling locations across the northern Gulf of Mexico.

**1079 APPLICATION OF EASILY-SYNTHESIZED INTERNAL STANDARDS FOR RAPID QUANTITATIVE ANALYSIS OF CYANOBACTERIAL BLOOMS FOR HEPATOTOXIC MICROCYSTINS BY MATRIX-ASSISTED LASER DESORPTION IONIZATION MASS SPECTROMETRY (MALDI-MS).**

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The ubiquitous freshwater cyanotoxins, microcystins, (MCs), pose a global public health threat with potent acute hepatotoxicity, persistence in drinking and recreational water, and potential chronic and neurotoxic effects. Over 80 congeners have been identified worldwide, yet most risk assessment and monitoring rely on one congener (MC-LR). To achieve sensitivity to recommended World Health Organization (WHO) guideline of 1 μg/L, analytical methods require substantial sample processing and concentration, resulting in undesirable time lags during SOT 2012 ANNUAL MEETING 231
bloom outbreaks. While existing ELISAs demonstrate capable congener detection, HPILC-MS requires individual standards and skilled technicians. In contrast, MALDI-MS requires little sample, short analysis time and demonstrates remarkably reduced matrix interference in raw samples; the major limitation for quantification application to surface water has been lack of an internal standard with similar crystallization and desorption properties. (S-hydroxyethyl-Cys(7))-MC-LR and -RR internal standards (IS) were synthesized within minutes from commercially available MCs at room temperature. Standard curves and limit of quantification (LOQs) were determined for spiked MC –LR, -RR and -YR. Quantitative analysis of MCs in raw surface water with IS was compared with ELISA and HPILC. Linear standard curves were reproduced across several orders of magnitude. Using the ratio of peak intensity of analyte to IS, LOQs were 5 μg L⁻¹, permitting quantification below the WHO limit after a 20-fold concentration. Limits of detection were consistently well below 0.1 μg L⁻¹. Eighty six to 94% of MC-LR was recovered from spiked raw waters. The minimal processing and concentration, easy synthesis of IS and ability to determine across concentrations of hydrophilic congeners will drastically reduce time needed to provide an estimate of total toxin concentration and congener-specific risk.

**1080 LOW-LEVEL KETOCONAZOLE INCREASES ESTROGENIC POTENTIAL OF IBUPROFEN IN H295R CELL LINE AND ORYZIAS LATIPES.**


Most of toxicity tests were made on individual chemicals, while pharmaceuticals found in the aquatic environment usually occur as mixtures. In the present study, we hypothesized that ibuprofen metabolism might be decreased due to CYP2C9 inhibition by low level ketoconazole exposure, leading to increase of estrogenic potential of ibuprofen exposure. After 48 hr exposure to 0.02-20 mg mL⁻¹ of ibuprofen alone or in combination with 5 ng L⁻¹ of ketoconazole in the human adrenocortical carcinoma (H295R) cell line, the production of 17β-estradiol (E2) and testosterone (T), the activity of aromatase, and the mRNA expression of steroidogenic genes and CYP2C9 gene were measured. In addition, concentrations of E2 and T in blood plasma, and the mRNA expression of steroidogenic genes were determined in male medaka fish after 14 days exposure to ibuprofen 0.02 and 0.2 mg L⁻¹ or in combination with 10 μg L⁻¹ of ketoconazole. Ibuprofen alone exposure increased E2 production, and enhanced both aromatase activity and expression of CYP11β2 mRNA in the cells. When 5 ng L⁻¹ of ketoconazole was added, the extent of increase in E2 production, aromatase activity, and expression of CYP11β2 mRNA became greater. In medaka fish, while ibuprofen exposure at 0.2 mg L⁻¹ did not cause any significant effects on E2 concentration compared to that of control, co-exposure to ketoconazole caused significant increase of E2 concentration. In addition, a combined exposure to ibuprofen and ketoconazole resulted in an elevated expression of CYP17 and CYP19β mRNAs in male medaka, compared to ibuprofen only exposure. The results of present study indicate that non-effective concentrations of ketoconazole can increase the potential for endocrine disrupting effects of ibuprofen exposure.

**1082 CLINICO-HEMATOLOGICAL RESPONSE TO EXPERIMENTAL INFECTION OF ESCHERICHIA COLI O157:H7 IN CLARIAS GARIEPINUS.**

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African catfish, Clarias gariepinus is one of the most suitable species for aquaculture in Africa. Information on pathogenesis of E. coli O157:H7 infection is scarce. This study therefore aims at elucidating information on pathogenesis and hematological response of C. gariepinus experimentally infected with E.coli. With the hope of giving a baseline information for possible production of a vaccine against E.coli. Hundred clinically healthy C.gariepinus of body weight 110-30 g were acclimated for 2weeks. 103, 104 and 105 cfu of E.coli were inoculated intraperitoneally into the test fish. Clinical signs and gross changes were observed throughout the experiment. Trend in bacteriological, hematological and plasma biochemical parameters were also observed using standard methods. Data generated were analysed using SAS package. The fish became lethargic and disoriented in swimming in the first 24hours. No significant differences were observed in PCV, Hb, RBC, platelet, lymphocyte, neutrophils, eosinopahils, total protein and albumin at 48hours. Significantly high WBC, globulin and monocyte counts were observed at 48hours in fish exposed to 105 cfu of E.coli. The significant increase at 48 and 96hours was observed in enzymes AST, ALT and ALP suggest liver damage. Significant decrease in WBC and platelet counts at 96 and 168hours suggests immune response to E.coli. At 36hours, further decrease was observed in platelet compared to the control. PCV and Hb dropped significantly from 168hours to 504hours but the reverse ensued in globulin and ALP when compared to control. At 336hours, a significant decrease was observed in AST, ALT and ALP. E.coli counts in log10 cfu/g were 5.079(27hr), 4.4(216hr) and 2.372(control). Although total protein was not affected at acute and chronic exposure to E.coli, the general physiology of the fish was adversely affected even at 105, this implies that C.gariepinus has a strong immune response which should be further investigated.

**1083 US EPA ECOLOGICAL STRUCTURE-ACTIVITY RELATIONSHIP (ECOSAR) MODEL—VERSION 1.1 RELEASE.**


The US EPA Office of Pollution Prevention and Toxics (OPPT) is responsible for implementing the Toxic Substances Control Act (TSCA). TSCA is the US law that regulates industrial chemicals in the US and OPPT evaluates both new chemicals entering commerce, as well as those chemicals that have been in existence for some time. In evaluating new and existing chemicals over the past 35+ years, OPPT has developed a strong knowledge base in structure-activity-relationships (SARs). The Ecological Structure Activity Relationships (ECOSAR) Class Program is a computerized predictive system that estimates aquatic toxicity. The program estimates a chemical’s acute (short-term) toxicity and chronic (long-term or delayed) toxicity to aquatic organisms based on their structural similarity to chemicals for which aquatic studies are available. Equations are available for fish, invertebrates and aquatic plants along with some terrestrial and marine organisms, where data were available. This poster highlights the new features of the ECOSAR v1.1 model which was released in September 2011 and is posted on the EPA website for free download. The new model includes expanded datasets, an enhanced user interface, and now contains ECOSAR class definition documents describing the boundaries of the class and training set chemicals which was previously not available. Through publication of the ECOSAR model, the US EPA provides public access to the same methods the EPA uses for evaluating aquatic toxicity of new industrial chemicals. These tools can be used by the public to prescreen new substances for environmental impacts and to inform R&D decision making. It can also be applied to prioritize existing chemicals for further testing to focus testing or identify greener alternatives. Disclaimer: This abstract represents the views of the authors and not necessarily the official position of the US EPA.
Anisakiasis is a zoonotic disease caused by parasites in fish provoked by larval stages of nematodes of the genus Anisakis, those can be present in people living in countries where fish is consumed raw or undercooked. Mugil inciliis was collected in the Bay of Cartagena, Colombia to investigate the possible influence of organochlorine pesticides in the histological changes of the liver, the spleen and existence of parasites in fish. The presence of organochlorine compounds were determined using the technique of headspace-solid phase microextraction (HS-SPME), showing amounts of organochlorine compounds in the muscle of M. inciliis as are the β-BHC, γ-BHC, heptachlor, aldrin, endosulfan, 4-, 4′-DDE and dieldrin, among others, indicating that the fauna of the Bay of Cartagena is exposed to these pollutants. Samples of liver and spleen were fixed, processed and embedded in paraffin. The most important changes in the liver and spleen were the presence of melanomacrophages and granulomas. Some morphological damage in the liver of M. inciliis was associated with the bioaccumulation of organochlorine compounds. Parasite abundance was discriminated as Anisakis sp (1.6%), Pseudoterranova sp (25.3%), and Contracaecum sp., (57.8%). Were found other parasites such as nematodes (15.3%) of genus Ascarocephly longa. There was no significant correlation between parasites and organochlorines. This study is the first in to correlate the presence of organochlorine compounds and histological damage in liver, spleen and the presence of parasites in fish from the Bay of Cartagena, Bolivar, Colombia.

Aryl hydrocarbon receptor (AhR) plays a profound role in the induction of cytochrome P450 1A (CYP1A) and dioxin toxicity. Our previous studies detected a significant positive correlation between accumulated levels of dioxins and related compounds (DRCs) with CYP1A expression levels in the liver of common cormorants (Phalacrocorax carbo) from Lake Biwa, Japan. This suggests that cormorant is activated by the accumulation of DRCs. Since legal and ethical concerns preclude the direct testing of DRCs in wild animals, the risk of DRCs has not been well assessed in wild populations. We have cloned two AhRs from the common cormorant (ccAhR1 and ccAhR2). To evaluate the species-specific responses to DRCs in cormorant, this study investigated the transactivation potencies of ccAhR1 and ccAhR2 by graded concentrations of individual DRCs including polychlorinated dibenzo-p-dioxins (PCDDs), furans (PCDFs) and coplanar PCBs (Co-PCBs) in C57-7 cels, where ccAhR1 or AhR expression plasmid and a luciferase reporter plasmid containing cormorant CYP1A3 promoter/enhancer were transiently transfected. For the congeners that exhibited ccAhR-mediated dose-dependent activities, 50% effective concentrations (EC50s) and 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) relative potencies were estimated. Based on the REPs, TCDD induction equivalency factors were determined. Results suggested that ccAhR1 has more transactivation efficacies for most of the tested DRC congeners than ccAhR2. Cormorant IEFs obtained from this study showed that TCDD may not be the most potent congener for ccAhR1 and ccAhR2. According to the TCDD-EC50 obtained, ccAhR1 seems to be less sensitive to TCDD than AhR of other avian species. This study suggests that our in vitro approach may be a useful alternative to wildlife testing in toxicological researches.

Environmental estrogens (EEs) are chemicals in the environment that can elicit adverse effects on estrogen (E2) signaling through binding with the estrogen receptors (ERs). In largemouth bass (LMB), the physiological actions of E2 are primarily mediated via three receptors (ERα, ERβ, and ERβ). First we used binding and transfection assays to test the ability of the LMB ERs to bind and respond to 4,4′-[(4-hydroxyphenyl)-propionitrile (DPN), a mammalian ERβ-specific agonist, 2,3-bis(4-hydroxyphenyl)-propionitrile (DPR), a mammalian ERβ-specific agonist, bisphenol A (BPA), pp′-DDE, and dieldrin (model EEs). Our data shows that PPT and DPN were not isoform-specific agonists for the LMB ERs. E2 binds and activates the LMB ERs similarly, whereas differences were observed in the binding and activation of the receptors by the xenoestrogens. In the binding assay, dieldrin was not able to fully displace E2 from ERβ, and in the transfection
assays it acted as a weak partial agonist of the LMB ERβ, and a full agonist of ERα. Second, we determined the effects of the ERα on ERβ-regulated LMB gene expression using an in vitro liver slice assay. Liver slices from male LMB were exposed for 48 h to E2 (0-1 μM), pp'-DDE (0-100 μM), BPA (0-100 μM) and dieldrin (0-100 μM). Induction of ERα, vitellogenin (VTG) and zona radiata proteins (ZRP) mRNA were used as biomarkers of exposure to E2. A significant induction of VTG, ERα and ZRP was observed with exposure to E2, pp'-DDE and BPA. Dieldrin did not up-regulate ERα, VTG or ZRP mRNA. Moreover, two month dietary exposure of male LMB to 2.8 mg/kg body weight dieldrin appeared to inhibit the E2-mediated induction of ERα, VTG and ZRP mRNA by E2, pp'-DDE and BPA in liver slices.

Taken together, these in vitro assays can be useful tools to screen the relative potency and efficacy of E2s for fish receptors, as well as effects on ER regulated genes. This research may not reflect EPA Policy.

1089 DIPHALLIA IN PLOCOPURPURA PANSÁ (MOLLUSCA: NEOGASTROPOD) FEMALES EXPOSED TO TRIBUTYRL CHLORIDE.

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Imposex is characterized by the development of male features, such as the penis and vas deferens, in female prosobranch gastropods and could be caused by organotin compounds. In this study, we experimentally tested the potential to induce imposex by tributylchloride (TBTCl) on Plcopurpura panza. Twenty females with similar size, without imposex, were collected from Olas Altas, Sinaloa, Mexico and were divided in two groups: one group was treated with TBTCl (10 μg Sn/L), and the other was used as a control. Each group was kept in 8-L glass beakers with artificial sea water for eight months and was fed every three days with squid bits. Organisms were searched monthly for imposex using stereomicroscopy. Among the exposed females, three presented imposex after two months: two developed a 1-mm long pseudopenis and the other showed three small warts (0.1 mm). Three months later, the pseudopenis of one imposexed female showed a spiral form, similarly to male pseudopenis and the other showed three small warts (0.1 mm). Three months later, males, three presented imposex after two months: two developed a 1-mm long pseudopenis and the other showed three small warts (0.1 mm). Three months later, males, three presented imposex after two months: two developed a 1-mm long pseudopenis and the other showed three small warts (0.1 mm).

1090 EFFECTS OF PARENTAL AND DIRECT METHYLMERCURY EXPOSURE ON FLIGHT ACTIVITY IN YOUNG HOMING PIGEONS (COLUMBA LIVIA).

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Mercury is one of the most common metals found in contaminated ecosystems. It occurs naturally, but high levels found in contaminated areas derive from human use practices. Among the most vulnerable species to exposure are birds that live, nest, or feed in or near these contaminated ecosystems. Conventional avian toxicology models have shown impairment of cognitive skills from exposure to levels higher than those generally considered environmentally relevant, but little work has been conducted on low-level exposure effects. Because of the known neurological effects of mercury on birds, it is hypothesized that effects upon migratory ability would be evident after exposure to low levels of this metal, and effects may be exacerbated in young birds. Difficulties in following mercury exposed birds once they migrate away from contaminated areas have left investigators with insufficient data to establish exposure levels causing injury of migratory species due to migration disruption. With the understanding that compromised flying efficiency and migratory orientation may lead to negative effects on migratory bird species, homing pigeons (Columba livia) were chosen as the test species. Breeding pigeons were exposed to -1.0 mg/kg/day methylmercury via the drinking water, and first round offspring were trained to home after fledging, while also continually exposed to methylmercury. The young pigeons were released individually for three flights, and flight times were assessed and compared to control young pigeon flight times from 3.5, 9, 21, 53, 65, and 98 air miles as well as two individual flights at 50 air miles from multiple directions. Results indicate that methylmercury exposed birds exhibit slower flight times than controls during the initial flight, and generally improve on successive flights at each distance and direction. This may suggest orientation impairment and allude to migration disruption in migratory species. These studies were funded in part by USGS and the Nevada Agricultural Experiment Station.

1091 EFFECT OF DEEP HORIZON LIGHT CRUDE OIL EMULSION WITH AND WITHOUT THE DISPERANT, COREXIT, ON EARLY FISH DEVELOPMENT.

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Louisiana light crude oil released into the Gulf of Mexico by the Deep Horizon (DH) incident underwent significant alterations by remediation attempts and weathering processes before reaching coastal marshes. These studies examined the effect of DH oil emulsions and Corexit dispersant concentrations alone and in combination upon development of fish embryos. Corexit alone at 0.0, 0.3, 3.0 and 100.0 mg/L did not alter the incidence of abnormalities or death in zebrasfish (ZF) embryos exposed through 8 days of development (near completion of organogenesis). Direct contact exposure of ZF embryos to DH emulsions “buttered” on a contact surface of 16cm2 (250mg) resulted in a high incidence of edema/axial deformities and subsequent mortality (40-90%) over a range of Corexit concentrations of 0.0, 3.0 and 100mg/L. Only the 100 mg/L Corexit treatment with emulsion resulted in a synergistic effect. Non-contact water exposures to emulsion (250 mg) resulted in axial changes alone and mortalities < 10% throughout the 0.0 to 100 mg/L. Corexit concentration range. Emulsion exposures of ZF during different developmental intervals indicate that early exposure (0-48hrs) is more inclined to affect genes involved in neurological system processes, visual perception, and response to abiotic stimuli while late exposure (48-96hrs) triggered genes associated with responses to a stimulus, especially a chemical stimulus. Exposure and development data suggest that an emulsified light crude effectively presents hazardous compounds to fish embryos under direct exposure conditions present in coastal marshes. Corexit had little effect on the developmental toxicity of oil emulsions except at the highest concentrations.

1092 RELATIONSHIPS BETWEEN CYTOCHROME P450 ACTIVITY AND EXPRESSION AND ALLELOCHEMICAL TOXICITY IN BUTTERFLYFISH (CHAETODON SPP.) OF DIFFERING FEEDING STRATEGIES.

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Cytochrome P450 monoxygenase (CYP) is the primary enzyme system for detoxification of xenobiotics including dietary chemicals and pollutants. Some herbivorous rious insects have the ability to detoxify toxic dietary chemicals by specific CYP forms. While thorough research has uncovered certain relationships between herbivorous insects and dietary chemicals, little is known about biotransformation and detoxification of allelochemicals derived from dietary products in marine organisms. Certain species of butterflyfish of the genus Chaetodon have been shown to feed on several species of chemically-defended corals including the soft coral Sinularia. This study examined the effects of the natural product, 5-episulupelidol (SE5) on the expression and catalytic activities of CYP3A, CYP2 in two butterflyfish species one of which is an obligate coral feeder and a generalist feeder that can feed on coral. Fish were gavaged 1.0 mg/kg and 3.0 mg/kg of SE5. Initial mortality indicated that C. multicinctus (obligate hard coral feeder), had 100% mortality in both doses. In contrast, 80% survival was observed in C. auriga (generalist feeder). Testosterone hydroxylase (TOH) (6 beta, 16 alpha, 16 beta) was 130-740 times lower in C. multicinctus relative to C. auriga. These results indicate an association between CYP2 (16-alpha TOH) and CYP3A (6 beta, 16 beta TOH) catalytic activities and detoxification of SE5 in butterflyfish with different feeding strategies which may provide a selective advantage in allowing generalists to feed on chemically defensed prey.

1093 HISTOPATHOLOGY AND GENOTOXICITY STUDIES IN Iguana iguana FROM A COAL MINING AREA IN COLOMBIA.


Colombia is the second largest coal exporter in Latin America. However, coal mining is threatening local ecosystems. Iguana iguana, known as iguana, is a common reptile in tropical areas and little is known about the effects of this activity on this species. The aim of this work was to describe histopathological lesions and to eval-
ulate the potential genotoxic damage in iguanas collected in La Loma, an important coal mine in the Department of Cesar, Colombia, comparing these results with those obtained for specimens captured in a natural reserve. Ten specimens from the coal mining area and eleven from the control site were euthanized and blood and tissue samples removed. The liver of iguanas from the control area presented normal characteristics, with some pigment laden macrophages. The presence of this pigment, possibly melanin, was greater in animals from the exposed area. Spleens from reference iguanas did not register tissue alterations, whereas samples from La Loma showed larger dense white pulp areas, several lymph follicles and extensive zones of B lymphocyte production, perhaps in response to foreign antigens or as an effect related to potential immunotoxicity. Regarding genotoxicity, the alkaline comet assay performed on whole blood cells evidenced that animals living near coal mines had greater percentages of DNA damage than those from reference sites. This was also shown by a significant increase in the number of nuclear buds in exposed iguanas. In summary, iguanas residing around coal production zones present greater risk of histopathological lesions in both the liver and spleen, as well as DNA damage in blood cells. Moreover, this species showed to be sensitive and useful to investigate the potential genotoxic damage in individuals. T en specimens from the Department of Cesar, Colombia, comparing these results with those obtained for specimens captured in a natural reserve. T en specimens from thirteen species were captured in marshes along the Dique Channel, a freshwater ecosystem in Colombia. A sample of 890 fish was obtained, of which 708 were analyzed for genotoxicity. In the present study, the effects of chronic exposure of AA on digestive organs including pancreas were evaluated in golden hamsters. A total of 90 female and 90 male hamsters were divided into three groups each, treated with AA at 0.06 mg/kg BW DON or 15ADON. After the absorption immediately. However, the bioavailability and emesis toxicity of these ADONs are still unknown. Acrylamide (AA) has been reported to show carcinogenicity in rats and mice, of which targets are various organs. On the other hand, a review and meta-analysis of epidemiologic studies concluded that there is no increased risk for common type of cancer from exposure to AA, but high occupational exposure showed relatively higher values of pooled relative risks for pancreatic and kidney cancers. Among rodents, certain kinds of chemicals have been demonstrated to induce pancreatic acinar cell tumors but not ductal ones in rats, and Syrian golden hamsters are sensitive to ductal carcinogenesis with N-nitrosobis(2-oxopropyl)amine. In the present study effects of chronic exposure of AA on digestive organs including pancreas were evaluated in golden hamsters. Trichothecene mycotoxin deoxynivalenol (DON) is a toxic secondary metabolite produced by Fusarium fungi in agricultural crops. In addition, acetylated derivative of DON, such as 3 and 15-acetyldeoxynivalenol (3ADON and 15ADON), are evaluated to have equivalent toxicity to DON because these ADONs convert to DON after absorption immediately. However, the bioavailability and emesis toxicity of these ADONs are still unknown. In this study, we compared the bioavailability and emesis toxicity between DON, 3ADON and 15ADON by oral administration test using pigs and the house musk shrew (Suncus murinus (SUR-Heris)). The pigs (10.7 ± 2.4 kg, male) were divided into a control group and mycotoxins (3 ADON, 15ADON or DON) feeding groups. The oral administration tests achieved with the diets containing 0.06 mg/kg BW DON or 15ADON. After the administration, pig serum was collected at 0, 5, 10, 20, 30 min and 1, 2, 3, 4, 8 hr. Then these sera were measured by LC/MS/MS. In ADONs feeding group, ADONs were not detected from all samples, suggesting that ADONs would be converted to DON in serum. However, Maximum drug concentration time...
The trichothecene mycotoxin deoxynivalenol (DON) is well known to cause food refusal in experimental animals. However, the relative anorexic potencies of structurally related 8-keto-trichothecene are not known. A simple food refusal bioassay employing the mouse was used to compare the effects of 8-keto-trichothecene following by oral and intraperitoneal (ip) exposure. The results suggested that, similar to DON, the anorexic effects of 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON) were transient (lasting only a few hours) and food intake recovered within 16 h. In contrast, the food refusal responses to nivalenol (NIV) and fusarenon X (FX) were markedly different, persisting from 36 to 96 h depending on administration route. For both ADONs, the no observed adverse effect levels (NOAEL) and lowest observed adverse effect levels (LOAEL) were 0.5 and 1 mg/kg bw for ip, respectively, and 1 and 2.5 mg/kg bw for oral, respectively. The NOAEL and LOAEL for FX were 0.025 and 0.25 mg/kg bw, respectively, for both ip and oral exposure. The NOAEL and LOAEL for NIV were 0.01 and 0.1 mg/kg bw, respectively, for ip and 0.1 and 1, respectively, for oral exposure. To summarize, the anorexic effects of 8-keto-trichothecene followed the rank order NIV>FX>DON=15-ADON=3-ADON, based on the NOAEL and LOAEL, with FX effects being greater when administered ip as compared to oral exposure. 3-ADON and 15-ADON caused acute anorexia, similar to DON, whereas, the anorexic effects being greater when administered ip as compared to oral exposure. The trichothecene mycotoxin deoxynivalenol (DON) is well known to cause food refusal in experimental animals. However, the relative anorexic potencies of structurally related 8-keto-trichothecene are not known. A simple food refusal bioassay employing the mouse was used to compare the effects of 8-keto-trichothecene following by oral and intraperitoneal (ip) exposure. The results suggested that, similar to DON, the anorexic effects of 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON) were transient (lasting only a few hours) and food intake recovered within 16 h. In contrast, the food refusal responses to nivalenol (NIV) and fusarenon X (FX) were markedly different, persisting from 36 to 96 h depending on administration route. For both ADONs, the no observed adverse effect levels (NOAEL) and lowest observed adverse effect levels (LOAEL) were 0.5 and 1 mg/kg bw for ip, respectively, and 1 and 2.5 mg/kg bw for oral, respectively. The NOAEL and LOAEL for FX were 0.025 and 0.25 mg/kg bw, respectively, for both ip and oral exposure. The NOAEL and LOAEL for NIV were 0.01 and 0.1 mg/kg bw, respectively, for ip and 0.1 and 1, respectively, for oral exposure. To summarize, the anorexic effects of 8-keto-trichothecene followed the rank order NIV>FX>DON=15-ADON=3-ADON, based on the NOAEL and LOAEL, with FX effects being greater when administered ip as compared to oral exposure. 3-ADON and 15-ADON caused acute anorexia, similar to DON, whereas, the anorexic effects of NIV and FX were more persistent.
1102 HEXABROMOCYCLODECANE (HBCD) STEREOSISOMERS IN US FOOD AND ADULT DAILY DIETARY INTAKE.

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Hexabromocyclododecane (HBCD) is a brominated flame retardant mixture used in polystyrene foams and as a replacement for Decabromodiphenyl ether. The commercial mixture has 3 major stereoisomers - α (~10%), β (~10%), and γ (~80%). HBCD exposure is associated with effects in animal and human studies. Food ingestion, especially of meat and fish, is a major route of exposure. This study was designed to measure HBCD stereoisomer levels in US food and estimate US HBCD daily intake. We previously measured total HBCD levels in 36 pooled food samples using high-resolution gas chromatography/low resolution mass spectrometry. Ten of these pools as well as 36 individual food samples were analyzed for HBCD stereoisomers using liquid chromatography tandem mass spectrometry. α-HBCD was detected in 13/36 new foods and all of the original pools; γ-HBCD in all 10 original pools but only 8/36 new foods; and β-HBCD was in 8/10 pools and 3/36 individual foods. Median levels for α-β- and γ-HBCD were 115, 16, and 59 pg/g ww, respectively. US Adult dietary intake of all 3 HBCD stereoisomers measured in these studies was estimated to be approximately 14 ng/day, primarily from meat consumption. Total HBCD levels are similar to those reported from European countries, and also show the stereisomeristic shift from γ- to α-HBCD. Further research is indicated to determine HBCD stereoisomer levels and daily intake from a larger and more representative sample of US food. (This abstract does not reflect NIH policy).

1103 LC-MS/MS DETERMINATION AND OCCURRENCE OF FUSARIUM MYCOTOXINS ENNATINAT, BEAUVERICIN AND FUSAPROLIFERIN IN PASTA.

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Mycotoxins are toxic secondary metabolites produced by filamentous fungi, mainly Aspergillus, Penicillium and Fusarium species under appropriate environmental conditions. In the last years, the called emerging Fusarium mycotoxins were acquired importance, since their severe toxic effects on animal and human health, as enzymatic inhibition, cytotoxicity and teratogenicity. In this work, a new, rapid, sensitive, reproducible and reliable method for the determination of the emerging mycotoxins: Enniatins ENs (EN A, EN A1, EN B and EN B1), beauvericin (BEA) and fusaproliferin (FUS) by liquid chromatography-triple quadrupole-tandem mass spectrometry and their occurrence in pasta samples has been developed. Besides, three extraction techniques have been studied: Ultra-Turrax, ultrasonic and microwave and the time, temperature and the extraction solvent have been optimized. The best results were for Ultra-Turrax extraction with 3 minutes of acetone-trile. The chromatographic determination has been performed in reverse phase with C18 column, and acetone/methanol (20:80 ammnium formate) mobile phase. The recoveries ranged from 86-112%, with relative standard deviation lower than 15%; Limits of detection were lower and ranged from 0.02-0.15 μg/kg. In addition, application to the analysis of 73 organic and non organic pasta samples purchased from Spanish and Italian supermarkets and risk assessment associated to daily intake were studied. The results obtained showed that the 84% of the samples were contaminated and the incidence of BEA, EN A, EN A1, EN B, EN B1 and FUS was 23, 49, 50, 74 and 23% respectively. The higher incidence and concentrations were obtained in organic pasta samples. Acknowledgments: This study was financially supported by the projects AGL2010/17024/ALI (Science and Innovation Spanish Ministry) and PLAT/2009-012 (Valencian Government, Spain).

1104 ASSESSMENT OF THE SURFACE STABILITY AND AFLATOXIN SorPTION CAPACITY OF MONTMORILLONITE CLAY FOLLOWING FERMENTATION AND HEAT TREATMENT.

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Aflatoxin B1 (AFB1), is commonly found as a contaminant of crops such as maize and has caused concern for food safety and public health. This mycotoxin is listed as a Group 1 carcinogenic by the International Agency for Research on Cancer and may act synergistically when consumed with other contaminants, e.g., fumonisin B1 (FB1). Uniform Particle Size NovaSil (UPSN) clay, calcium montmorillonite, has been shown to bind AFB1, in both in vitro and in vivo studies, thus reducing its bioavailability. During a previous intervention trial in Ghana, UPSN was added to common Ghanaian dishes including Koko, maize porridge that is both fermented and boiled, as a strategy to reduce AFB1, and FB1, exposure in people. Our objective was to evaluate the stability of UPSN during the processing and preparation of Koko as demonstrated by its ability to sorb AFB1, prior to ingestion. Portions (50g) of maize meal were spiked with AFB1, in triplicate, at concentrations ranging from 5 to 1000 ppb with and without UPSN additive and were then used to prepare Koko. Extractions of AFB1, followed a modified procedure based on the USDA- FGIS Single Filtration Procedure for Corn using All assays immunofluorometric assays followed by fluorescence detection (Series 4 VICAM Fluorometer). UPSN significantly bound AFB1, with less than 10% recovered from samples containing the clay, while there was greater than 85% recovery in samples without clay demonstrating that UPSN was able to reduce AFB1, levels by more than 75%. These results confirm our recent cross-over study in Ghana and suggest that UPSN clay-AFB1 complex are stable under cooking and food preparation conditions. Importantly, this work confirms that UPSN can be used as a food additive to reduce exposure to AFB1, in populations at risk for aflatoxicosis and hepatocellular carcinoma. This research was supported by USAID LAG-G-00-96-90013-00 and NIH RO1MD005819-01.

1105 EVIDENCE FOR FUMONISIN INHIBITION OF CERAMIDE SYNTHASE IN HUMANS.

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Fumonisins are mycotoxins found in corn worldwide. Fumonisin B1 is the most common of the fumonisins. It is the cause of several farm animal diseases and is carcinogenic in rodents. Fumonisin B1 is poorly absorbed and is excerted primarily in feces and small amounts can be detected in urine. The mode of action is the inhibition of ceramide synthase a key enzyme in the biosynthesis of sphingolipids. Inhibition of ceramide synthase causes an accumulation of sphingoid bases and sphingoid base 1-phosphates in tissues and blood. In mice gavaged with fumonisin B1, sphinganine 1-phosphate (Sa1P) accumulates in red blood cells in a dose-dependent manner and there is an increase in the ratio of Sa1P:Sphingosine-1-phosphate (So1P). The purpose of the present IRB approved study was to determine the relationship between fumonisin B1 in the urine and the Sa1P/So1P ratio in blood spots collected from women living in communities consuming large amounts of corn potentially contaminated with fumonisin B1. In the human study approximately 640 urine and blood spot samples were collected and analyzed from three locations in rural Guatemala (Chimaltenango, Escuintla and Jutiapa) in March and June of 2011. Corn samples (n=57) from the same locations have been analyzed for fumonisins. The level of fumonisin in corn collected from local markets was significantly higher in Chimaltenango and Escuintla than Jutiapa. The urinary fumonisin B1 and the Sa1P/So1P ratio were also significantly higher in Jutiapa compared to Chimaltenango or Escuintla. The preliminary results show that fumonisin B1 in urine is significantly correlated with the increase in the Sa1P/So1P ratio in the blood spots. These results are consistent with the conclusion that fumonisin B1 disrupts sphingolipid metabolism in humans consuming large amounts of fumonisin B1 contaminated corn. Research supported by NIH grant # 1 RC4 HD067971-01.

1106 AGE AND GENDER ARE SUSCEPTIBILITY FACTORS IN DEOXYNIVALENOL-INDUCED ANOREXIA.

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Deoxynivalenol(DON, vomitoxin), a trichothece mycotoxin, is a common cereal grain contaminant that is resistant to processing and can enter human and animal food. Regulatory standards have been established for DON based on its capacity to cause anorexia and suppress growth in the mouse. The aim of this study was to test the hypothesis that age and gender will affect the susceptability of mice to DON-induced feed refusal. Here we employed a mouse anorexia bioassay previously described by our lab to compare the effects of DON exposure to 1), weanling males (3 wk) vs adult males (4wk), 2) adult males (11 wk) for elderly male (22 months) and 3) adult females (11 wk) vs adult males (11 wk). Briefly, mice were acutely exposed to 0, 1 and 5 mg/kg bw via IP injection and food intake was monitored 36 h later.
post exposure to the toxin. Elderly mice were extremely sensitive to DON as evi-denced by a markedly lower food intake and significantly lower body weight gain of adults compared to those of adult and elderly males. Adult female mice were the least sensitive to DON exposure at recovery after doses. These data suggest the following rank order of susceptibility to DON-induced anorexia: elderly males > adult males > weanling males > adult females. This indicates age and gender might be important factors to consider when conducting risk assessments for DON and other trichothecenes.

1109 CONSUMPTION OF FOLATE DEFICIENT DIET DID NOT INCREASE NEURAL TUBE DEFECTS IN LM/Bc MICE EXPOSED TO FUMONISIN B₁
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Fumonisins B₁ (FB₁), a mycotoxin produced by Fusarium verticillioides and F. proliferatum. It is found in corn and evidence suggests it is a possible risk factor for neural tube defects (NTD) in populations consuming large amounts of contami-nated corn-based foods. The mechanism(s) underlying NTD induction by FB₁ in the sensitive LM/Bc mouse is not well characterised. The purpose of this study was to compare the folate status and neural tube defect (NTD) incidence in LM/Bc mice fed moderate and high levels of folate as a part of the general toxicology study performed at Creighton University. The folate deficient diet is known to increase NTD incidence. The study was conducted to determine if the moderate level of dietary folate in the LM/Bc diet could reduce the NTD incidence.

1110 SAFETY ASSESSMENT OF AGARICUS SUBRUFESCENS SP AND THEIR PRODUCTS OF THERAPEUTIC INTEREST OR FOR DISEASE PREVENTION.
F. E. Creppy¹, S. Moukhel¹ and C. Ferandon¹. ¹Toxicology, University Bordeaux 2, Bordeaux, France and ²MycoSa, INRA, Villenave d’Ornon, France.

Agaricus subrufescens is a mushroom native to Brazil and France. It is found in corn and evidence suggests it is a possible risk factor for neural tube defects (NTD) in populations consuming large amounts of contaminated corn-based foods. The mechanism(s) underlying NTD induction by FB₁ in the sensitive LM/Bc mouse is not well characterised. The purpose of this study was to compare the folate status and neural tube defect (NTD) incidence in LM/Bc mice fed moderate and high levels of folate as a part of the general toxicology study performed at Creighton University. The folate deficient diet is known to increase NTD incidence. The study was conducted to determine if the moderate level of dietary folate in the LM/Bc diet could reduce the NTD incidence.

R,Monatin is a naturally occurring substance identified in Sclerochitin ilicifolius, a plant native to South Africa. To contribute data relevant to evaluation of the safety of R,Monatin salt during gestation days 6-21. The fetuses were examined for external, visceral, and skeletal malformations and variations. There were no fetal malformations or developmental variations that were attributable to R,Monatin salt at any exposure level, nor were there any test article-related effects on intrauterine survival. Maternal toxicity, as evidenced by lower maternal body weights, body weight gain, and food efficiency, was observed at 50,000 ppm. A developmental effect, in the form of lower mean fetal body weight, was noted in the 50,000 ppm group in the presence of maternal toxicity. Therefore, based on the results of this study, the no-observed-adverse-effect level (NOAEL) for maternal and embryo-fetal toxicity in Sprague Dawley rats resulting from dietary exposure to R,Monatin salt during gestation days 6-21 was 30,000 ppm (approximately 2,564 mg/kg bw/day) based on reductions in maternal and fetal body weights.

1111 SAFETY ASSESSMENT OF GENETICALLY MODIFIED, HERBICIDE-TOLERANT CANOLA DP-073496-4: 13-WEEK DIETARY STUDY IN RATS.
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A 90-day rat feeding study was conducted as part of the pre-market safety assessment of genetically-modified (GM), herbicide-tolerant canola DP-073496-4 (73496). Metabolic inactivation of the herbicidal active ingredient glyphosate is
conferring by genomic integration and expression of a gene-shuffled acetyltransferase coding sequence, from this study the gene was nutritionally comparable to PMI® Certiﬁed Rodent LabDiet® 5002 were formulated by total functional replacement (e.g., protein, ﬁber, and energy equivalency basis) of de-hulled (DH) soybean meal, hulls, and oil with meal and oil from DH canola seed from untreated (73/496) and herbicide-treated (73/496+Gly) plants, the non-transgenic near-isogenic control, and four non-transgenic commercial varieties. To evaluate the potential health effects from long-term consumption of 73/496 canola, the experimental diets were fed to young adult SD rats (12/sex/group) for at least 91 consecutive days. Compared with rats fed diets containing meal and oil from the near-isogenic control canola, no diet-related adverse effects were observed in rats fed diets containing meal and oil from 73/496 or 73/496+Gly canola with respect to standard nutritional performance metrics and OECD 408 parameters (body weight/gain, food consumption/efﬁciency, mortality, clinical signs of toxicity, ophthalmological observations, neurobehavioral assessments, organ weights, and clinical and anatomic pathology). No health hazards were identiﬁed. These results support the nutritional equivalence and comparative safety of meal and oil from DH canola seed from GM herbicide-tolerant 73/496 canola and conventional, non-transgenic canola.

1112 NEONATAL PIGLETS AS AN ANIMAL MODEL TO EVALUATE SAFETY OF NEW INGREDIENTS IN INFANT FORMULA.

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Although breast-feeding is recommended for the ﬁrst year of life, infant formulas constitute the majority of the diet for most American infants. Infant formulas consist of two major types: dairy-based using bovine milk protein and soy-based made from soy protein isolates. Preclinical studies are a vital ﬁrst step to assess the safety and quality of ingredients, which may be new to infant formulas. Neonatal piglets are used as an animal model to evaluate the safety of baby formula ingredients, because the physiology of digestion is similar to that in humans. In addition, piglets thrive in the laboratory environment without maternal support just days after birth, which provides a practical means for infant formula testing. In the present study one-day old domestic Yorkshire-Crossbred piglets were fed with either Purina ProNurse or VetOne multispecies milk replacers for 3 weeks, based on manufacturer’s recommended feeding regimens. Growth rate, clinical observations, and clinical pathology parameters were evaluated. On arrival, animals were acclimated to feeding bowls, which were supplied with liquid feed via a customized infusion system. They were offered formula each day, every hour, for 14 hours. At receipt, animals weighed approximately 2 kg and clinical signs were unreproducible among groups. Hematological parameters during the ﬁrst 3 weeks of life showed signiﬁcant differences when compared to adult pigs. Most notable were dramatic increases in reticulocytes, reticulocyte percentage, and mean cell volume (MCV) with moderate decreases in lymphocyte counts. Increased average red cell size (MCV) and reticulocyte values correspond to accelerated erythropoietic production observed in suckling piglets which generally subsides by 2 to 6 months of age. In conclusion, this piglet design, optimized for growth and tested with different milk replacement formulas, demonstrated important differences in the infant data as compared to adults and conﬁrmed the practical use of this animal model for infant formula testing.

1113 RESPONSES OF TILAPIA TO CONTAMINATED DIETS SUPPLEMENTED WITH NOVASIL.

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The aquaculture industry is one of the fastest-growing food sectors in the world. However, like all farm-raised animals, ﬁsh are susceptible to food-borne toxins, including afatoxin B1 (AFB1). AFB1 is a mycotoxin mainly produced by the fungus Aspergillus parasiticus, which is well known for its adsorption to other crops. The high percentage of plant-based material in the feed of warm-water species warrants further investigation of species sensitivity to AFB1. Rainbow trout (Oncorhyncus mykiss) have been shown to be one of the most sensitive species to AFB1, developing tumors when exposed to levels as low as 0.4 ppm. Nile tilapia, Oreochromis niloticus, is a popular warm-water species known for their high resistance to disease. However, tilapia have susceptibility to AFB1 and demonstrate a decrease in body weight when exposed to AFB1. Enterosorption therapy using natural clay, such as NovaSil (NS) is among the novel strategies to reduce exposure to afatoxins in both farm-raised animals and humans. The objective of this study was to evaluate the capacity of NS clay to remediate the toxic effects of a chronic exposure to AFB1 in tilapia. Several parameters were tested at the end of 10 weeks to determine the effects of AFB1 and NS. Weight gain and mortalities were determined on a weekly basis. 1% NS caused a 10.87% and 8.58% increase in total body weight gain when compared to the 1.5 ppm and 3.0 ppm AFB1-exposed groups. Lipoygenase concentration in the plasma, and oxidative radical production in neutrophils and macrophages were determined. NS-supplemented diets showed an increase in oxidative radical production from neutrophils in blood compared to the AFB1-exposed groups (Supported by CONACYT ID No. 2010-020 and NIH 1RO1MD005819-01).

1114 SAFETY OF CALCIUM AND SODIUM BENTONITE CLAYS AS ENTEROSORBENTS FOR AFTOXIN.

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Bentonites can potentially protect against afatoxiscosis. NovaSil (NS) clay has been shown to sorb afatoxins in the gastrointestinal tract and to diminish both their bioavailability and their adverse effects in multiple and different model systems. Similarly, Na-bentonite has been shown to reduce exposure to afatoxins in farm animals however concerns about its potential interactions with nutrients warrant further research. We investigated the afatoxin sorption capabilities and the physiologival effects of a Ca-bentonite (Uniform Particle Size NS, UPSN) and a Na-bentonite (NaB) on young Sprague-Dawley rats were fed ﬁctions free of clay (control) or containing either UPSN or NaB at 0.25% and 2% (w/w) for 13 weeks. Growth performance, serum and blood biochemical parameters were measured along with serum vitamins (A and E), iron and zinc. Both clays showed similar AFB1 sorption characteristics based on isothermal analysis. Feed conversion efﬁciency and ﬁnal body weights were unaffected in either sex at the doses tested. Neither UPSN nor NaB-dependent differences in relative organ weights or gross appearance were observed. In both sexes of NaB groups, serum Na and K ratios were increased. Serum K was decreased in males of UPSN and NaB groups. Serum Zn was reduced in the 2% UPSN group, while this reduction was observed at both inclusion rates of NaB. Serum vitamin A was reduced in both NaB groups when compared to control. Serum vitamin E was increased in males and females of UPSN and NaB groups. None of these differences were dose-dependent and parameters fell within the normal clinical ranges. These results suggest that dietary inclusion of UPSN and NaB does not result in overt toxicity. Research supported by USAID LAG-G-00-96-90013-00 and NIH 1RO1MD005819-01.

1115 ETHANOL PRODUCTION FROM FUMONISIN-CONTAMINATED MAIZE GRAIN.

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The use of mycotoxin-contaminated grain in ethanol production can have multiple implications, including initiating co-product quality, dried distiller’s grains and solubles (DDGS), a co-product of maize ethanol processing, are an important livestock feed component. Due to the conversion of mass to ethanol and carbon dioxide during fermentation, the quantity of DDGS obtained from maize ethanol processing is approximately one-third of the mass of the original grain. Therefore, the concentration of fumonisins (a class of mycotoxins produced primarily by Fusarium verticillioides) is typically higher in DDGS than in the original grain. This is a result of fumonisin heat stability and lack of volatility under fermentation conditions. Fumonisins have detrimental health effects in a variety of livestock and, for this reason, their concentration in DDGS is concerning. The accepted “rule of thumb” for mycotoxin concentration in DDGS is a 3x increase from the original grain. A combination of ELISA and HPLC analysis of NDA-derivatized fumonisins was performed over three years of field trials revealing strong correlations between ground grain fumonisin levels and levels found in resultant DDGS (R ≥ 0.80 for all trials). The same analyses showed fumonisin concentration in DDGS to be 2X, rather than the aforementioned 3X levels in the original grain. This suggests that fumonisins may undergo structural alteration or degradation during fermentation. Analyses of naturally infected grain with zero, low (<4 ppm), and high (>4 ppm) levels of fumonisin contamination showed a trend of decreased ethanol production with increasing levels of fumonisins in the fermentation broth (R ≥ 0.80). Fermentation of fumonisin-contaminated maize can result in negative impacts on process efﬁciency and on the quality and subsequent marketability of DDGS. Direct effects of fumonisins on yeast activity need to be carefully evaluated.
Boric acid (BA) is a dietary component found naturally in the environment. Low levels reduce the incidence and mortality of prostate cancer. Our studies have shown that low doses of BA significantly inhibit proliferation of DU145 prostate cancer cells. At similar doses we see inhibition of calcium release from the ryanodine receptor (RyR) in the endoplasmic reticulum (ER) in response to RyR agonists.Stored calcium in DU145 cells was reduced by 22% when treated with 10 μM BA for 1 hour. Altered cellular calcium homeostasis can lead to acute ER stress and, as a result, unfolded protein response (UPR) can be triggered due to unfolded or misfolded protein accumulation. In this project our objective is to identify if physiological levels of BA influence ER stress and UPR in DU145 cells. This research may be a key to understanding the mechanism by which BA slows the proliferation of these cells. Preliminary analysis of DU145 cells treated with BA indicates the presence of ER stress and activation of the UPR. Multiple genes, including calreticulin, HERP, and EDEM1, which are controlled by the ER stress response element I (ERSEI) and II (ERSEII) and the UPR response element (UPRE), respectively, are significantly upregulated with 10 μM BA treatment. The UPR is detected through analysis of the activation of the three UPR branches: PERK, ATF6, and IRE1.

Further research will include using western blot analysis, real time pcr, and immunofluorescence to study the activation and regulation of the plethora of proteins and genes involved in these UPR branches.

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1116 BORIC ACID INDUCES ER STRESS IN DU145 PROSTATE Cancer CELLS.
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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 studies have shown that low doses of BA significantly inhibit proliferation of DU145 prostate cancer cells. At similar doses we see inhibition of calcium release from the ryanodine receptor (RyR) in the endoplasmic reticulum (ER) in response to RyR agonists. Stored calcium in DU145 cells was reduced by 22% when treated with 10 μM BA for 1 hour. Altered cellular calcium homeostasis can lead to acute ER stress and, as a result, unfolded protein response (UPR) can be triggered due to unfolded or misfolded protein accumulation. In this project our objective is to identify if physiological levels of BA influence ER stress and UPR in DU145 cells. This research may be a key to understanding the mechanism by which BA slows the proliferation of these cells. Preliminary analysis of DU145 cells treated with BA indicates the presence of ER stress and activation of the UPR. Multiple genes, including calreticulin, HERP, and EDEM1, which are controlled by the ER stress response element I (ERSEI) and II (ERSEII) and the UPR response element (UPRE), respectively, are significantly upregulated with 10 μM BA treatment. The UPR is detected through analysis of the activation of the three UPR branches: PERK, ATF6, and IRE1.

Further research will include using western blot analysis, real time pcr, and immunofluorescence to study the activation and regulation of the plethora of proteins and genes involved in these UPR branches.

1117 PROCAGULANT AND PROTHROMBOTIC EFFECTS OF HERBAL MEDICINE, DISPACUS ASPER ON HUMAN PLATELETS.

Despite the growing popularity of herbal medicines and food supplements, their adverse effects on cardiovascular homeostasis remain largely unknown, especially regarding their pro-thrombotic risks. Here, through screening of the extracts from 21 herbal teas widely consumed, we discovered that Dispacus asper (DA), previously known to have analgesic and anti-inflammatory efficacy may induce procoagulant activity in platelets, a critical promoter of thrombosis. Dispacus saponin C (DSC) was identified as the key active ingredient for DA-induced procoagulant activities through activity-guided purification. Washed platelets were separated from healthy male donors’ blood. Procoagulant activity was measured. Exposure of phosphatidylserine (PS), microparticle (MP) generation, phospholipid translocation and intracellular calcium were measured by flow cytometry. ATP levels were applied to luciferin/luciferase assay. Mitochondrial membrane potential (ΔΨm), Bax translocation, cytochrome c release and caspase-3 activity were measured. In vivo venous thrombosis model, SD rats were treated with DSC (10 and 25 mg/kg, i.v.). DSC- and DA-induced procoagulant activities were achieved by the exposure of PS and MP generation that were caused from the alteration in activities of scramblase and flippase. These events were initiated by increased intracellular calcium and ATP depletion. Notably, DSC induced a series of apoptotic events including the disruption of ΔΨm, mitochondrial translocation of Bax, cytochrome c release, and caspase-3 activation. Key roles of apoptosis and caspase activation were demonstrated by the reversal of DSC-induced PS exposure and procoagulant activities with the pretreatment of caspase inhibitors. These results were also confirmed in vivo where the rats exposed to DSC exhibited ΔΨm dissipation and PS exposure in platelets. Most importantly, DSC or DA treatment led to increased thrombus formation in rat venous thrombosis model, demonstrating that herbal medicines or natural products like DA or DSC might have prothrombotic risks through procoagulant activation.

1118 SAFETY AND TOXICOLOGICAL EVALUATION OF A NOVEL, WATER-SOLUBLE UNDENERATURED TYPE II COLLAGEN (NATUC-II™).
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1RoEL, NutriToday LLC, Mississauga, ON, Canada, 2Stouss Pharmaceutical University, Tokyo, Japan, 3Ruiyende & Co, Tokyo, Japan and 4EuroNutri/Product Safety Labs, New Jersey, NJ.

The present study was conducted to examine the safety of a novel, water-soluble undenatured type II collagen (NATUC-II), derived from chicken sternum cartilage. The presence of epopeptide in NATUC-II was confirmed by using a commercial kit, Native Type Collagen Capture kit (Axarta Biologies, LLC (WA 98073-2308, USA)). The present study evaluated the broad-spectrum safety of NATUC-II using a variety of toxicological assays including acute oral, acute dermal, primary skin irritation, and primary eye irritation toxicity. Under the conditions of the study, the acute oral LD50 of NATUC-II was found to be greater than 5,000 mg/kg body weight in rats, while the single dose acute dermal LD50 of NATUC-II was greater than 2,000 mg/kg body weight. The primary dermal irritation index (PDII) of NATUC-II was found to 1.8 and classified as slightly irritating to the skin. In primary eye irritation studies, the maximum mean total score (MMTS) of AC was observed to be 7.3 and classified as minimally irritating to the eye. Furthermore, two experiments were conducted to assess the potential of NATUC-II (5000 mg/mL was selected as the highest concentration) to induce mutations with and without metabolic activation at the mouse lymphoma thymidine kinase locus using the cell line L5178Y. No biologically relevant increase of mutants was found after treatment with NATUC-II. Also, no dose response toxicity was observed. Furthermore, colony sizing showed no clastogenic effects induced by NATUC-II under the experimental conditions. These studies demonstrated the broad spectrum safety of NATUC-II. (This study was sponsored by Ryuendo Co., Ltd, 2-12-1 Minami-ike-bukuro, Toshima-ku, Tokyo 171-0022, Japan)

1119 REVAMPING THE LAB EXPERIENCE IN A TOXICOLOGY COURSE.
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As a junior professor, it is difficult to create and implement Toxicology course-related laboratory activities that require the students to think critically about an experiment. This is compounded when resources and funding are limited. Using student feedback, a professor can revamp the laboratory experience from “cookbook” to “inquiry.” Lab activities used the first time the course was taught were taken from various sources such as books and articles. At the end of the course students indicated that the lab experiences did not give them a sense of what a toxicologist does and none of them seemed inclined to pursue graduate school. Improving students’ feedback and perception of toxicology required revamping the lab such that the activities were inquiry-driven. After establishing a baseline of knowledge, including serial dilutions and dose-response, the students designed and implemented an experiment that was completed during the semester. This lab experience yielded positive student feedback and nearly 25% of the class indicated they were considering pursuing graduate school in toxicology. Knowing of the successes and pitfalls of revamping a toxicology lab experience will allow the toxicology education community to improve students’ feedback and perception of toxicology, as well as encourage critical thinking.

1120 COMMUNITY RESEARCH RALLY BRINGS TOXICOLOGY EDUCATION TO A RURAL COMMUNITY IMPACTED BY ASBESTOS EXPOSURES.
J. C. Pflue1, K. Serve1, K. Rowe2 and B. Black3. 1Biological Sciences, Idaho State University, Pocatello, ID and 2Center for Asbestos Related Diseases, Libby, MT.

Educational outreach is a critical component of research using human populations that have been impacted by toxic exposure. The community of Libby MT has suffered health issues as well as psychological and economic impacts due to amphibole asbestos exposure from the mining of asbestos-contaminated vermiculite. Several strategies have been used by researchers over the years to increase the education and interaction with residents of Libby and surrounding areas. One of the most successful endeavors has been the Libby Research Rally. This annual event is free and open to the public, and in the fall of 2010, over 300 people attended from a community of under 10,000, ranging from young children to elderly adults. The 2011 rally will occur on October 13. The Rally consists of a) hands-on learning activities for all ages regarding asbestos and its toxic effects, physiology of the lung, and the kinds of testing that researchers are doing, b) booths manned by the researchers themselves, who answer questions about the objectives, methods, and current status of the research, c) open discussion panels of experts from EPA, ATSDR, physicians and researchers to field community questions and concerns. Participation is encouraged through the use of gifts, gift, prizes, and fun food. The Rally is hosted by the Center for Asbestos Related Diseases (CARD) in Libby, under sponsorship from federal grants supporting research in the community. The positive response and feedback from this event has demonstrated its success in improving community interaction with research and providing a forum that allows individualized access to research and toxicology information.
1121 EUROPEAN MODULAR EDUCATION AND TRAINING PROGRAM IN SAFETY SCIENCE FOR MEDICINES.

1Human & Environmental Toxicology, University of Konstanz, Konstanz, Germany.
2R&D, Sanofi, Vitry-Sur-Seine, Paris, France. 3R&D – Alderley Park, Alderley Park, Macclesfield, United Kingdom and 4R&D, GlaxoSmithKline, Ware, United Kingdom.

SafeSciMET presents a new and unique pan-European education and training network, developing and establishing a comprehensive modular Safety Sciences for Medicines Education & Training Program. SafeSciMET is a joint effort between academia and pharmaceutical industry and bridges crucial gaps in the education and training of scientists evaluating the safety of drug candidates and new medicines. SafeSciMET delivers a new, high quality and sustainable program for education and training in Safety Sciences for Medicines, with emphasis on actual industry case studies. The program encompasses training as a one week on-site course followed by one week worth of home assignments in translational and integrated safety assessments as needed in drug development and usage.

SafeSciMET courses are open to all scientists from industry, academia and regulatory agencies. The applicants should have an MSc degree in a Life Science discipline or equivalent. In addition, applicants are expected to have at least one year's work experience in a related discipline. For other participants no formal entrance requirements are set, although a scientific back-ground with at least an honors degree level is recommended.

SafeSciMET ensures that Europe's biomedical education landscape has maximum support in revolutionizing the conventional drug discovery and development paradigms. SafeSciMET provides novel communications in translational safety sciences, an aspect found largely lacking in today's educational programs. It leads to a new generation of safety scientists who are able to perform holistic and critical evaluations of the safety of drug candidates and new medicines. Linking animal and human data more effectively, in vitro and in vivo, will contribute to a better understanding of drug safety and how to bridge and make best use of animal and human data collected in drug development and safety assessment.

1122 USE OF BISPHENOL—A IN LABORATORY-BASED UNDERGRADUATE LEARNING EXPERIENCES.

M. E. Kirkpatrick. Psychology and Neuroscience, Wheaton College, Norton, MA.

Sponsor: T. Dodds-Butera.

Undergraduate education provides unique opportunities to expose students to current issues in toxicology in meaningful ways in both the laboratory and the classroom. In particular, students gain valuable experience developing research questions with a specific focus on hypothesis-testing and experimental design. Two projects were designed to involve students in toxicology research examining the impact of the proposed endocrine-disruptor, bisphenol—A (BPA), on hormone-dependent reproductive behaviors in female rats. Project 1: Independent Research: Three students worked with a faculty mentor to develop and execute a series of experiments focused on the behavioral impacts of BPA on estrogen-dependent reproductive behaviors in female rats. BPA, acting as an environmental estrogen, was hypothesized to affect the normal display of sexually receptive and protechnic behaviors. Different experimental paradigms were utilized to explore the effects of varied BPA doses, acute vs. chronic BPA exposure and possible role of endogenous hormone levels on the impact of BPA exposure. The student experience was extremely positive and resulted in presentation of data in both academic and professional meetings. Project 2: Classroom-based research project: Based on the success of the independent research project, a toxicology-based project will be added to be baviorial endocrinology unit of an upper-level laboratory behavioral neuroscience course. Students will work as a class to develop a simple experimental design testing the impact of acute BPA exposure on estrogen-dependent reproductive behaviors. Students will surgically remove ovaries from adult female rats and test the ability of ovarian hormones to reinstate spontaneous reproductive behaviors both in the presence of BPA and alone. Previous versions of this teaching module have been extremely successful and published in the Journal of Undergraduate Neuroscience Education. The addition of a toxicology-based hypothesis will serve to strengthen the activity and emphasize the application to lab research to “real-world” applications.

1123 COLLECTION OF WEB RESOURCES FOR THE TEACHING OF TOXICOLOGY.

N. Reynolds. Biology, Washington College, Chestertown, MD.

It is becoming increasingly clear that we need to enhance our education of toxicology at the undergraduate level. In order to aid in this process the Undergraduate Education Subcommittee for Web Resources is gathering toxicology teaching materials for instructor use. The committee has finalized criteria for the submission and review of these resources and is in the process of collecting the following resources: syllabi, lectures, lab activities, and assessment tools. These resources are currently being reviewed by the members of the subcommittee and organized in ToxChase to create a searchable database. I will highlight the resources collected by the group and discuss how they can be used by instructors for the development of new courses in toxicology at the undergraduate level. In addition, I will demonstrate how the group is utilizing ToxChase for the purposes of both resource organization and accessibility.

1124 DEVELOPMENT OF A CRITICAL THINKING TOOL FOR UNDERGRADUATES RELATED TO ONLINE DATABASE SEARCHES.

S. M. Ford. College of Pharmacy & Allied Health Professions, St. Johns University, Jamaica, NY.

An essential skill for students in scientific disciplines is information literacy, which includes identifying, locating, evaluating, and using needed resources. Standard 2 of “Information Literacy Competency Standards for Higher Education” (Association of College & Research Libraries, 2000) states “The information literate student accesses needed information effectively and efficiently.” Students enter college with a diverse set of skills for composing term papers and may be ill-prepared for literature searches required in science courses. The current learning instrument was developed to provide students in a 2nd year toxicology course with a self-paced assignment designed to improve information retrieval competencies and to promote critical thinking skills related to library research. Students are first oriented in the classroom to the resources available through the University library. They are then given the assignment - a “Library Scavenger Hunt” - comprised of ten questions which can be answered using the university's online databases. Questions guide students to compare search results using different strategies and databases, and to find answers to specific questions. The final question is a short test passage drawn from current events with instructions to formulate a question and a search strategy to answer it. Students have 7-10 days to finish the assignment, which is evaluated by the instructor and returned with feedback. At its completion, students: a) are familiar with the logic and logistics of the databases, b) have learned how to perform more effective searches, c) know how to obtain the articles retrieved by their searches, and d) have hands-on experience with different databases. By using a problem-based learning strategy, students are better prepared to execute sophisticated searches for their term papers and other assignments. The project is further described at http://stjohnscampusguides.com/Library/ScavengerHunt and was supported by a Department of Education Title III grant (P031A050301) to St. John's University.

1125 TOXICOLOGY AND PUBLIC HEALTH: A CONCEPTUAL FRAMEWORK FOR POLICY UTILIZING A LOGIC MODEL.

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Background: Current threats to funding for various public health agencies may impact the discipline, education, and practice of toxicology. Addressing methods of presentation and evaluation of programs are needed for clarification of the value of targeted funding in toxicology. A logic model provides an illustration of an underlying conceptual framework and the logical connection within and between systems. Methods: A logic model was utilized to analyze the policy of decreasing spending for public health measures that impact selected programs for toxicology. An underlying assumption was that a range of toxicology programs serve core functions and provide essential public health services. Inputs included stakeholders and resources impacted by the policy to decrease public spending. The logic model also considers negative outcomes, when decreased funding levels lead to the elimination of access to various types of toxicology programs. Results: Immediate impact of decreased public spending would deny access to certain essential public health services, particularly significant to vulnerable populations. Short and long-term outcomes would include decreased utilization of appropriate resources when funding is cut for clinical toxicology services, and an accompanying increase in costs and imposition on emergency services and resources, respectively. In addition, there is an impact on the future strategies and directions for community environmental health, an absence of some preventive services, and obstacles presented for educating future toxicologists when a broad range of toxicology programs are considered. Conclusions: Logic models can be effective in establishing conceptual frameworks for shaping policy for public health, specifically addressing toxicology programs. The visual frame of reference clarifies the basis of fiscal policy, and gives perspective to long term impacts on budget, despite the short term reduction in spending.
1126 JACKSONVILLE ASH SITE TOXICOLOGY TRAINING TO IMPROVE KNOWLEDGE OF THE LOCAL COMMUNITY.

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Introduction: For more than 60 years, the City of Jacksonville, Florida burned its solid waste in incinerators depositing lead, arsenic, polyaromatic hydrocarbons (PAHs), dioxins in landfills near residences, playgrounds, parks and schools. Contaminants in the surface water, ground water, sediments, contaminated fish and shell fish in contaminated creeks are of concern to the community.

Methods: The major goal of this project was to plan, develop, and implement a sustained community-based and culturally sensitive environmental health and toxicology educational program through proactive outreach and training of community stakeholders (train-the-trainer approach) impacted by the Jacksonville Ash sites. A pre-and post-test was developed to measure knowledge gained as well as a participant satisfaction survey. In all steps of the development of the Toxicology materials, the Duval County Health Department and community leaders had direct input.

Results: The majority of participants strongly agreed that the Community Toxicology Curriculum is a useful tool for promoting awareness of potential environmental risks in their community. Suggestions for improvement included using terminology more appropriate for the lay community, developing a website and a brochure for the communities impacted by the ash sites. Based on the pre/post test, there was a 24% average learning gain for the "train-the-trainer" session and a 46% average learning gain for the community resident training session.

Conclusion: Racial and socioeconomic disparities of minorities living near hazardous waste sites have historically and continue to raise health concerns regarding the disproportionate impact of probable and potential exposures to toxic substances emitted from these sites. One of the key gaps is the lack of knowledge regarding the toxicology and health impact of environmental contaminants amongst the community. Grant funded by ATSDR 5 R01/TS000108-02

1127 EXPLORING TOXICOLOGY: TEACHING HOW BASIC TOXICOLOGY IS PRESENT IN EVERYDAY LIFE.

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The Molecular & Environmental Toxicology (MET) graduate program has participated in toxicology outreach events to promote a better understanding of toxicology to children and members of the local community. Graduate students provide learning opportunities through several activities which include a demonstration of "toxin" uptake in carnations and a game called Toxic Land. In the carnation activity, colored dye is added to the water of white carnations. As the carnations take up the water, the dye accumulates in the petals. Learning goals for this activity are age dependent and include the concept that plants take up "toxins" present in the water and soil. The concept of differential uptake rates is exemplified by the use of different dye colors. For more advanced groups, the concepts of bio-accumulation and partitioning are also presented. Toxic Land is a game that takes participants on a path through global events related to toxicology. During their journey, participants are given cards containing pertinent events that happen in daily life and are asked to determine if they will have positive or negative effects on people, animals, and the environment. These cards allow the participants to move forward and backward along the Toxic Land path while learning about toxicology at a personal level. The learning goals for Toxic Land include that participants are informed about toxicologically relevant events that have occurred at a global level and discuss how every day events have a toxicological effect on people, animals, and the environment. Both the toxin uptake activity and Toxic Land will be evaluated to determine if the learning goals of each event are achieved at after school programs in the Madison area. Elementary and middle school students at these after school events will fill out surveys, with questions specific to our learning goals, after participating in each event. The MET program will utilize the assessments to improve the activities and continue to inform the public about toxicology.

1128 RESOURCES FOR TOXICOLOGY K–12 EDUCATION OUTREACH: UPDATING THE SOT K–12 WEBSITE.


The Society of Toxicology’s Education K–12 subcommittee is updating our website to increase our support for K–12 education activities, to share resources across the membership and with the public, and to increase our effectiveness as we interact with educators, parents and students. One main goal is to work with the Society membership to create the most useful resource Website to help support excitement for toxicology through K–12 education outreach. Ideally, this Website would be accessible to the public, easily searchable and contain hands-on activities, PowerPoint presentations and recommendations for increasing K–12 education outreach. Many SOT members are active in toxicology outreach, and many more would like to be but don’t know where to start. Working with Society members, we will update the SOT Website to include links to successful activities, such as past Annual Meeting activities and ongoing Regional Chapter outreach activities. This resource Website will serve as a tool for those interested in getting involved in K–12 education outreach, as well as for those interested in learning more about what toxicologists do. The World Wide Web is a powerful tool that can educate and provide education and information. We want to utilize it to make K–12 education outreach available to members and educators across the globe. This presentation is an opportunity to make meaningful impacts throughout the Society to increase understanding of our efforts, to share the importance of our work, and to encourage future scientists who are dedicated to this work. We need your excitement and interest – get involved, contact us! The views expressed in this abstract are those of the author’s and do not necessarily represent the views or policies of the US Environmental Protection Agency or other affiliations.

1129 TEACHING TOXICOLOGY TO TEENS IN TUCSON.

M. Lindsey. Southwest Environmental Health Sciences Center, University of Arizona, Tucson, AZ. Sponsor: S. Lau.

Rationale and Scope: Tucson has a large Superfund site located in the Sunnyside School District (SUSD), where many of the student’s parents and grandparents were affected by illnesses associated with TCE contamination of the drinking water. The contamination occurred from improper waste disposal at the Tucson International Airport between the 1940s to the 1970s. A citizen’s group, the Unified Community Action Board (UCAB), was formed in the 1980s to monitor the contamination clean up. This project developed and implemented curriculum to improve high school education around environmental health and toxicology by focusing on the TCE contamination. Approach: In 2005, the UCAB requested the author, a board member; assist them teach the youth in Tucson about the issue of TCE contamination. They supported a grant application, awarded by the US EPA in 2008 (#NE09031010), to develop curriculum using an Understanding by Design (Wiggins & McTighe, 1998) approach. The UCAB developed the Essential Understandings to be taught. Teachers from the SUSD wrote interdisciplinary lessons, addressing toxicology in integrated science, biology, and chemistry lessons and environmental public health in social studies, government, and language arts lessons. (http://coep.pharmacy.arizona.edu/tce/). Results: Subsequently the author worked with some teachers to implement lessons in the high schools. In 2009 the school board mandated use of the lessons to teach about the TCE contamination. Students have created digital stories from family interviews; have demonstrated clean up methodologies to the UCAB, and have developed posters for public meetings, such as the opening of treatment plants. The author and one of the teachers presented the curriculum at the National Science Teachers Association in 2009, removing Trichloroethylene from the Groundwater. To achieve full implementation of the curriculum, an online training program for all teachers is being developed to facilitate teaching the lessons in 2012 spring semester. References: Wiggins, G., & McTighe, J. (1998). Understanding by Design.

1130 TOXICOLOGY K–12 EDUCATION OUTREACH: AN UNDERSERVED OPPORTUNITY WITH ENORMOUS POTENTIAL BENEFITS.


The Society of Toxicology’s Education K–12 subcommittee is partnering with K–12 education liaisons in each Regional Chapter in an effort to 1) increase our support for K–12 education activities in every region; 2) share resources across regions; and 3) increase our effectiveness as we interact with educators, parents and students. Forming this interaction will facilitate a functional understanding by the public of the essential role of toxicology in a diverse range of activities and challenges that affect our health and our environment. Summarized below are the goals of this partnership: Development of education sub-committees within Regional Chapters with the goal of mentoring students and conducting outreach activities as well as training others interested in participating in outreach activities; Establish K–12 educator advisory bodies to shape outreach efforts and identify outreach opportunities and to increase and assess effectiveness; Identify and partner
The mission of the (U.S.) National Library of Medicine (NLM) is to collect, organize, preserve, and disseminate health-related information. NLM’s efforts to assist first responders and others include three tools for emergency response that are provided free to users. These tools in NLM’s “disaster-response triad” include WISER (Wireless Information System for Emergency Responders), REMM (Radiation Emergency Medical Management), and CHEMM (Chemical Hazards Emergency Medical Management). WISER, REMM, and CHEMM are designed to be easy to use, intuitive, and able to provide accurate, trustworthy information when and where it is needed, e.g., on-site for first responders at an emergency. WISER’s focus is to assist first responders in hazardous material incidents involving chemicals, while REMM and CHEMM enable first responders, first receivers, and other healthcare providers to plan for, respond to, recover from, and mitigate the effects of mass-casualty incidents, and chemical and biological events, respectively. Each tool includes extensive educational and training resources. NLM’s efforts to provide easy access to this information include the development of downloadable and Web-based versions of WISER, REMM, and CHEMM, and the development of WISER and REMM apps for iPhone, BlackBerry, and Android smartphones. This poster focuses on presenting an overview of CHEMM’s educational and training components, which have been enhanced to evolve as CHEMM’s public release in July, 2011. WISER’s and REMM’s educational and training content will also be noted. (CHEMM and REMM are the result of collaborative efforts between the U.S. Department of Health and Human Services, Office of the Assistant Secretary for Preparedness and Response (ASPR) - Office of Preparedness and Emergency Operations (OPEO) and NLM’s Division of Specialized Information Services (NLM SIS)).

1133 INTEGRATING TOXICOLOGY AND ENVIRONMENTAL HEALTH INTO BASIC AND ADVANCED NURSING EDUCATION.

B. Satler. Environmental Health Education Center, University of Maryland School of Nursing, Baltimore, MD. Sponsor: T. Dodd-Butera.

The integration of toxicology and environmental health into nursing has been initiated with a number of activities. A multidisciplinary initiative was created to teach nursing faculty basic environmental health and toxicology principles. The University of Maryland created the first graduate program in environmental health in a nursing school. Nursing researches have been awarded applied research grants in environmental health including healthy homes and research on agricultural exposures, and the National Children’s Study. Nurses are including environmental exposure assessments in patient electronic health records; engaged in environmentally preferable purchasing decisions; and advocating for legislation to decrease environmental exposures from toxic chemicals.

All nurses must take pharmacology. A side-by-side comparison of the basic scientific principles of toxicology and pharmacology was created for nurses. Translational articles are being written by nurses. There is a distinct disconnect between the science that is published in environmental science and toxicology journals and what is published in medical and nursing journals. While some of the science has direct implications for clinical practice, it is rare that the translational articles appear in the journals that clinicians read.

The American Nurses Association (ANA) has created a set of environmental health principles and in 2010 the ANA created a distinct nursing practice standard for Environmental Health thus firmly placing environmental health in the nursing profession’s domain. In 2009, the Alliance of Nurses for Healthy Environments was created and an educational roadmap was developed for the integration of environmental health and toxicology into nursing education and a new webtool was created: www.enviRN.org. Nurses are the predominant employees in the health care sector. One in every one hundred Americans is a Registered Nurse. The benefits of having nurses to address environmental health, with a basic understanding of toxicology, can have a significant benefit.

1134 OUTREACH AND EDUCATION PROGRAMS DESIGNED TO IMPROVE THE DIAGNOSIS, TREATMENT, AND REPORTING OF PESTICIDE-RELATED ILLNESSES IN CALIFORNIA.


Although 1000-2000 cases of pesticide-related illness are reported annually in California, under-reporting remains a concern. To help address this problem, the Office of Environmental Health Hazard Assessment (OEHHA), as mandated by California law, has developed and implemented a program of medical education to inform physicians and other health care professionals of the diagnosis and the treatment of pesticide illnesses and the requirement to report them. OEHHA’s program consists of on-site presentations by staff; an on-line course in English and Spanish (www.medepesticide.org) that provides an overview of the recognition, management and reporting of pesticide illness; and a specialized on-line course on the California Medical Supervision Program, a cholinesterase monitoring surveillance program established in the 1970s to prevent acute illness among agricultural pesticide mixers, loaders, and applicators who handle the more toxic organophosphorus or carbamate pesticides. Medical professionals who have taken the courses include physicians, registered nurses, medical students and residents, physician assistants, and emergency first responders. Continuing medical education credits are offered for on-line courses. Other educational materials, including posters and brochures, specifically target the general public, and provide brief descriptions of symptoms and instructions for obtaining medical attention. Another specialized course, which will soon be available on-line, is for medical professionals who staff the California Poison Control System call centers. The call centers receive and log phone calls related to pesticide exposures and California’s Urban Pest Eradication Programs, and electronically report them to OEHHA, the California Department of Pesticide Regulation, and the local county health and agricultural departments. OEHHA is continuing to explore ways to enhance its educational offerings and outreach to inform the health care community of the availability of these resources.
1135 RISK AND DECISION MAKING: A WORKSHOP FOR ENVIRONMENTAL PROFESSIONALS.


In the early 1990s, a training workshop for environmental professionals was developed in USEPA Region 9. The workshop was successfully taught throughout Region 9, especially in California through collaboration with the California Department of Toxic Substances Control and other California regulatory agencies. We have updated the manual to introduce changes in risk assessment methods, including the evaluation of vapor intrusion into indoor air, benchmark dose, new cancer guidelines, inhalation toxicity criteria (USEPA RAGS Part F), ecological risk assessment, screening risk assessments, conceptual site models and data quality objectives. At the same time, we have maintained the popular workshop format in which students evaluate the information and draw conclusions, rather than being lectured on the "correct" answer. We have also maintained the case study approach to simulate realistic environmental issues. After an introduction to the case study facility, environs, and contaminants, students must plan a sampling strategy. Students are introduced to principles of toxicology and learn how to develop toxicity criteria based on hypothetical animal study results. Exposure pathways are identified by students who learn to develop quantitative estimates of exposure. Once students determine the pathways, they must determine appropriate alternatives. At the end of the evaluation process, role playing is used to develop risk communication skills. This workshop has proven to be effective for training new employees and providing continuing education for more experienced employees. It has been successful in training staff in consulting and in military organizations, as well as in a variety of regulatory settings. Participants in the workshop benefit from exchanging knowledge and viewpoints of a range of skills and backgrounds (supervisors, project managers, toxicologists, geologists, engineers, public participation experts, etc.). The workshop format fosters these exchanges and provides a dynamic learning environment for students.

1136 CONTRIBUTION OF PHYSOCHEMICAL PROPERTIES TO AUTOPHAGY PERTURBATION.

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Autophagy refers to the catabolic processes in eukaryotic cells that deliver cytoplasmic materials to lysosomes for degradation. This highly conserved process is involved in the clearance of long-lived proteins and damaged organelles. Consequently, autophagy is important in maintaining cellular homeostasis, providing nutrients to maintain cellular function under starvation, and promoting cell survival in various stress conditions. Multiple mechanisms have been shown to regulate autophagy, such as mTOR pathway modulation. However, the link between lysosomal functional impairment and the ensuing impact on the autophagy process has not been fully explored. Basic lipophilic compounds can accumulate in lysosome via pH partitioning and then perturb lysosomal function. We hypothesized that those compounds can disturb the autophagy process. Twelve drugs that have previously been shown to accumulate in lysosomes were selected. Cytotoxicity was evaluated using an ATP depletion assay and autophagy was assessed with LC3 immunofluorescent staining. All twelve drugs induced cytosolic vacuoles and increased staining of LC3. The connection between the increase in autophagosome staining and lysosomal dysfunction was studied using transcriptomic analysis. Gene expression profiles from all tested drugs revealed a downward trend of expression of plentiful long-lived proteins, including structural cytoskeleton and associated proteins, and extracellular matrix proteins, indicating a retardation of protein turnover. Interestingly, various antioxidant response element containing genes, including glutathione S-transferases, NADPH dehydrogenase, and quinone 1, were up-regulated, suggesting activation of the Nrf2 transcription factor. Our data indicate that lysosomal accumulation due to the basic lipophilic nature could contribute to the perturbation of autophagy process.

1137 ASSESSING SELECTIVITY OF COVALENT IRREVERSIBLE B-LACTAMS AND B-LACTAMASE INHIBITORS USING ACTIVITY-BASED PROTEIN PROFILING.

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A significant number of drugs and candidate drugs acylate catalytic amino acids (ser, thr, cys) in enzyme targets resulting in irreversible inactivation. Optimizing this mechanism of action requires balancing chemical reactivity (to minimize off-target binding) with target affinity. Assessing off-target binding using traditional one-target-one-assay approaches can be a challenge due to the large size and structural diversity of some enzyme families, e.g. serine hydrolases (SerH) contain > 200 members. Activity-Based Protein Profiling (ABPP) uses an enzyme class-specific and active site-directed probe to detect enzymes in the catalytically active state. ABPP can be used to assess inhibitor selectivity across an enzyme family using pure enzymes as well as complex biological samples (Nature Biotechnology, 2003, 21:687). Both b-lactams and B-lactamase inhibitors (BLIs) bind irreversibly to a catalytic serine in their bacterial enzyme targets, so off-target binding to the mammalian SerH proteome can be studied using ABPP. A biochemical fingerprint of active SerH in rat tissue extracts (brain, lung, liver, heart, kidney, testes) was generated using the probe compound fluorophosphorodihydroxyamine and utilizing SDS-PAGE with in-gel fluorescence scanning. Pretreating recombinant enzymes (thrombin, trypsin) and tissue extracts with tool compounds (thrombin and trypsin inhibitors, PMSF) blocked labeling by the probe, demonstrating specificity. Marketed BLIs (sulbactam, tazobactam, clavulanate) and a b-lactam (faropenem) did not affect the SerH biochemical fingerprint in rat tissues, consistent with their clean safety profile. In vivo BLI in clinical development (MK-7655) also showed a clean ABPP profile. This method for assessing off-target activity has application as an early in vitro screen to aid in series prioritization and lead optimization. In addition, ABPP can be performed in vivo to enable translational assessment of off-target binding.

1138 AUTOMATED IMAGE-BASED ACTION POTENTIAL AND CALCIUM TRANSIENT ANALYSIS FOR CARDIAC SAFETY ASSESSMENT.

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Currently available analysis for cardiac safety assessment rely on scarcely biological relevant high throughput assays or utilize complex and time consuming electrophysiological techniques that analyze only few cells or conditions at a time. The first approach downsize the importance of multiple ion channel activity on the Action Potential and the second is of scarce utility in the early phase of the drug development process. To enable High Throughput measurements of parameters relevant to cardiac activity, we have developed a Kinetic Image Cytometer (KIC) for simultaneous dynamic imaging and automated cell-by-cell analysis of intracellular Action Potential and Calcium Transient. The instrument automatically electrically field-stimulates and records fluorescence emitted from intracellular voltage and calcium probes from hundreds of cardiomyocytes per well in multi-well format. The associated software segments the acquired images and measures fluorescence dynamic on individual cells and performs statistical analyses on the entire cell population or gated subsets. Post-fixation staining and single-cell tracking enable direct correlation between dynamic parameters and cell subtype. Here we report the use of KIC for measuring the effect of Na+, K- and Ca2+ channel blockers (e.g. flecainide, E-4031, dofetilide and verapamil) and activators (NS 1463 and Bay K 8644) in hiPSC (human induced pluripotent stem cell)-derived cardiomyocytes. RH421 (Action Potential) and Fluo-4 (Calciump Transient) fluorescent probes were loaded simultaneously and acquired from the same field of view for each of 96-well plates. Post-recording staining with atrial, ventricular and nodal markers in mixed populations identifies kinetic characteristics and drug effects that are related to specific cardiac cell subtypes. Our results suggest that this new high content approach can have a profound impact in the field of early in vitro cardiac safety assessment.

1139 IDENTIFICATION OF DRUGS CAUSING CLINICALLY SERIOUS DRUG-INDUCED LIVER INJURY (DILI) USING IN VITRO APPROACH WITH PRIMARY HUMAN HEPATOCYTES.

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Clinically serious DILI is a major cause of early termination of drug development and of FDA regulatory action against marketed drugs. The FDA's guidance expresses specific concerns about clinically serious DILI. In this study, we evaluated the utility of primary human hepatocytes in assessing it. The unique aspects of these study are: 1) a large number of drugs surveyed to predict serious DILI, 2) multiple doses used so that a cumulative measure of drug effect can be quantified by area under the curve (AUC), and 3) individual mechanistic endpoints evaluated to associate mechanism(s) with serious DILI. Based on the FDA Drug Labeling on DILI criteria (w/ or w/o liver failure (ALF) reports, 82 drugs were divided as serious DILI or not. Serious DILI drugs are those withdrawn from market or labeled with a Black Box Warning due primarily to hepatotoxicity as well as those implied as
causing DILI in the Warnings and Precautions or Adverse Reaction with associated ALF confirmed in at least two countries. Human hepatocytes were treated at seven concentrations and luminescence and fluorescence assays performed with four endpoints (ATP content, GSH depletion, Caspase 3 activity and ROS) at 24 hours. The data was normalized and AUC calculated. Assay performance was evaluated with Receive Operating Characteristic (ROC) and the results quantitatively reported as sensitivity with 95% confidence intervals. We found that only ATP content and Caspase activity had moderate prediction potential for serious DILI. However, ROS normalized with ATP content predicted serious DILI very well with 89 and 88% of sensitivity and specificity respectively, indicating that oxidative stress is one of the critical factors to cause serious DILI and specifically combined toxic endpoints (ROS/ATP) performed better than individual ones. The current assessment of this in vitro system indicates that it could provide a useful application in preclinical drug development.

**1140 A NOVEL HIGH-CONTENT SCREENING ASSAY TO PREDICT HUMAN DRUG-INDUCED LIVER INJURY.**


Clinical and nonclinical toxicity are major causes for failure of drug development programs in the pharmaceutical industry, as well as of drug withdrawals. Although adverse drug reactions manifest themselves in different ways and organs, drug-induced liver injury is relatively frequent. DILI has traditionally been difficult to predict because of the multitude and variety of risk factors involved, and because of the lack of sensitivity of animal models. Inspired by recent publications on high content screening cytotoxicity assays, we have developed a multi-parametric quadprobe assay with high predictivity towards human DILI. The assay is performed on primary human HepG2 cells and focuses on early molecular markers such as nuclear area, plasma membrane integrity, lysosomal activity, mitochondrial membrane potential, and mitochondrial area, in order to provide an integrated view of cytotoxicity. The assay is compatible with formaldehyde fixation to allow high throughput. We used a validation set of >60 marketed drugs classified according to their potential to cause hepatotoxicity: safe, moderately toxic, or severely toxic. When analysing the data with respect to total Cmax, our assay shows >80% correct predictions, a sensitivity of >65%, and no false positives, when incubating cells for 24h with drug. Corresponding numbers for 72h incubation with drug are 80% correct predictions, a sensitivity of >70%, and three false positives. False negatives comprise compounds which cause immune system-mediated DILI or which require extensive metabolism, both of which HepG2 cells are not covering. Hierarchical clustering using the multi-parametric data allows cytotoxicity similarity matching of new chemical entities with reference compounds/known drugs for better risk assessment during drug discovery. In summary, our newly developed high content imaging assay classifies drugs with potential to cause DILI, shows low numbers of false positives, and is believed to be a powerful screening tool for risk assessment during drug discovery.

**1141 ASSESSMENT OF TIME-KINETICS OF ENDOTHELIAL BARRIER INJURY BY IMPEDANCE TECHNOLOGY.**


Vascular leak syndrome (VLS) is an undesired side effect of some therapeutic monoclonal antibodies and therapeutic cytokines. Reduced integrity of the endothelial monolayer, i.e. separation of cells for a certain time then allows for a leakage of fluid from the circulatory system to the interstitial space finally resulting in interstitial edema, decrease in microcirculatory perfusion and organ damage. Soluble factors in the blood including IL-1beta, IL-2, and Thrombin have been shown to induce VLS by multiple mechanisms which lead to an endothelial cell contraction and disassembly of the interendothelial junctions. In the present study we designed an in vitro system based on human umbilical vein endothelial cells (HUVEC) as a model for the vascular wall to assess the potential of drug candidates to induce VLS. Drug-induced effects affecting monolayer integrity were then monitored by measuring electrical resistance over time on an xCELLigence® system able to capture morphological changes of cells producing a time-lapse impedance pattern termed ‘cell index’ (CI). Cells were cultured under conditions allowing cell-cell contacts confirmed by the expression of tight- and gap-junction proteins such as ZO-1, Claudin-5, and VE-Cadherin, and a constant CI. Thrombin decreased CI significantly and reversibly. Altered expression of ZO-1, Claudin-5 and VE-Cadherin measured by immunohistochemistry confirmed that the effect on CI was due to intracellular gaps following Thrombin exposure. To exclude unspecified cytotoxic effects co-treatment of non-endothelial cells (fibroblasts) was performed. The obtained results were compared to the current standard assay measuring flow-rate of FITC-dextran, respectively. Our results in a vitro approach chosen to assess VLS and we suggest real-time label-free impedance monitoring of vascular integrity as a simple, reliable and fast tool to assess the risk for VLS following drug treatment.

**1142 PREDICTING DRUG-INDUCED HEPATOTOXICITY IN ZEBRAFISH LARVAE: UNDERSTANDING THE COMPLEXITY.**

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Zebrafish larvae represent an attractive lower animal test model to fill the gap between high throughput in vitro cellular assays and conventional preclinical animal testing. The model may in particular be useful in predicting human liver toxicity, as a huge drawback of cellular hepatotoxicity assays remains the single cell system they are based on and the consequent limited predictive capacity. It is however important to acknowledge the complexity of this whole organism. A phenotypic darkening of the liver may arise as part of an overt toxic or stress response. In addition, understanding the effects of hepatotoxins on the liver of a vertebrate larva is essential in order to study critical hepatotoxicity pathways. Testing thoroughly characterized reference compounds against a well defined endpoint and correlating with their known mechanisms of action may answer these questions. We show the systematic investigations of this approach. First, the expression of a liver specific protein fabp10 during development of control larva by in situ hybridization was explored. Subsequently, larvae were treated with 10 well characterized hepatotoxin and 5 non hepatotoxic compounds. The hepatotoxic compounds (acetaminophen, amiodarone, tetracycline, nefazodone, tamoxifen and others) could be identified by a statistical significant change in fabp10 expression or staining pattern of the liver region. Different patterns were observed: enlarged or reduced size, reduced intensity and absence of staining. Interpretation of these patterns required high throughput histopathological analysis of zebrafish larvae. After characterization of the histology of the liver region, treated larva were assessed for pathological effects on the liver, which were correlated with the staining patterns.

**1143 INVESTIGATION INTO THE ROLE OF OCTN2 IN DRUG-INDUCED TOXICITY.**

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Cardiotoxicity is a leading cause of drug attrition at all phases of development and may be caused by a number of interrelated mechanisms. One such mechanism may involve the organic cation/carnitine transporter subtype 2 (OCTN2). Mutations in the OCTN2 gene result in primary systemic carnitine deficiency characterized by cardiomyopathy in man, and some xenobiotics are thought to cause secondary carnitine deficiency via inhibition of OCTN2. Drug-transporter interactions may have clinical implications due to altered disposition of a given drug and/or disruption of carnitine homeostasis. We therefore investigated, if cardiotoxic and non-cardiotoxic drugs inhibit OCTN2-mediated carnitine uptake and/or exhibited OCTN2-dependent cytotoxicity. 68 compounds (47 cardioxins, 21 non-cardioxins including non-toxic controls) were tested at a concentration of 100 μM. Inhibition of OCTN2-mediated 4H-acetyl-L-carnitine uptake was determined in HEK-hOCTN2 or empty vector HEK-control cells in 40 min incubations. Cytotoxicity was determined at 40 min and 24 hrs using an ATP viability assay. Inhibition and toxicity, expressed as percentage of control (0.5% DMSO), were defined as <80%, >carnitine uptake was Na+-dependent and inhibited by unlabeled L-carnitine (IC50 7 ± 1.5 μM). 9 compounds were excluded from the inhibition analysis due to confounding toxicity. Out of the remaining 59 compounds 14 inhibited OCTN2 activity: these were 10/39 (26%) cardioxins, 4/20 (20%) non-cardioxins including the known non-toxic substrate milderanort. 30/68 compounds exhibited cytotoxicity (24/47 cardioxins) after 24 hrs, but both HEK-hOCTN2 and HEK-control cells were equally sensitive. This suggests that OCTN2 does not enhance drug cytotoxicity and that none of the 30 toxins are potential substrates. These results suggest that OCTN2 may not be considered as a polyspecific drug transporter. Nevertheless, given the clinical impact of dysfunction of carnitine homeostasis and the observation of OCTN2 inhibition amongst cardioxins, in vitro evaluation of OCTN2-drug interactions may be useful for cardiotoxic hazard identification.
Mitochondrial toxicity is frequently investigated as a potential mechanism of drug-induced adverse events. Mitochondrial membrane permeability transition (MMPT) is an important endpoint of mitochondrial toxicity. As an early event in mitochondrial mediated apoptosis, MMPT leads to release of cytochrome c from mitochondria to cytosol thereby triggering downstream caspase activations. In this study, we assessed MMPT and its association with viability and mitochondrial membrane potential (MMP) in MolR4 cells exposed to known mitochondrial toxicants. Cells were exposed to various doses of the mitochondrial uncoupler carbonyl cyanide P-(trifluoromethoxy) phenylhydrazone (FCCP), the alternative electron acceptor menadione (MD) or doses of the mitochondrial uncoupler carbonyl cyanide P-(trifluoromethoxy) phenylhydrazone (FCCP), the alternative electron acceptor menadione (MD) or the complex III inhibitor antimycin A (AmA) for up to 24 h. MMPT was evaluated by cytometric assay potentially valuable in addressing mitochondrial toxicity in drug development.

Small interfering RNAs (siRNA) are used as a tool to knock-down gene expression in a sequence-specific manner, opening new possibilities for the development of siRNA as human therapeutics. While safe and effective delivery of siRNA to the target organ is the major challenge, there is also potential for side effects induced by the siRNA molecules themselves. These potential side effects can be grouped in three classes: (1) off-target silencing of genes; (2) interference of endogenous RNA silencing pathways or competition for the RISC complex; (3) undesired activation of innate immune responses, mediated by pattern recognition receptors (PRR) recognizing nucleic acids commonly found in pathogens, like Toll-like receptors 3, 7 and 8 (TLR3, TLR7, TLR8). While TLR3 and TLR7 are present in endosomes and detect siRNA based on the presence of short sequence motifs rich in uridine, TLR3 binds in a sequence-independent manner to dsRNA and is present in endosomes as well as on the cell surface of immune and non-immune cells. Here, we describe the establishment of a panel of in vitro assays to monitor the activation of TLR3 for safety evaluation during the development of therapeutic siRNAs. A combination of tests performed on human dendritic cells, NK cells and endothelial cells (eg. HUVEC cells) allow the evaluation of the risk of triggering innate immune responses due to the binding of siRNAs to TLR3 molecules present on the surface of these cells. Furthermore, the activation of other endosomal and/or cytoplasmic PRRs caused by potential internalization of siRNAs can also be assessed. PRR-mediated responses were assessed by measuring cytokine secretion and gene expression analysis upon stimulation of cell populations with specific activators or siRNAs. While dendritic and NK cells were found to be suitable models to analyze the siRNA activation of the innate immune system by various mechanisms, HUVEC cells were intensively characterized and selected to evaluate TLR3-specific mediated responses.

Mitochondrial toxicity is one of the leading causes of drug attrition at all phases of development. It is therefore important to understand the expression of transporters in the heart relative to transporter-rich tissues such as the liver and kidney. Next generation sequencing of the transcriptome (RNA-seq) offers a global snapshot of the RNA population enabling high-resolution comparisons between genes and across samples. Here we use RNA-seq to identify the relative expression of 47 ABCs and a subset of 70 SLCs with potential for drug transport. Total RNA was extracted from the atria, ventricles, kidney, liver and gastrocnemius of male Wistar rats (n = 2). Sequencing of samples was carried out on an illumina platform and reads mapped using Genomic Workbench (CLC bio). Gene expression values were filtered using a minimum cut off of 10 reads/gene. Values were normalised using the trimmed mean of M-values method. Hierarchical clustering identified 53 out of 117 genes expressed with an organ-specific bias with respect to either heart, kidney or liver (10, 28 and 15 respectively). Within the heart 7 genes represented transporters with potential for drug transport (members of SLC16, SLC22, SLCO, ABCBC and ABCB families). To determine whether any of these were heart-specific rather than muscle-specific, expression levels of each transporter were compared to skeletal muscle. As a result 4 transporters appeared to exhibit a heart-specific bias with regard to expression levels (>50% greater normalised RPM). It is therefore important to assess the drug-transporter interactions of these genes to further determine a role for transporters in cardiotoxicity.

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had increased by 50% or more during a 24-30 hour exposure. Data were rejected at lower values, which indicate excessive cytotoxicity. The cell permeability dye Ethidium monazide (EMA) was also assessed as a measure of cell viability. Compound exposure regimes producing any more than a 4 fold increase in EMA staining have a higher risk of producing misleading micronucleus induction data. Progenotoxins produced expected positive results in the 39 exposure protocol. The trials results demonstrate a high concordance of results between laboratories, and high reproducibility within and between laboratories. Discordant results were not associated with particular compounds, suggesting that the protocol is highly transferable. The ability of the MNT to predict the results of other in vitro and in vivo genotoxicity endpoints reflected findings from an earlier validation of a tube-based assay. In summary, in the three cases presented, MEA assays using hCMs or rCMs had excellent predictivity for cardiovascular outcomes, and were more predictive of results of other standard safety assays: three examples.


Using MEAs and cardiomyocytes derived from human induced pluripotent stem cells (hCMs) or rat fetus (rCMs), we evaluated the effects of three internal compounds from different programs whose in vitro cardiovascular outcomes were not predicted by standard in vitro safety assays. hCMs were purchased from CDI, and rCMs were isolated from E18 rats. Spontaneous field potentials (FPs) were recorded on MEAs following an equilibration period. FP beating rate (BR), waveform morphology and conduction time (CT) were analyzed with custom software written in Matlab. The first compound prolonged QT interval in safety pharmacology (SP) telemetry and EP studies (12% at 0.6 μM). A development compound in a second program reduced heart rate in humans at lower exposures than predicted based on SP telemetry studies. Mechanistic studies showed the compound was a direct inhibitor of sinusoidal node automaticity. The compound inhibited BR in hCM MEA assays by 27, 72 and 86% at 3, 10 and 30 μM, respectively, while inhibiting BR in rCMs by only 38% at 30 μM. The MEA assays were more predictive for heart rate slowing, and hCMs were more sensitive than rCMs. A development compound in a second program reduced heart rate in humans at lower exposures than predicted based on SP telemetry studies. In summary, in the three cases presented, MEA assays using hCMs or rCMs had excellent predictivity for cardiovascular outcomes, and were more predictive than standard assays. hCMs were more sensitive than rCMs depending on the mechanism.

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Drug-induced organ toxicity is a major problem in new drug development and continues to be a significant issue in late stage attrition of development compounds. In vitro screening assays developed upon pathways and mechanisms linked to drug toxicity are widely used to predict some of these adverse effects in vivo. However, these cell-based models, however, do not take into consideration factors that regulate in vivo accumulation and distribution of drugs. Our in vitro screening data shows that 1) compounds that cause low-dose cytotoxicity in cell-based assays often exert such effects in cell lines independent of their in vivo organ expression; and 2) different cell lines showed different sensitivity toward certain compounds. This difference in sensitivity among cell lines may be partially due to differential expression levels of transporters. There is increasing evidence supporting their roles in drug disposition, therapeutic efficacy and adverse drug reactions. In this study, we profiled the gene expression levels of annotated transporters, which include approximately 2000 human and 1000 rat transporters, in five human cell lines and three rat cell lines using Affymetrix microarrays. We also evaluated the cytotoxicity of over 400 compounds with known rat in vivo outcome data in two liver cell lines, HepG2 and THLE. The relationship between differential expression levels of transporters among cell lines and differential sensitivities of compounds was investigated. Our preliminary data shows that differential expression levels of transporters can explain the differential sensitivity of, at least, some compounds in different cell backgrounds. This analysis will enable us to improve our in vitro predictivity by choosing the right cell model.

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Drug-induced organ toxicity is a major problem in drug development and continues to be a significant issue in late stage attrition of development compounds. In vitro screening assays developed upon pathways and mechanisms linked to drug toxicity are widely used to predict some of these adverse effects in vivo. These cell-based models, however, do not take into consideration factors that regulate in vivo accumulation and distribution of drugs. Our in vitro screening data shows that 1) compounds that cause low-dose cytotoxicity in cell-based assays often exert such effects in cell lines independent of their in vivo organ expression; and 2) different cell lines showed different sensitivity toward certain compounds. This difference in sensitivity among cell lines may be partially due to differential expression levels of transporters. There is increasing evidence supporting their roles in drug disposition, therapeutic efficacy and adverse drug reactions. In this study, we profiled the gene expression levels of annotated transporters, which include approximately 2000 human and 1000 rat transporters, in five human cell lines and three rat cell lines using Affymetrix microarrays. We also evaluated the cytotoxicity of over 400 compounds with known rat in vivo outcome data in two liver cell lines, HepG2 and THLE. The relationship between differential expression levels of transporters among cell lines and differential sensitivities of compounds was investigated. Our preliminary data shows that differential expression levels of transporters can explain the differential sensitivity of, at least, some compounds in different cell backgrounds. This analysis will enable us to improve our in vitro predictivity by choosing the right cell model.


It has been proposed that many marketed drugs with clinical safety issues disrupt mitochondrial function. Knowing the primary mechanism of a compound on mitochondrial function early in development could prevent later stage attrition. However, comprehensive knowledge about the ability of a compound to perturb mitochondrial function and then identify the primary mechanism of mitochondrial perturbation has not been fully elucidated. To address this gap, a custom built mitochondrial function profiling platform was developed and used to test compound effects on electron transport chain complexes (I-IV), NADH oxidation, ATP synthesis (complex V), TCA (glutamate, succinate, pyruvate) and fatty acid (acyetyl-CoA, acetyl carnitine, butyryl-CoA, octanoyl-CoA, palmitoyl-CoA, palmityl carnitine) substrates oxidation, mitochondrial membrane potential, uncoupling (induction of the respiratory burst), mitochondrial permeability transition, calcium loading potential, citrate synthase activity, and oxidative stress (acetonate activity). 56 marketed drugs and 22 known mitotoxins were profiled. Several classical mitotoxins displayed characteristic effects (e.g., Antimycin A inhibited complex III); however, at higher concentrations, Antimycin A also inhibited complex V. Our results indicate there are relatively few potent (IC50 less than 1 μM) marketed drugs that had effects on any mitochondrial endpoint (9%) and none of these were electron transport chain inhibitors. However, mitochondrial uncouplers and substrate
1153 STRATEGIES TO IDENTIFY THE POTENTIAL SAFETY LIABILITIES OF COVALENT, IRREVERSIBLE INHIBITORS.


Covalent irreversible inhibition is an attractive strategy for pharmacological targets as it may offer unprecedented levels in selectivity, thereby reducing the chance of any undesired side-effects. Such an approach, however, requires the inhibitor to possess sufficient levels of electrophilicity that enable it to bind covalently with nearby nucleophilic residues, yet not too reactive that it binds non-selectively to other cellular proteins. Measurement of off-target protein binding is usually performed once a lead compound has been selected, typically through the identification of radiolabelled protein adducts. Such an analysis, however, is expensive and not applicable to the design stages of the drug discovery process.

We investigated three in vitro assays to determine the off-target reactivity of electrophilic compound groups; two were based on intrinsic thiol-reactivity, one utilizing Ellman’s reagent to detect untreated thiol and the other utilizing fluorescently-labeled glutathione to follow reaction kinetics. A third assay involved measuring activation of the Nrf2 transcription factor, which plays a critical role in inducing the Antioxidant Response Element (ARE). Using luciferase reporter methodology, levels of Nrf2 activation were measured in HEK293 cells on treatment with a variety of electrophilic compounds. Levels of Nrf2 activation correlated well with electrophilicity of the test compound, as measured by reaction with glutathione and detection by Ellman’s reagent. These three assays are being utilized to measure direct electrophilicity as well as to determine a functional cellular readout of thiol reactivity. As a result, several structural-activity relationships have been developed for cysteine-reactive functional groups, including maleimides, acrylamides and cyanamides. Application of all three approaches in compound screening may be useful in determining the optimal balance between target and off-target covalent binding.

1154 GAMMA-SECRETASE MEDIATED HEPATOTOXICITY: USING MULTIPARAMETER IN VITRO METHODS TO ASSESS TOXICITY AND INTERPRET MECHANISM.

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In vitro and in vito hepatotoxicity evaluations were conducted on a novel gamma-secretase modulator (GSM); BIO-021169 (BIBO042, MW of 499.5). Three structurally-related molecules (BIBO042, BIO-017708, and BIO-021882) and two structurally-unrelated GSMs (BIO-026501 and BIO-027436) were evaluated using the CellCiphr® system in primary rat hepatocytes and human hepatoma cells (HepG2). All three BIBO042-related compounds were potent hepatotoxicants in vitro. The most sensitive effects in primary hepatocytes were: mitochondrial membrane potential changes and apoptosis/nuclear size changes (activity concentration 50% (AC50) for the most sensitive indicator was ~10.5 M M or 5.4 μg/mL). In HepG2 cells, stress-kinase activation, mitochondrial membrane potential and reactive oxygen species generation leading to cell cycle arrest, nuclear size changes and cell death was observed. The structurally unrelated molecules were significantly less hepatotoxic in primary hepatocytes and had a maximum tolerated concentration of 105.6 and 195.6 μM, respectively. However, in HepG2 cells, these compounds were slightly more potent with a cytotoxicity threshold of 26.1 and 70.8 μM (BIO-026451 and 027436, respectively) and were observed to have effects on both the mitochondria and activation of stress-kinases similar to BIBO042. This data correlates with in vivo studies with BIBO042 in rat and cynomolgus monkey where the doselimiting toxicity was hepatic and was observed at or near exposures (based on Cmax) in the range determined in vitro. CellCiphr analysis appeared to link toxicity in hepatocytes to direct effects on the mitochondria, perhaps leading to activation of stress-linked kinases and the intrinsic apoptotic cascade. Gamma-secretase is known to be expressed in the mitochondrial membrane and it appears that activity of this complex (particularly the presenilins) can have effects on mitochondrialf function and basal metabolic state, potentially linking the pharmacologic activity of this molecule to the toxicity observed.

1155 NONINVASIVE REAL-TIME MEASUREMENT OF OXIDATIVE METABOLISM IN VITRO ALLOWS SENSITIVE DYNAMIC READOUT OF DRUG RESPONSE.

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Disruption of cellular oxidative metabolism pathways is recognized as playing a critical role in a large number of drug safety-related problems. There are many methods for evaluating changes in cellular metabolism or mitochondrial function in vitro, but these assays are typically either invasive or require sampling at discrete intervals. Here, we report on the development of a noninvasive real-time measurement system for oxidative metabolism based on readouts of oxygen concentration at defined locations relative to the cells in culture. Specifically, fluorescence-based sensors were integrated into standard multi-well cell culture formats and their utility was evaluated in several experimental conditions mimicking effects on the oxidative metabolism. Importantly, this measurement method is not only suitable for static two-dimensional cultures, but is particularly useful for flow-based cell culture environments where human liver monolayers are often compared to 60% density with the Ames Assay. flow based liver cell culture environment. The concentration measurement results represent a simple yet highly sensitive and dynamic readout of an in vitro drug response. Because the measurement is conducted in real-time, this system is also amenable to determining the dynamics of the drug response and is not limited to discrete sample intervals; furthermore, it allows selection of an appropriate concentration and time point for detailed offline analysis during the experiment.

1156 NEXT GENERATION DEL ASSAY: CYTOTOXICITY AND GENOTOXICITY DETECTION WITH DEL-XG.

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Generic instability is a hallmark of carcinogenesis. Furthermore, cells from patients carrying mutations conferring cancer prone phenotypes show a higher level of genetic instability, including DNA deletions. We have previously constructed and/or used assays (DEL assays) that select for DNA deletion events in yeast, human cells and in vivo in the mouse. In fact, the original yeast-based DEL Assay detects 92% of known human carcinogens as compared to 66% detection with the Ames Assay. Deletions occur by homologous recombination between two copies of a duplicated DNA sequence to delete the interrupting sequence together with one of the repeats. DEL events in all three formats are inducible by a wide variety of carcinogens including carcinogens that are negative in many other short-term tests. Furthermore, many cancer prone mutations highly elevated the frequency of DNA deletions in mice adding to the solid correlation between DEL events and carcinogenesis. Here we introduce the next generation DEL assay: a novel dual-read out Saccharomyces cerevisiae screen DEL-XG. Based on the original DEL assay, DEL-XG simultaneously assesses the compound’s genotoxicity and cytotoxicity properties. DEL-XG test strain cells contain a lacZ gene sequence with a region of homology interrupted by an auxotrophic gene; during an exposure to a genotoxic event (carcinogens, ionizing radiation, etc.) the sequence is excised and the lacZ gene recombines to a functional beta-galactosidase genotype that with an addition of the X-Gal substrate produces a detectable indole product. Here we compare the efficacy of the DEL-XG assay to the original DEL Assay. DEL-XG is a rapid and economical way to screen large chemical libraries for toxicity and carcinogenicity properties and enabling a screening “triage”: compounds with higher toxicity profiles will be subjected to additional screening as a first priority.

1157 ASSESSMENT OF HERG CHANNEL FUNCTION USING STEM CELL-DERIVED CARDIOMYOCYTES.


The vast majority of drugs withdrawn from the market due to association with TdP arrhythmia interfere with the Lkf repolarization current mediated through the HERG. Consequently, the ICH S7B guidelines recommend that all new chemical entities should be subjected to HERG repolarization assay, typically using cell lines.
that recombiantly express hERG protein. However, in the last decade it has become evident that not all hERG channel inhibitors result in TdP and not all compounds that induce QT prolongation and TdP necessarily inhibit hERG. In order to better understand and assess the different kinds of drug liabilities associated with hERG channel inhibition and modulation we have used a panel of drugs and compounds which (i) directly bind and inhibit hERG channel function (overt inhibitors); (ii) compounds which inhibit hERG as well as other channels and therefore compensate for the IKr block (covert inhibitors) and (iii) compounds which interfere with the trafficking of hERG channel protein to the plasma membrane (trafficking inhibitors). We have assessed the activity of these compounds using human induced pluripotent stem cell-derived cardiomyocytes (iPSC) together with a system that can measure the beating activity of the spontaneously beating cardiomyocytes. Our data clearly shows that overt hERG channel inhibitors disrupt the periodicity of beating of iPSC cardiomyocytes leading to plateau oscillations and a signature waveform that is typical of this class of compounds. Covert hERG channel inhibitors at physiologic concentrations do not appear to affect cardiac function and therefore appear to be safe. hERG trafficking inhibitors display a time-dependent effect on the periodicity of beating that manifests several hours after compound dosing. In summary, the results clearly show that dynamic monitoring of iPSC cardiomyocyte beating can be used in a predictive way to assess various types of hERG channel modulators and provide additional information to electrophysiological methods.

1160 UNBIASED APPROACH FOR THE DOSE-RESPONSE ANALYSIS OF SIGNAL TRANSDUCTION PATHWAYS.

H. N. Williams, J. Vrana, C. Kinzer and J. Boyd, Chemistry, West Virginia University, Morgantown, WV.

High throughput multiplex assays capable of quantifying signaling protein activities have led to a major increase in the availability of large datasets describing signal transduction networks. Computational models are often used to organize and interpret these large datasets into tractable biological knowledge. Data driven models often yield unanticipated results, and can be advantageous because prior biological understanding of the network need not be relied upon, minimizing variability and error that can result when biological information obtained from different treatment conditions, timescales, species, and cell types is used. In this study, computational modeling was used to assess the signal transduction networks associated with stress response. A unique aspect of this study is that the signal transduction networks were assessed from a toxicological point of view; that is, the protein networks were constructed based on changes in protein activities in response to increasing doses of inhibitors, independent of time. The phosphorylation response of HepG2 cells exposed to increasing doses of deguelin, potassium cyanide (KCN), staurosporine, SB202190, and SB202474 alone and in binary combinations was assessed via principal component analysis for 8 highly networked signaling proteins (MEK, ERK, AKT, JNK, p38, p53, HSF27, and p90RSK). Phosphorylation was quantified using a bead based multiplex ELISA assay. This data was then used to frame cellular network responses with regard to dose for 2 disparate toxicological examples: exposure to single-target vs. multi-target inhibitors and mixtures with greater than additive toxicity. Our results indicate that dose-to-dose protein activity is significantly different from the overall response; single target inhibitors lead to a more highly correlated protein response than multi-target inhibitors; and mixture additivity may be the result of low dose, non-toxic thresholds of the individual constituents. Overall this approach provides unique and unbiased toxicological insight into the signal transduction networks associated with stress response.

1161 ESTIMATING TEMPORAL BIFURCATIONS ASSOCIATED WITH CELLULAR SIGNAL TRANSDUCTION.

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The pharmacodynamic response of cells to xenobiotics is primarily coordinated by signal transduction networks, which typically follow a fundamental motif, the phosphorylation / dephosphorylation cycle mediated by kinases and phosphatases. Defining the temporal scale of critical signal transduction events post-dose is difficult, but may be possible by exploiting the intricate inter-relationship that exists between mitochondrial-driven energy metabolism and kinase response. Kinase signaling is an energy demanding process, and its reliance on phosphorylation results in the consumption of substantial amounts of available ATP. Further, it has been shown that ATP-production actually governs the majority of ATP-consuming processes in mammalian cells, and regulation is primarily driven by oxidative phosphorylation within mitochondria. Therefore, if one monitors the energy production processes in the cell, key time points of significant deviation may point toward critical kinase signaling events. Monitoring these responses without potentially disrupting sensitive intracellular activity is imperative; thus we have developed an extracellular approach to determine kinase signaling events on a temporal scale by estimating ATP production with kinetic data collected from real-time oxygen
consumption and NADH production assays. These data sets allow for stoichiometric
determination of ATP production in real-time. Preliminary results will be pre-
sented with data collected from HepG2 cells dosed with MEK inh II or TDZD-8
(GSK3β inhibitor) monitored over 24 hours at 10 minute intervals. We found key
bifurcations at 20 minutes and 500 minutes post-dose for MEK inh II, and bifur-
cations at 40 minutes and 600 minutes post-dose for TDZD-8. We then measure the
phosphorylation response of several proteins at these time points, and relate them
to 24 hour viability. In this study, we demonstrate the ability to estimate crit-
ical signaling events via temporal response using an extracellular, in vitro means of
estimating phosphate generation by exploiting stoichiometric relationships between
NADH and O2 to produce ATP.

1162 IDENTIFICATION OF EARLY SIGNAL TRANSDUCTION RESPONSES PREDICTIVE OF SURVIVAL.

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The phosphorylation/dephosphorylation cycle, mediated by kinases and phos-
phatases, is responsible for the coordination of many cellular responses to xenobi-
otics. This simple cycle is embedded in networks which use positive and negative
feedback to generate extremely diverse functions like signal amplification, re-
versible/irreversible switches, and oscillations. One key irreversible switch is apop-
tosis, and both the signaling network and infrastructure surrounding the decision
to flip this switch is complex. Multiple signals generated by either external or inter-
nal stresses converge upon the mitochondria and result in outer membrane perme-
abilization, which is considered the point of no return for apoptosis. To determine the
drivers of apoptosis and nanofibrosis in vivo, we exposed HepG2 cells to several doses (1-100 μM) of a protein inhibitor (MEK inh II) and determined 24
hour viability. Next, we estimated the temporal response of protein phosphoryla-
tion by calculating the phosphate available for cellular signaling from oxygen con-
sumption and NADH generation in vitro. The results showed that apoptosis was induced when ATP production was severely hindered and was in general agreement with 24 hour viability measurements. Overall, this new approach can reasonably predict 24 hour viability at very early time points post-dose (+1 hour), by simply interpreting the dynamic activity of signal transduction networks in response to xenobiotics.

1163 MECHANISTIC INVESTIGATION OF B-RAF INHIBITOR-INDUCED HYPERPLASIA AND DEVELOPMENT OF A 3D HUMAN IN VITRO MODEL.

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The Raf proteins play a role in the MAP/ERKs kinase signaling pathway, which regulates cell division, differentiation, and secretion. B-Raf mutations have been reported in various types of cancers in human; therefore selective inhibitors of B-Raf have been developed for tumor therapy. Although cell line and tumors that harbor the B-Raf mutant can be sensitive to these inhibitors, hyperplasia in several normal squamous epithelial tissues has been reported in animal studies. Here we re-
ported the development of an in vitro 3D model to replicate the proliferative effect seen in rodent studies and to better understand the biology. The 3D model culture of Reconstructed Human Epidermis (RHE) cells made by SkinEthics was evaluated in our study because of its histological similarity to that of the human epi-
dermis. RHE that was cultured in vitro was treated with DMSO or B-Raf inhibitor
and W. Hu.ing B-Raf inhibitor-induced hyperplasia and demonstrates the human relevance of
these findings from a two week study in rats.

1164 KINASE ACTIVITY PROFILING IN INVESTIGATIVE TOXICOLOGY.

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Molecular toxicology aims to link gene and protein expression profiles to estab-
lished end-points of toxic responses. These methods provide informative snapshots of what is occurring in response to toxicants but do not capture the dynamic nature of the responses. Peptide microarrays can assess functional consequences of trans-
scriptional/translational changes linked to pathogenesis. We study signal transduc-
tion mechanisms by monitoring signaling kinases, using dynamic peptide microar-
rays in order to find underlying mechanisms of toxic responses. Total protein was extracted from renal cell carcinoma and normal kidney tissues from patients treated with either of the anti-angiogenic drugs, sorafenib and sunitinib. Equal amounts of total protein (10μg) were analyzed for kinase activities on dynamic PanChip pep-
tide microarrays comprising 144 or 256 different peptides. Our results show that kinase activity profiles can be generated robustly from tissue extracts. Experiments showed signal dependence on protein extract concentration, ATP concentration and finally, modulation of the peptide phosphorylations by the drugs. Different phosphorylation profiles were obtained between tumour and healthy tissue from the same patient, showing higher activities in the former. Investigating both chemotherapeutics, similar profiles were found in tumour tissue, while clear differ-
ences were detected in the corresponding normal tissue. These are potentially linked to differences in toxicity between the drugs. Importantly, phosphorylation of peptides representing PDGFβR and EGFR was inhibited more in tumour than after sunitinib treated versus sorafenib treated patients. The observations indicate that this method could identify appropriate target proteins, but also off-target proteins possibly in-
volved in adverse drug reactions. This novel molecular approach has a good poten-
tial application in clinical and preclinical toxicological research. Clearly it provides
the means to study the dynamics of the adverse effect of pharmaceuticals, whether from cell lines or (animal or patient derived) tissues.

1165 LYSOSOME FUNCTION IMPAIRMENT AS A POTENTIAL MECHANISM OF RETINOPATHY.

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The retina is a highly structured tissue formed by seven different cell types arranged in layers. The photoreceptor cell is a specialized type of neuron in the retina that is capable of absorbing photon and converting light into signals that can stimulate bi-
ological processes. There is a constant renewal process for photoreceptors consisting of intermittent shedding of the distal tips of the photoreceptor outer segment and subsequent phagocytosis in the retinal pigment epithelia (RPE) cells. Rebuilding process is essential for vision and the survival of photoreceptors and RPE cells. Drugs with basic moity have the potential to accumulate in lysosomes and impair its functions including the phagocytosis process, which could hinder clearance of outer segment of the retina and ultimately induce retinopathy. To determine the prevalence of this cellular mechanism in retinal toxicity, a collection of proprietary compounds associated with retinal toxicity were put through a battery of in vitro tests using a human retinal pigmented epithelium cell line, ARPE. The tests included phagocytosis assay, Lysotracker staining, Bafilomycin A (BFA) rescue exper-
iments, and autophagosome staining. The compounds that induced retinopathy were identified to accumulate in the lysosomes. This accumulation coincided with phagocytosis inhibition and an increase in autophagosome staining, suggesting a blockage of the membrane trafficking process related to lysosomal functional im-
pairment following compound accumulation. A correlation between the phyto-
chemical properties and in vitro lysosomal pathway effects was also established. In addition, cytotoxicity of selected compounds was determined, and it was shown that treatment of BFA, further suggesting the involvement of lysosomes in the toxicity observed. This data reveals the importance of physiochemical properties and lysosome accumu-
lation as a common mechanism for drug induced retinopathy and demonstrates the potential usefulness of an in vitro screen in predicting this liability.

1166 NEW MODEL OF NONCLINICAL CARDIAC RISK ASSESSMENT.

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Drug-induced inhibition of the cardiac hERG potassium channel is assumed to predict delayed cardiac repolarization (DR). The consequent QTc prolongation is a
powerful predictor of torsade de points (TdP), a rare but potentially lethal iatro-
genic outcome. Drugs with effective therapeutic plasma concentrations (ETPC)
within 30-fold of their hERG IC50s are thought to be dangerous despite the fact that multiple ion channel effects (MICE) can mitigate DR. Here we demonstrate that a logistic regression model, which integrates MICE, predicts TdP with much greater certainty than the hERG safety ratio (hERG IC50/ETPC) alone. Safety ratios were calculated for 41 drugs (20 +TdP and 21 -TdP) from multiple classes by dividing their hERG, Nav1.5 and Cav1.2 IC50 values by each drug’s ETPC. Two logistic regression models were constructed; one using the hERG IC50/ETPC ratio alone (Model 1), the other integrating hERG IC50/ETPC + Nav1.5 IC50/ETPC + Cav1.2 IC50/ETPC data (Model 2). The predictive power of each model was evaluated by performing leave-one-out cross validations. Each model’s accuracy for discriminating +TdP and -TdP drugs was determined by comparing their receiver-operator characteristics (ROC, true vs. false positive rates). Model 1 had a 52% False Positive Rate associated with a 90% True Positive Rate and a ROC area under the curve (AUC) of 0.74. Model 2 significantly improved accuracy showing a 14% False Positive Rate associated with a 90% True Positive Rate and a ROC AUC of 0.88. We propose that models that incorporate quantitative drug effects on multiple cardiac ion channels will be robust nonclinical predictors of cardiac risk.

Cardiovascular (CV) toxicity is a leading contributor to drug withdrawal and late-stage attrition. Earlier and broader screening is a validated approach to build-in CV safety, as demonstrated with hERG screening to reduce drug-induced arrhythmia. There is an urgent need for novel in vitro assays to extend this success to contractility, heart rate, hypertrophy, structural damage, and non-hERG arrhythmia. Recent advances in label-free cellular impedance technology now enable tracking of spontaneous synchronized beating of cultured cardiomyocytes. Analysis of beating allows integrated detection of electrical and mechanical aspects of contraction. Here, we evaluate impedance cardiomyocyte assays against criteria required for drug screening. The throughput and sensitivity allowed for rapid assay development and identification of critical optimization parameters with rat neonatal cardiomyocytes including cell seeding density and serum levels. Once optimized, consistent stable beating for at least three days was straightforward to achieve. In tests of ligands spanning a breadth of target classes, the potency values showed excellent precision, wide dynamic range, and consistency across multiple experiments. Impedance data could be analyzed for beat rate, amplitude, and beat rise slope with each parameter yielding similar precision. Potency values calculated by beat rate and amplitude were highly correlated for most compounds although selected compounds displayed unique profiles indicative of different mechanisms. Evaluation of a set of drugs selected based on established CV activity in humans revealed concordance between impedance and clinical findings for 20 of 21 compounds. Thus, impedance assays combining features including sensitivity to contractive activity, versatility data analysis, and robust/translatable data can be utilized to address critical gaps in CV drug screening.

Cardiovascular toxicity remains one of the leading causes of late-stage drug attrition and drug withdrawals. Drugs of the tyrosine kinase inhibitor class have received particular attention in this regard (Force et al. (2011) Nat Rev Drug Discov 10, 111-26). The cytochrome P450 isoform 2J2 (CYP2J2)-epoxyeicosatrienoic acids (EETs) pathway regulates key aspects of cardiovascular function and plays an important cardioprotective role under stress conditions linked to mitochondrial function (Karragoda et al. (2009) J Mol Cell Cardiol 46, 867-75). We investigated the hypothesis that this pathway may be linked to drug-induced cardiotoxicity. We validated a CYP2J2 inhibitor assay based on commercially-available CYP2J2 super-somes (BD biosciences) and substrate (Promega). A known reference compound (terfenadine) gave an IC50 value of 315 nM (mean pIC50 6.50 ± 0.02; n = 34). IC50 data (n = 2) for 114 clinically-relevant drugs annotated for organ toxicity were generated. A number of inhibitors were identified, where at a threshold of IC50 <1 μM, 6 were associated with cardiotoxicity, compared to 2 that were associated with hepatoxicity, 1 associated with both toxicities and 1 with low organ toxicity. Strikingly, 3 of 4 cardiotoxic tyrosine kinase inhibitors tested were potent 2J2 inhibitors with IC50 values of 79, 336 and 872 nM (imatinib, sorafenib, dasatinib), the remaining kinase inhibitor IC50 value was 10 μM (sunitinib). Drugs were tested for ATP depletion in the cardioid-derived rat H9c2 cell line and in H9c2 cells adapted for increased mitochondrial aerobic activity (using a galactose energy source to circumvent the Crabtree effect (Marroquin et al. (2007) Toxicol Sci 97, 539-543)). The 3 cardiotoxics also potent CYP2J2 inhibitors were more cytotoxic to the H9c2 cells with a galactose energy source compared to normal H9c2 cells. These data suggest a potential association between using 30 μM as a cut-off of BR20 could correctly flag all 17 compounds. These results suggest the beat rate change is a more sensitive predictor and more translational biomarker of potential ECG alterations. Hence, including the beat rate analysis adds additional predictive value to this cardiomyocyte arrhythmogenesis model in assessing the likelihood of compounds to cause ECG alterations in the down-stream in vivo studies.

1169 HEMODYNAMIC FLOW AND HETEROCELLULAR CELL COMMUNICATION ARE NECESSARY FOR PREDICTING HUMAN VASCULAR DRUG RESPONSE IN PRECLINICAL VASCULAR IN VITRO SYSTEMS.

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Pre-clinical animal models are poor predictors of the human response and fail to deduce vascular cell-specific responses and mechanisms. Moreover, current in vitro systems do not incorporate fundamental in vivo parameters; such as hemodynamic forces and complex, heterotypic cell-cell interactions, i.e. endothelial and smooth muscle cell. In this study, molecular and functional responses to multiple FDA-approved drugs (statins, Ca2+ channel blockers, NSAIDs, thiazolidinediones, NO-donors) were investigated in a human primary vascular cell system. The technology imposes MRI-human-derived, region-specific, vascular hemodynamic patterns onto human primary vascular endothelial cells that are co-cultured with vascular smooth muscle cells. The cells are physically separated by a thin porous membrane, which allows for both interaction between and separation of both cell types and collection of secreted factors. Results: 1) compared to traditional culture methods, hemodynamic culture enhances human primary vascular cell arrival and re-establishes vascular bed specific endothelial and smooth muscle cell morphology and molecular phenotype, consistent with biology in intact arteries. 2) Molecular responses in the technology correlate to human drug responses whereas traditional culture responses are often misleading. 3) Functional responses (e.g., nitric oxide signaling, vasoactivity, permeability) varied depending on the nature of the hemodynamic flow pattern, e.g., small arteriole versus large conduit artery. Thus drug class-specific, differential responses were observed and correlated to human clinical response. Finally, non-human species cells (e.g., rat, dog, monkey) and hemodynamics can be utilized for comparison to human to assist in validating vascular cell-specific, vascular bed-specific properties of compounds, improving decision making of both positive and negative effects at the molecular and functional levels. NHLBI R43HL102951, R44HL102955
some forms of drug-induced cardiotoxicity, inhibition of the CYP2J2-EETs pathway and mitochondrial dysfunction, particularly for these cardiotoxic tyrosine kinase inhibitors.

**1171 IN VITRO SAFETY PHARMACOLOGY PROFILING IN EARLY DRUG DISCOVERY: MITIGATION OF ADRS.**


In vitro safety pharmacology has been applied in different forms to drug discovery. However, recent developments in assay technologies, automation and in silico sciences transformed a panel of target-based assays into a powerhouse of early safety profiling with a unique ability to support mitigation based on off-target SAR. In vitro safety pharmacology applies a panel of selected human targets associated with clinical ADRs to lead selection and optimization. The panel covers a broad pharmacology-chemistry space, employs biochemical and functional assays and has the capacity and quality to provide information on pharmacological promiscuity and predicts major liabilities. In silico tools are also available to predict off-target characteristics and link pharmacology with chemical structures. Information generated in the safety pharmacology panels should be considered together with PK/PD data and correlated with clinical observations obtained from marketed drugs with similar safety pharmacology profile. We will demonstrate the various utilities of in vitro safety pharmacology in the prediction of clinical ADRs.

**1172 AEROSOL DELIVERY TO A MICROFLUIDIC HUMAN BREATHING LUNG-ON-A-CHIP FOR INHALATION TOXICITY.**

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In vitro model systems that accurately predict drug efficacy, bioavailability and toxicity in humans are needed to replace costly and time-consuming animal studies to speed development and regulatory approval of new and safer medical products. Although advances have been made in cell culture models, these methods, in many cases, still fail to accurately predict toxicity in humans mainly due to insufficient re-construction of the key structural and mechanical features of the whole organ. Recently, we demonstrated a biomimetic microfluidic device that reproduces the alveol-capillary interface of the human lung under physiologically relevant cyclic mechanical strain and flow conditions. But aerosol drug delivery to cells at an air interface in a physiologically relevant manner constitutes a challenge, especially on the microscale. We address this challenge and report delivery of aerosolized microdroplets into a microfluidic device with human cells at an air-liquid interface. A commercial prescription nebulizer generates aerosolized liquid droplets. A design incorporating a miniature impactor delivered the respirable fraction to the alveolar epithelial monolayer. We have demonstrated cell vitality after aerosol delivery. Using this method, drugs, particles and toxins can be administered to human lung cells cultured at an air interface in microfluidic channels, where the environment closely mimics the in vivo mechanical and structural environment. Integration of this aerosol delivery system with the breathing lung-on-a-chip microdevice provides a new method to measure pulmonary absorption, efficacy and toxicity of aerosolized drugs, particles and toxins. Ultimately, aerosol drug delivery to biomimetic cell culture devices should provide a more accurate model for drug and toxicity screening.

**1173 PROTEOMIC ANALYSIS OF CARBONYLATED PROTEINS IN THE KIDNEYS OF TRICHLOROETHENE-EXPOSED MRL+/+ MICE.**

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Trichloroethene (TCE), a common environmental and occupational pollutant, is associated with multi-organ toxicity leading to diseases like cancer and autoimmune diseases. Kidney is one of major target organs affected as a result of TCE exposure. Our previous studies have shown that exposure to TCE causes increased protein oxidation (protein carbonylation) in kidneys of MRL+/+ mice, and suggested a potential role of carbonyl stress in TCE-mediated toxicity. However, identity of carbonylated proteins and potential changes in their structural and functional properties remain unknown. To assess the impact of chronic low dose TCE exposure on protein oxidation, particularly to identify the carbonylated proteins in kidney, we treated with TCE at the dose of 2 mg/kg via drinking water for 36 weeks and kidney protein extracts were analyzed for protein carbonyls and carbonylated proteins identified using proteomic approaches (2D gel, Western blot, MALDI TOF/TOF MS/MS, etc.). TCE exposure led to significantly increased protein carbonyls in the kidney protein extracts (20,000 g pellet fraction). Interestingly, among 18 identified carbonylated proteins, 10 were found only in the kidneys of TCE-treated mice, whereas other 8 were present in the kidneys of both control and TCE-treated mice. The identified carbonylated proteins represent skeletal proteins, chaperones, stress proteins, enzymes, plasma protein, and proteins involved in signaling pathways. These findings provide a map for further exploring the role of carbonylated proteins in TCE-mediated nephrotoxicity.

**1174 PROFILING PROTEIN CARBONYLATION IN A MURINE MODEL OF ALCOHOLIC LIVER DISEASE.**

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Hepatic oxidative stress is a well recognized result of sustained ethanol consumption. The resulting formation of lipid peroxidation end-products has been proposed to play a role in pathogenesis of alcoholic liver disease (ALD). These reactive electrophiles are well documented to form covalent adducts with protein side-chains (e.g. protein carbonylation) potentially leading to altered protein activity, structure and/or localization. To investigate the role of protein carbonylation in ALD, a 6-week ethanol feeding regimen was employed in C57BL/6 mice. Ethanol-fed mice displayed a 2-fold increase in hepatic TBARS while immunohistochemical analysis for the reactive aldehydes 4-hydroxynonenal (4-HNE), 4-oxononal (4-ONE), malondialdehyde (MDA) and acrolein (ACR) revealed a significant increase in the staining of modified proteins in the ethanol-treated mice. Increased protein carbonyl content was confirmed utilizing subcellular fractionation of liver homogenates followed by biotin-tagging through hydrazide chemistry. This revealed approximately a 2-fold increase in aldehyde-modified proteins in microsomal and cytosolic fractions. Novel targets of protein carbonylation were identified utilizing avidin binding followed by 2-dimensional liquid chromatography tandem mass spectrometry (2D LC-MS/MS). Our results have identified 414 protein targets for modification by reactive aldehydes in a mouse model for ALD; these data have also confirmed the presence of novel in vivo sites of protein modification by 4-HNE, 4-ONE, MDA and ACR. Bioinformatic analysis was also performed on a composite list of protein identifications and elucidates key pathways associated with the pathogenesis of ALD including lipid homeostasis, fatty acid metabolism, amino acid metabolism and protein acetylation. (R37 NIH/AA009300 & F31 AA018660).

**1175 PROTEOMIC IDENTIFICATION OF CARBONYLATED PROTEINS IN RAT HIPPOCAMPS AFTER 1-BROMOPROPANE EXPOSURE.**

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1-Bromopropane (1-BP), an alternative to ozone-depleting solvents, is neurotoxic both in animals and humans. Previous proteomic analysis of hippocampal tissues from rats identified alteration of protein expression and pathways involved in oxidative stress, suggesting that oxidative stress may play a role in 1-BP neurotoxicity. The present study investigated the contribution of oxidative stress to 1-BP neurotoxicity at protein level. Male F344 rats (n=9/group) were exposed to 1-BP at 0, 400, or 1000 ppm, 8 hrs/day for 1 week or 4 weeks. Protein carbonyl assay, two-dimensional gel electrophoresis, immunoblotting, and matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (MALDI-TOF-TOF/MS) were utilized to analyze quantitatively protein carbonylation in the rat hippocampus. Advanced glycation end products (AGEs) level was detected in the hippocampus and plasma by ELISA assay. Hippocampal protein carbonylation increased significantly after 1-BP exposure at 1000 ppm for 4 weeks, demonstrating 1-BP-associated induction of oxidative stress. MALDI-TOF-TOF/MS identified 10 carbonylated proteins whose levels significantly increased (p<0.05; fold change ≥ 1.5) with 1-BP exposure. The identified proteins were involved in biological processes such as glycolysis, ATP production, tyrosine catabolism, GTP binding, guanine degrada-
Reactive dicarbonyls, such as methylglyoxal (MG), are present in blood and react with arginines (R) of target proteins, leading to diabetic micro- and macrovascular complications. Normal MG plasma concentrations are estimated to reach 4.5 μM, with these values tripling as diabetic complications progress. Dicarbonyls irreversibly modify R residues, resulting in a net loss of positive charge via hydroimidazolone formation. Shotgun LC/MS/MS proteomics analysis of human serum, reacted with 5 mM MG (18 hrs at 37°C), produced adducts on 7 different plasma proteins: albumin, transferrin, fibrinogen, haptoglobin, apolipoprotein A-1, α1-acid glycoprotein and plasminogen (PLG). Although PLG was the least abundant plasma protein modified by MG, it was second only to fibrinogen in the number of modified peptides (R504, R561, R610, R637, R712, R638, R712, R677, R779, and R789). Albumin, present at concentrations -104 greater than PLG, yielded far fewer modified sites (R218, R257, R410, R428, R485). The findings indicate that Rs within the tail region of PLG are particularly susceptible to MG-mediated modifications. While the basis for this is unclear, molecular modeling revealed that R561 is solvent exposed, rendering this specific site highly susceptible to MG modification. Modified R561 in PLG is of particular biological interest because of its role in the thrombolytic cascade, being the site of cleavage from plasminogen to plasmin. Consistent with the data obtained from human serum, reactions of PLG with as little as 50 μM MG (48 hrs at 37°C) reproduced the modified R561 adduct. Modification of PLG was further confirmed by western blot analysis using an anti-MG antibody. The data demonstrate that R561 is a hot spot on PLG, the modification of which may disrupt the thrombolytic cascade thereby contributing to vascular complications associated with diabetes. The functional significance of the MG-mediated R561 adduction of PLG is under investigation. (R24DK083948, ABRC, ES016652, T32ES070791, P30ES006694).

253 DICARBONYL ADDUCTION OF PLASMINOGEN: POSSIBLE ROLE IN DIABETIC CARDIOVASCULAR COMPLICATIONS.

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254 POSTTRANSLATIONAL MODIFICATION AND INACTIVATION OF MITOCHONDRIAL COMPLEX I IN KAINATE NEUROTOXICITY.

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Chemoconvulsants such as the excitoxotic glutamate analogue, kainate, can result in acute and chronic hippocampal neurotoxicity resembling damage associated with temporal lobe epilepsy (TLE). We aimed to investigate the role of mitochondrial oxidative stress in the rat kainate model of TLE, specifically its contributions to mitochondrial dysfunction and consequential development of spontaneous seizures (ie. epileptogenesis). We hypothesized that persistent mitochondrial oxidative stress decreases mitochondrial protein function and bioenergetics [mitochondrial redox status (CoASH/CoA), bioenergetics (ATP/ADP), complex I (CI) activity and post-translational modifications to CI subunits] that contribute to epileptogenesis. To address this theory, indices of mitochondrial function were measured in hippocampus of saline and kainate (11mg/kg) treated rats shortly after administration (48hr), prior to the development of epilepsy (1wk), and during epilepsy (6wk). The CoASH/CoA ratio decreased ~70-80% at all time points while CI activity and the ATP/ADP ratio decreased 20-30% at 48hr and 6wk. Furthermore, arginine 76 (Arg76) was oxidatively modified to a semi-glutamatic aldehyde (GSA) product (ie. carboxylation) on the 75kDa subunit of CI, Ndufs1 (75kDa) modification was identified with an Agilent 6510 QTOF LC/MS system and MS/MS peptide data was dually analyzed with Mascot 2.2 (Matrix Science) and SpectraMill (Agilent). A correlation was observed between total mitochondrial carboxylation via biotinylated carbamoylation, specific Ndufs1 carboxylation at Arg76, and inhibition of CI activity. Molecular modeling studies reveal that Arg76 is located in the active site of CI and its carbonylation impacts local protein folding and decreases active site interaction energy (zRank score) by 15%. This irreversible modification has been considered a biomarker for oxidative stress-induced cellular damage and may be a future therapeutic target for chemoconvulsant neurotoxicity. (RO1NS039578).

255 POSTTRANSLATIONAL MODIFICATION AND REGULATION OF GLUTAMATE CYSTEINE LIGASE BY S-GLUTATHIONYLATION.

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The glutathione (GSH) antioxidant defense system plays a critical role in maintaining cellular redox homeostasis and counteracting the deleterious effects of oxidative stress. GSH can also be utilized in disulfide exchange reactions resulting in formation of mixed protein-glutathione disulfides and S-glutathionylation of proteins is gaining recognition as an important signal transduction mechanism during oxidative stress. 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. While cellular GCL activity is highly sensitive to relative GCL subunit expression levels, we and others have demonstrated that post-translational modifications of the GCL subunits may play a major role in the acute regulation of GCL activity. In this study, purified recombinant proteins were utilized to demonstrate that GCLC and GCLM are both direct targets for reversible S-glutathionylation in vitro. S-GLutathionylation increased GCLC activity -2-fold, yet had little effect on GCLM holoenzyme activity. Furthermore, while S-glutathionylation prevented GCLM holoenzyme formation and activity, it did not dissociate the GCLC holozyme complex. The masking of relevant cysteine residues may account for these apparent discrepancies as prior formation of GCLM holoenzyme significantly reduced S-glutathionylation of both GCLC and GCLM subunits. Mass spectrometry analysis identified multiple residues on both GCL subunits that are modified and may be functionally relevant based on in silico molecular modeling. In aggregate, these findings demonstrate that GCLC activity and GCLM holoenzyme formation and activity can be regulated via direct post-translational S-glutathionylation of the GCL subunits in vitro. This novel post-translational regulation of GCL activity could significantly affect cellular redox homeostasis and signal transduction during periods of oxidative stress.

257 PLASMA PROTEIN NITRATION: A USEFUL BIOMARKER FOR DETECTING UNDIAGNOSED COPD IN TOBACCO SMOKERS.

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Chronic obstructive pulmonary disease (COPD) affects millions of Americans, with as many as 10 million undiagnosed cases. Early detection of COPD improves long-term prognosis and health, suggesting a need for a simple screen for detecting COPD in high-risk subjects, particularly current and past smokers. We evaluated nitrotyrosine in 25 individual plasma proteins as markers for early detection of COPD. A custom antibody microarray platform was developed to analyze the levels of 3-nitrotyrosine modifications on 24 proteins. Samples from 282 smokers were analyzed, including 193 with COPD and 89 without COPD (nonCOPD). We observed a consistent increase in nitrotyrosine levels in smokers with COPD that is presumably associated with chronic inflammation. When using only the nitrotyrosine protein data, a classification accuracy of 77% in an independent test set could distinguish between smokers with and without COPD. A more favorable classification could be achieved by combining clinical measures with the nitrotyrosine data. In these analyses, age, BMI, systolic and diastolic blood pressures, years smoking and carboxyhemoglobin levels were included. The final classification model was derived from an iterative variable selection routine which found that four variables (age, BMI, nitrated ceruloplasmin and nitrated surfactant protein A) could classify nonCOPD from COPD with an accuracy of 86% in the independent sample set. These data were also examined using receiver operator characteristic (ROC) curves. The ROC curves produced area-under-the-curve values that ranged between 0.55 and 0.69 for the four individual variables, but was 0.81 for the combined variables. Thus, nitrated plasma proteins have promise as biomarkers for identifying subjects that are at high risk for having undiagnosed COPD. Supported by NIEHS Exposure Biology Program, U54 ES016015.
1180 2D-LC-MS ANALYSIS OF OXIDIZED LIPID SPECIES: APPLICATION TO THE STUDY OF CARDIOLIPIN IN APOPTOSIS AND DAMAGED TISSUE.

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Oxidized lipid species are important signaling molecules that usually exist in low abundance in biological tissues. Along with their inherent stability issues, these oxidized lipids present themselves as a challenge in their detection and identification. These oxidized lipid species can co-chromatograph with non-oxidized species making their detection extremely difficult. In this study, a normal-phase and reverse-phase two dimensional HPLC-mass spectrometric system was applied to separate oxidized phospholipids from their non-oxidized counterparts, allowing unambiguous detection in a total lipid extract. We have utilized bovine heart cardiolipin as well as commercially available tetratenoyleoyl cardiolipin oxidized with cytochrome c and hydrogen peroxide as well as with lipoxygenase to test the separation power of the system. We found that oxidized and nonoxidized phospholipid species can be clearly separated from in this two dimensional system. We utilized two biologically relevant model systems, namely rotenone treatment of lymphocytes to induce mitochondrial damage and cell death as well as traumatic brain injury, to separate and identify cardiolipin oxidation products, critical to the cell death pathways in these tissues following cellular stress/injury. We identified oxidized linoleic acid chains (50%) as the preferred moieties for oxidation in cardiolipin, followed by arachidonic (31%) and docosahexaenoic acid chains (18%). The ability of the two-dimensional HPLC-mass spectrometric system to detect and characterize oxidized lipid products will allow new studies to be formulated to probe the answers to biologically important questions with regard to oxidative lipomics and cellular insult. Supported by NIOSH OH008282; NIH U19 A068021, HL70755, HL094488, ES020693, ES021068.

1181 CELL-BASED ANALYSIS OF LIPID PEROXIDATION AND LIPID PEROXIDATION-DERIVED PROTEIN MODIFICATIONS USING FLUORESCENCE MICROSCOPY.


Oxidative stress plays an important role in the progression of several diseases including inflammation, atherosclerosis, aging and age-related degenerative disorders. Reactive oxygen species damage membrane bound lipids including unsaturated fatty acids like linoleic acid and arachidonic acid to form lipid electrophiles, which can rapidly react with proteins and DNA to form adducts. Cell-based measurements of lipid peroxidation and protein carbonylation by fluorescence microscopy provide a powerful platform to quantitate lipid peroxidation in cells and also monitor spatial distribution of damage caused by lipid peroxides. Here, we used 2 different approaches to measure lipid peroxidation in cells. 1) A ratiometric determination of lipid peroxidation using a fluorescent ratiometric lipid probe for live cells which is incorporated into cellular membranes and emits at 590 nm. Under oxidative stress conditions, the oxidation of the dye results in a shift of fluorescence to a peak emission of 510 nm. 2) In a click chemistry based approach, unsaturated fatty acid analogs like linoleic acid alele or arachidonic acid zide are incorporated into the cellular membranes and probe modifications from oxidation, like LINE and DODE can readily modify DNA or proteins. The modified proteins are then analyzed by click reaction using fluorescent tagged alkynes or azides. Using these approaches, we measured lipid peroxidation caused by oxidants like tert-butyl hydroperoxide, cumene hydroperoxide, menadione, hemin and lipopolysaccharide in BPAE and RAW macrophage cells. The oxidants produced 2-3 fold increase in lipid peroxidation and protein modifications when compared to controls. The lipid peroxidation and the lipid peroxide-derived protein modifications were successfully inhibited by using anti-oxidants like tt-tocopherol and mixed tocopherols. The 2 strategies described here to measure lipid peroxidation and the derived protein modifications provide powerful tools to measure oxidative stress in cells.

1182 MALDI MASS SPECTROMETRIC IMAGING OF CARDIOLIPIN AND ITS OXIDATION PRODUCTS DIRECTLY FROM TISSUE SECTIONS IN A LUNG OXIDATIVE DAMAGE MODEL.

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Molecular understanding of lipid functions and signaling mechanisms has created the need for tissue selective assessment for localization of phospholipids and their oxidation products while maintaining tissue integrity. Cardiolipin (CL) is a mitochondria-specific anionic phospholipid that plays key roles in normal mitochondrial functions but also in cell death pathways. The molecular diversity of endogenous CL in some tissues and the low abundance of its individual species, particularly oxidized derivatives, makes in situ analysis challenging. Current Mass Spectrometry protocols using lung homogenates (LC-MS) have greatly enhanced our ability to explore molecular species of CL and its oxidized products. However location-specific information on oxidation cannot be determined from homogenates assuming that oxidative lipidomics analysis of acute lung injury will directly benefit from MALDI-MS imaging, we assessed possible advantages of this frontier technology in characterization of structural lipid changes in the lung. MALDI-MS imaging was performed on thin lung sections from both naive mice and ones receiving glucose oxidase (GOX) intratracheally as a model of oxidative injury. Scanning was at the diameter of the laser beam (20 microns), and each location scanned generated a complete mass spectrum that correlated with tissue location. A strong increase in oxidative degradation products of CL was observed by MALDI-MS imaging. Light microscopy confirms the preservation of tissue structure (without fixation). Serial sections were homogenized and analyzed by LC-MS to confirm the CL species identified by MALDI-MS Imaging. Assignment to an anatomical region suspected to be injured (e.g. airways) has been achieved. Supported by NIOSH OH008282; NIH U19 A068021, HL70755, HL094488, ES020693, ES021068, CCGS-P30 CA047904.
pression from preconfluency to postconfluency. We demonstrate that the pro-oxidant menadione causes dissociation of GSTA1-JNK complex and JNK activation in preconfluent Caco-2 cells only, whereas postconfluent cells are predominantly resistant to menadione-mediated complex dissociation. Our results provide novel evidence that GSTA1 plays a direct role in regulating JNK activation through complex formation. Preconfluent cells are more sensitive than postconfluent cells to menadione-induced cytotoxicity. Additionally, menadione induces transient JNK activation and following the removal of stimulus GSTA1 re-associates with JNK and significantly reduces cellular cytotoxicity. Modulated levels of GSTA1 affect GSTA1-JNK complex association and N-acetyl cysteine blocks menadione-induced JNK activation in Caco-2 cells. These results suggest that the mechanism of menadione-mediated JNK activation involves the production of reactive oxygen species and that the level of intracellular GSH plays an important role in preventing menadione-induced GSTA1-JNK complex dissociation.

1185 MENADIONE-INDUCED OXIDATIVE AND NITROSATIVE STRESS IN C2C12 MYOTUBES.

PS

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Skeletal muscle cells are subject to frequent oxidative challenges due to rapid changes in energy demand and oxygen flux, high metabolic activity, and the presence of high levels of heme-containing proteins. Nitric oxide synthases (NOS), which contain heme, can reduce redox-cycling compounds, which then reduce molecular O₂ to superoxide anion radical(O₂•-), depleting molecular O₂ and causing free radical-mediated oxidative injury and peroxynitrite formation. O₂ reacts with NO to form peroxynitrite, thus potentially decreasing bioavailable NO. Here, we study the effect of the redox-cycler, menadione (Mn), on C2C12 myotubes and the involvement of NOS in Mn-induced oxidative stress using a general and an NOS-specific inhibitor, L-NAME (3 mM) and ETPI (100 μM), respectively. Quantitative real time PCR of myotubes treated with Mn (10 μM) for 24 hours showed slightly increased nNOS expression (~25%), which increased to >2.3-fold with NO inhibitors. Such behavior may indicate that the cells compensate for decreased bioavailable NO with an increase in nNOS expression. Immunohistochemical analyses with Mn +/- NOS inhibitor treatment demonstrate increased nNOS protein expression when compared to myotubes, untreated or treated with NOS inhibitor alone. The expression of cavelin 3 (Cav3), a protein shown both to be necessary for myotube formation and to cause inhibition of NO synthesis by nNOS, was significantly decreased (73%) with Mn treatment. In the presence of ETPI, Cav3 is dramatically decreased (82%), indicating that myotubes may actively down-regulate NOS-inhibitory proteins to increase bioavailable NO. Additionally, Akt1, NFKB1 and INOS were also decreased by 40-50%. With Mn, GPX1 expression was decreased significantly but SOD1 expression was not affected. With NO inhibitors, GPX1 levels were restored whereas SOD1 levels increased more than 2-fold. Further studies are being performed exploring the mechanism involving Mn-induced oxidative/nitrosative stress (Supported by NIH GM052419 to SSM and LJRR).

PS

1186 DETERMINATION OF NITRITE USING A MODIFIED FERROUS OXIDATION-XYLENOL ORANGE (FOX) ASSAY: AN INTERFERENCE TURNED INTO A USEFUL METHODOLOGY.

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The Fe²⁺-oxidation-xylanol orange (XO) method of determination of hydroperoxides (ROOH, where R = H, alkyl or aryl moiety), popularly referred to as the FOX assay, is known for its robustness, sensitivity, and adaptability for high throughput screening. The assay is based on the principle that under moderately acidic conditions (pH=1-2) where there is negligible autoxidation of Fe²⁺, there is stoichiometric oxidation of Fe²⁺ to Fe³⁺ by ROOH and the Fe³⁺ so formed can be measured photometrically at 560 nm following complex formation with XO. A number of variations have been sought in the past to expand the application of this basic methodology to ROOH coming from oxidation of lipids, proteins, and nucleic acids. During the course of customizing the FOX assay, we noticed that NO₂⁻ present as an inadvertent contaminant in peroxynitrite (PN) interferes with the determination of ROOH. The NO₂⁻ interference could be abolished when ammonium sulfamate (AS), a chemical scrubber that removes HNO₃, or its putative NO²-carrier ([H₂NO₃]⁺, was added prior to the reaction of Fe³⁺ with ROOH. While the AS pretreatment increased the yields of Fe³⁺-XO complex, it was realized that the FOX assay with some modifications could be used to determine NO₂⁻ with detection limits that are similar to those reported for typical ROOH. The formation of Fe³⁺-XO complex was linear over the NO₂⁻ concentration range of 1 to 20 μM and the addition of AS completely abolished the increase in A₅₆₀ nm at all concentrations of NO₂⁻. These results indicate the specificity of the FOX assay for NO₂⁻ through use of AS-treated controls. These findings are important and timely in view of an ever increasing interest in the physiology of NO₂⁻ and numerous pathological states (urinary tract infections and pediatric acute kidney injury) wherein simultaneous determination of NO₂⁻ and H₂O₂ is indicated. [Support from NSF (HRD1043316 and HRD 0847742) and the US Department of Education (P031B040030) is acknowledged].

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1187 ACETAMINOPHEN INDUCES THE TRANSCRIPTION OF THE ANTIOXIDANT PROTEINS THIOREDOXIN 1 (TRX-1) AND GLUTAREDOXIN 1 (GRX-1) IN THE BRAIN AND LIVER OF BALB/C MICE.

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Tissues and organs express different levels of antioxidants, such as glutathione (GSH). Exposure to environmental toxins and/or pharmaceutical drugs induces differential organ-specific responses and/or damage. Acetaminophen (APAP) is an analgesic and antipyretic drug that can cause severe liver damage when high doses are administered. The damage is caused by the depletion of intracellular GSH and an excess of N-acetyl-p-benzoquinone imine (NAPQI) metabolite that reacts with cellular and mitochondrial proteins producing cell damage and tissue necrosis by mechanisms involving oxidative stress. In this study, we analyzed the response to an intraperitoneal (i.p.) injection (300 mg/kg) of APAP at 1, 2 and 3 h after administration in the livers, brains, and kidneys of BALB/c male mice. We observed a significant decrease in the levels of GSH in the livers and kidneys, and an increase in the transcription of Nrf2 and Grx-1 in the liver. In the brain, we found an increase in the transcription of Grx-1 and Trx-1 associated with NP-kB nuclear migration. In the kidney, the up-regulation of these antioxidant proteins was not observed, which coincides with the significant increase in lipid peroxidation.

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1188 COMPARISON OF N-ACETYLCYSTEINE, S-METHYL-CYSTEINE AND AMINOMETHANESULFONIC ACID FOR THE ABILITY TO ATTENUATE ACETAMINOPHEN-INDUCED CHANGES IN PLASMA INDICES OF OXIDATIVE STRESS IN THE RAT.

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Three sulfur-containing compounds representing an analog of cysteine (N-acetyl-cysteine = NAC, S-methylcysteine = SMC) or homolog of taurine (aminomethanesulfonic acid = AMSA) were compared for the ability to attenuate changes in plasma indices of oxidative stress induced by acetaminophen (APAP) in a rat model. Sprague-Dawley rats (225-250 g), assigned to groups of n = 6, were treated with a single, 800 mg/kg, i.p. dose of APAP. The treatment compounds (2.4 mmol/kg, i.p.) were administered 30 min before APAP. At 6 h after APAP administration, the rats were sacrificed by decapitation and their blood collected over sodium heparin. Control groups received only physiological saline (PHS, 2 mL, i.p.) or only APAP. The plasma fractions were assayed for malondialdehyde (MDA), lipid oxidation (NO₂, reduced (GSH) and oxidized (GSSG) glutathione contents, and for antioxidant enzymes (catalase, glutathione peroxidase, superoxide dismutase), glutathione reductase and glutathione S-transferase activities. APAP elevated the plasma levels of MDA and NO₂, reduced those of GSH, and GSSG and inhibited the activities of antioxidant and GSH-related enzymes to a significant extent (all changes by ≥±3%, p<0.001) compared to corresponding values for rats receiving only physiological saline. Without exceptions, these alterations were attenuated by a sulfur-containing treatment compound, with the order of potency usually decreasing in the order NAC>SMC>AMSA. These results suggest that small molecular weight sulfur-containing compounds (1-3 carbon long) containing a sulfonate or a sulfonic acid group (AMSA) or the cysteine structure plus a S-acyl (SMC) or N-acetyl (NAC) substituent can offer different degrees of protection against oxidative
1189 ALL-TRANSRETINOIC ACID AFFORDS CYTOPROTECTION AGAINST REACTIVE OXYGEN SPECIES-INDUCED RENAL INJURY.

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Ischemia reperfusion injury (IRI) and chemical-induced nephrotoxicity are major etiologic factors of acute kidney injury during which reactive oxygen species are released. 11-Deoxy-16,16-dimethyl-prostaglandin E2 (DMD-PGE2) protects against 2,3,5-tris(glutathione-5)-phylloquinone (TGHQ) induced ROS-dependent cell death in LLC-PK1 cells. Immuno blotting and proteomics analyses revealed that DMD-PGE2 cytoprotection was associated with a time-dependent increase in retinol binding protein (RBP) synthesis, suggesting that retinoid signaling is engaged during this process. Pharmacological manipulations that abolished the ability of DMD-PGE2 to induce RBP abrogated its cytoprotective effects, further indicating that RBP is necessary for DMD-PGE2 mediated cytoprotection. While both all-trans-retinoic acid (ATRA) and 9-cis-retinoic acid (9-cisRA) possess biological activity, pretreatment with ATRA, but not 9-cisRA, afforded cytoprotection in LLC-PK1 cells, following TGHQ treatment. Moreover, the cytoprotective kinetics of ATRA and DMD-PGE2, were identical, with maximal RBP induction and cytoprotection occurring at 12 and 24 hours, respectively. Chemical hypoxia was established in LLC-PK1 cells to recapitulate IRI conditions by exposing cells to 0.1 or 1 μM antimycin A for 1 hr followed by glucose-free media. Consistent with the effects of ATRA on TGHQ-induced cytopotoxicity, ATRA pretreatment completely protected hypoxic cells from cytoxicity as assessed by a mitochondrial dehydrogenase enzyme activity assay (MTT). Moreover, ATRA significantly reduced 8-oxo-deoxyguanosine levels in human kidney HK2 cells after TGHQ challenge. Collectively, these data reveal that ATRA protects renal cell injury, at least in part, via suppression of ROS-mediated oxidative damage. Therefore, ATRA may provide an effective therapeutic strategy in chemical-induced renal injury or pathological conditions where ROS contribute to the disease progression. (ES006694, ES016578).

1190 ALIPHATIC ALCOHOLS MAY PRODUCE NEPHROTOXICITY VIA OXIDATIVE STRESS(OS) AND INFLUENCE SIRT1 AND FOXO3A EXPRESSION IN VIVO.

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Most tissues survive in hostile environments by activating stress-responsive transcriptional regulators that coordinately increase production of protective factors. Sirt1 is a key player in this pathway which dictates the regulation of important intracellular proteins such as, p53 and FOXO3A; FOXO3A inturn regulates antioxidant enzymes, such as, catalase & MnSOD. This study explored whether a series of aliphatic alcohols (methanol, ethanol, isopropanol & t-Butanol) produce OS and influence key members of the Sirt1 pathway (FOXO3A, p53, AROS & HIC1). To explore the underlying mechanisms, LD40 doses (25% soln in H2O) of these alcohols were administered orally via gavage to mice. Sirt1, p38 and ERK1/2 inactivation was observed following Cr6+ exposure. Western blot analysis revealed Butanol decreased Sirt1 expression, whereas it increased p38 phosphorylation and ERK1/2 activation, as indicated by ERK1/2 phosphorylation. Cr6+ induced ROS generation since 5 min until 1 h, and at 6 h ROS level was as a control. ROS were induced in the cells simultaneously treated with alpha-tocopherol only at 5 and 10 min, and notably in presence of alpha-tocopherol ERK1/2 was not activated. Our results showed a clear relationship between the induction of ROS and the activation of ERK1/2 in NRK-52E cells; these effects was associated with the internalization of occludin and changes in the Dextran-FITC flux.

1191 ROLE OF OXIDATIVE STRESS AND MAPK SIGNALING PATHWAY IN OCCLUDIN SUBCELLULAR DISTRIBUTION IN THE NRK-52E CELLS.

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Dicluroracetate (DCA) and trichlororacetate(TCA) are byproducts formed during the process of drinking water chlorination and were previously found to induce phagocytic activation and oxidative stress in the hepatic tissues of B6C3F1 male

1192 METABOLISM-LINKED MACROPHAGE ERYTHROCYTE COCULTURE ASSAY TO PREDICT THE HEMOLYTIC POTENTIAL OF 8-AMINOQUINOLINE ANTIMALARIALS IN G6PD-DEFICIENCY.

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Existing preclinical toxicology procedures have been poorly predictive of the hemolytic potential of 8-aminoquinoline antimalarial drug candidates that are subsequently found to cause hemolytic anemia in patients with hereditary deficiencies of erythrocytic glucose-6-phosphate dehydrogenase (G6PD). A major limitation of existing in vitro approaches to predict hemolytic potential is that they do not reproduce the in vivo response, namely premature removal of oxidatively-damaged erythrocytes from the circulation by macrophages in the spleen. We hypothesize that an ex vivo human G6PD-deficient erythrocyte-macrophage co-culture system can overcome this limitation and provide a screening tool to support 8-aminoquinoline antimalarial drug development. To test this approach, we incubated human normal and G6PD-deficient erythrocytes ± pooled human liver microsomes (HLM) with primaquine (500 μM) for 1 hr at 37°C. After the incubation the erythrocytes were collected and co-incubated with J774A.1 murine macrophages for 24-48 hr to determine phagocytosis. Phagocytic uptake was assessed by 51Cr/PK4H6 labeling of erythrocytes. Uptake of erythrocytes exposed to primaquine in the absence of HLM was not significantly different from uptake of controls. In the presence of HLM, primaquine induced a significant increase in phagocytosis of normal erythrocytes, and this response was enhanced significantly in G6PD-deficient erythrocytes. These results suggest that a metabolism-linked human erythrocyte-murine macrophage co-culture system can serve as a valid model for assessing parent drug effects on human G6PD-deficient erythrocytes. Supported by UMiss subcontract (USAMRMC W81XWH-07-2-0095).

1193 INDUCTION OF PHAGOCYTIC ACTIVATION BY MIXTURES OF DICHLOROACETATE AND TRICHLOROACETATE IN MICE AFTER SUBCHRONIC EXPOSURE.

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Dichloroacetate (DCA) and trichloroacetate(TCA) are byproducts formed during the process of drinking water chlorination and were previously found to induce phagocytic activation and oxidative stress in the hepatic tissues of B6C3F1 male
mice after subacute and subchronic exposure. In order to determine the effects of mixtures of DCA and TCA on phagocytic activity, groups of male Balb/c mice were treated daily, by gavage, with 3 different mixtures (Mix I, Mix II and Mix III) of the compounds for 13 weeks. The concentration of each compound in Mix I, II and III corresponded respectively to that producing 15%, 25% and 35% of maximal induction of various biomarkers of phagocytic activation, including superoxide anion (SA) and tumor necrosis factor (TNF)-alpha production and myeloperoxidase (MPO) activity in the subchronic studies. The mice were euthanized at the end of the treatment period and peritoneal lavage cells were collected and assayed for the levels of SA and TNF-alpha, as well as for MPO activity. While the effects on all of the biomarkers in response to Mix I and II were additive, they were less than additive in response to Mix III. DCA- and TCA-induced phagocytic activation by the compounds was previously suggested as an adaptive response to protect against their long term hepatotoxicity, hence the resultant effects may be viewed as adaptive/protective in response to Mix I and II, and as a protection failure in response to Mix III. (Supported by NIH/NIEHS grant # R15ES013706-01A2)

1194 AGE-RELATED AND CIRCADIAN CHANGES IN THE REDOX CYCLE IN MALE SPRAGUE DAWLEY RATS.

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It is apparent that a major protective role against the reactive chemical intermediates, which are generated by bireduction and cause oxidative stress by redox cycling, is provided by the ubiquitous glutathione (GSH) redox cycle. In this study we have investigated the age related and circadian fluctuations in the levels of GSH and those of lipid peroxides in normal male Sprague Dawley rats. Normal rats (~200 g) were divided into different groups of 4-5 animals each. For circadian changes the groups animals were killed at 4 hours intervals within a 24 hour cycle. Blood and the organs studied exhibited distinct circadian variation both in GSH concentrations and peroxidation of polyunsaturated fatty acids. There was a great variation among organs in the periodicity and amplitude of the fluctuations in GSH concentrations. Liver displayed the highest variation (50%) followed by stomach (~37%), heart (~25%) and kidney (~19%). The changes in other organs were significant but of less magnitude. For age-related changes various tissues of animals of different age groups (1-36 months) were used. At all ages the GSH content in the liver was 3-10 times higher that of other tissues. In the oldest group (36 months) of rats the GSH content of all tissues studied were lower (35-60%) than that in 2.5 month old rats. The lipid peroxide levels increased by age in all tissues studied. Implications of such variations and caution in interpretation of experimental results in response to the exposure of animals to xenobiotics are significant. The studies also indicate that general characteristics of aging tissue may include a decrease in GSH content and increase in lipid peroxidation showing a decrease in redox cycling in senescence.

1195 GELSOIN’S FUNCTIONS IN HEAVY ACUTE RADIATION INJURY MOUSE AND CELL MODELS.

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Background Many factors involved in pathological process of heavy acute radiation injuries remain to be revealed to reduce mortality during treatment. Actin is suspected releasing to tissue gap and blood pool from damaged tissues and cells during radiation damage and would result in a secondary pathologic effect including dynamic change of blood flow, blood vessel wall damage and other cytotoxicity. Gelsolin as one of main actin scavenging proteins had been found with antiinfection and antioxidation functions. Understanding potential function of gelsolin in radiation damage might be helpful to find new way to reduce pathological damage and improve survival rate of heavy acute radiation syndrome. Methods BALB/c mice whole body and cultured human intestinal epithelial cells (HEC) in vitro were exposed to a 137Cs γ-ray source with different doses and a fixed dose rate. The changes of plasma GSN (pgGSN) level in peripheral blood of the mice and cytoplasmic (cGSN) level in cultured cell line were respectively determined. A recombinant pgGSN was injected into irradiated mice body through tail vein after irradiation and prothrombin time (PT), activated partial thromboplastin time (APTT), glutathione (GSH) and malonaldehyde (MDA) indexes which could respectively indicate coagulation and oxidative damage in tissue were analyzed at different times post radiation and pgGSN given. Results Radiation induced pgGSN level decrease in peripheral blood of the mice and cGSN level increase in cultured HEC with a dose-dependent manner. Giving of recombinant pgGSN to radiated mice after radiation, bleeding effect ameliorated as indicated by PT and APTT and antioxidative ability increased as indicated by GSH and MDA indexes. Conclusions GSNs might have involved in pathologic proceeding of acute radiation damage and recombinant pgGSN supplement to the heavy injured mice might act some protective functions by improving coagulant and antioxidative mechanisms.

1196 INCREASED OXIDATIVE STRESS STATUS IN NEURONAL CELLS EXPOSED TO XENOESTROGEN BISPHENOL A.

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Bisphenol A (BPA) is a semi-persistent organic pollutant in industrial and urban environments. Epidemiological studies have shown that urinary levels of BPA are positively associated with higher incidence of cardiovascular disease, diabetes, and abnormal concentrations of liver enzymes in non-institutionalized US populations and increased expression of oxidative stress markers in postmenopausal Korean women. Male albino rats exposed to low levels of BPA were shown to have decreased antioxidant enzymes. To understand the significance of oxidative stress in BPA-induced toxicity, in the present study, we examined formation of intracellular peroxides and superoxide anion (O2·−) in murine GT-17 hypothalamic neurons exposed to low levels of BPA. It was found that GT-17 neurons exposed to BPA (25-100 μM) have increased levels of intracellular H2O2 or other peroxides (DCF fluorescence) when compared to control, vehicle-only exposed cells. The production of peroxides in BPA exposed neurons was comparable to that observed in cells exposed to menadione (25 μM; positive control) and was found to be linear for periods up to 8 h. When co-incubated with N-acetyl-L-cysteine (NAC; 5 mM), in both BPA- and menadione-exposed cells, there was little or no increase in the generation of peroxides. In contrast, exposure to 1,4-Diamino-2-butanone (DAB), a putrescine analogue, is highly toxic to various cell lines and peroxides in BPA exposed neurons was comparable to that observed in cells exposed to menadione (25 μM; positive control) and was found to be linear for periods up to 4 h. Co-incubation with Trol ox (0.5 mM) also markedly decreased the production of O2·− in these neuronal cells. Taken together with results of our previous studies wherein it was shown that BPA phenolate radical has strong pro-oxidant properties, these results suggest that BPA induces the cellular oxidative stress presumably by promoting oxidation of cellular redox carriers and increased production of intracellular O2·− and peroxides.

1197 PRO-OXIDANT ACTIVITY AND CYTOTOXICITY OF 1, 4-DIAMINO-2-BUTANONE TO MAMMALIAN CELLS AND TRYPANOSOMA CRUZI.

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1,4-Diamino-2-butanone (DAB), a putrescine analogue, is highly toxic to various microorganisms including Trypanosoma cruzi. However, little is known about the molecular mechanisms underlying its cytotoxic properties. Here we show that DAB undergoes aerobic oxidation in phosphate buffer, pH 7.4, catalyzed by Fe(II) and Cu(II) ions yielding NH4+ ion, H2O2 and 4-amino-2-oxobutanal. Propagation of DAB oxidation by superoxide radical was confirmed by the inhibitory effect of added SOD and stimulatory effect of xanthine/xanthine oxidase. EPR spin trapping studies with 5,5-dimethyl-1-pyrroline-1-oxide (DMPO), revealed the intermediary of an adduct attributable to DMPO-HO· radical, and with α-(4-pyridyl-1-oxide)-N-tert-butylnitrone (POBN), a 6-line adduct assignable to POBN-DAB radical. DAB (0.05 - 10 mM) showed to be more toxic to RKO (human colon carcinoma) cultured cells (IC50 ~0.3 mM DAB, 24 h incubation) than to LLC-MK2 cells (IC50 ~ 1.5 mM DAB, 24 h incubation), derived from Rhesus kidney epithelium. Changes in redox balance and stress response pathways were induced by increased expression of Nrf-2, HO-1, NQO1 and xCT proteins. Caspase 3 and PARP cleavage products suggested DAB-triggered apoptosis in RKO cells. Similarly, DAB (0.05 - 5 mM) treatment of trypomastigotes, the infective stage of T. cruzi, led to a decrease of cell viability (IC50~0.2 mM DAB, 4 h incubation), and thiol redox imbalance dependent on the DAB concentration, parallel to an increase in T. cruzi SOD activity. Accordingly, host cell invasion by trypomastigotes was also hampered by DAB administration. Altogether, these data support the hypothesis that DAB exhibits a toxicological profile comparable to the current large spectrum of microorganisms, including T. cruzi. Support: FAPESP, CNPq, INCT Redoxoma, and NIEHS 5P01ES013125.
**1198 SULFUR DIOXIDE PROMOTES A PRO-OXIDANT SHIFT IN IL-10-DEFICIENT MICE WITH AIRWAY INFLAMMATION.**

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**RATIONALE:** Inhaled sulfur dioxide (SO2) at low concentrations (1-5 ppm) can produce bronchospasms in asthmatics, linked to increased oxidative stress, airway inflammation (AI), and airway hyperresponsiveness, potentially because they are deficient in IL-10 production. We tested this hypothesis in IL-10-deficient C57Bl6 (C57), and IL-10 knockout (+/-) mice, with experimentally-induced AI. METHODS: AI was induced by sensitization and challenge with ovalbumin (OVA), +/- inhaled SO2 (either during sensitization or post-sensitization (PS) with simultaneous OVA challenge, 1 ppm for 6 hr/day over 10 days). Bronchoalveolar lavage fluid (BALF) was analyzed for leukocyte counts; a pro-oxidant/anti-oxidant balance (PAB) assay provided an index of oxidative stress, and heme oxygenase-1 (HO-1) in lung homogenates was measured by ELISA, as a stress indicator. RESULTS: BAL macrophages remained unchanged across OVA and SO2 treatments, except for IL-10+/- mice, in which OVA + SO2 increased maces by 35% (P<0.05). As expected, BAL eosinophils (eos) were significantly increased by OVA challenge, but with OVA + SO2, eos were decreased in C57 mice (40%; P<0.05), and increased nearly 3-fold in IL-10/- mice (P<0.05). In IL-10/- mice, HO-1 expression was positively correlated with eos (Rsq=0.72; P<0.01), but not in C57 mice. OVA-challenge did not alter the PAB in C57 mice, and remained unchanged with SO2 during sensitization, but increased nearly 2-fold toward pro-oxidative, with SO2 + OVA PS (P<0.05). In the absence of IL-10, this balance was further shifted toward pro-oxidative, by another 14% (P<0.05). CONCLUSIONS: The results indicate that IL-10 is a critical factor in the eosinophilic and oxidative stress responses of the airway to SO2 inhalation, and its lack may be why asthmatics experience detrimental effects at low SO2 concentrations.

**1199 SELENOPROTEIN P REGULATES PCB3 METABOLITE-INDUCED CYTOTOXICITY IN HUMAN KERATINOCYTES.**


We previously showed 2-(4-chlorophenyl)-benzo-1.4-quinone (4-ClBQ), a metabolite of PCB3, forms a semiquinone radical, which in presence of oxygen generates superoxide leading to oxidative stress-induced DNA damage, inhibition in proliferation, and enhanced toxicity. This study was designed to investigate whether specific oxidative stress responsive genes regulate cellular responses to 4-ClBQ treatments. Microscopic observations showed significant change in the cytoskeleton of 4-ClBQ (1-3 μM, 1-5 days) treated human immortal keratinocytes, which was accompanied with a decrease in proliferation and an increase in cytotoxicity. 4-ClBQ treatments exhibited a G2-delay. Flow cytometry measurements of MitoSOX and MitoTracker oxidation demonstrated elevated levels of mitochondria (e.g. CuZnSOD, EcSOD, GPx-3, and DUSP1) in 4-ClBQ treated cells. Twenty-four hours after 2-hour exposure to 4-ClBQ, the expression of CuZnSOD, EcSOD, GPx-3, and DUSP1 was significantly decreased in all the down-regulated genes. SEPP1 is a major Se-protein that is known to reduce phospholipid hydroperoxide as well as transport Se. Sodium-selenite pre-treatment among all the down-regulated genes. Results suggest that 4-ClBQ-induced decreases in GPx1 mRNA in spite of up-regulation of other antioxidant enzyme mRNAs. This change was also observed in soleus skeletal muscles of rats treated with MeHg. MeHg-induced decrease in GPx1 mRNA was not suppressed by the co-addition with antioxidant Trolox but by the pretreatment with sodium selenite, suggesting that the decrease in GPx1 mRNA was due to MeHg-induced relative intracellular selenium deficiency. This notion was supported by the inhibition study of nonsense-mediated mRNA decay (NMD), which recognizes a UGA codon for selenoproteins on GPx1 as a nonsense codon and degrades GPx1 mRNA in selenium-deficient condition. In contrast, thioredoxin reductase 1 (TrxR1), another antioxidant selenoprotein, was likely skipped by NMD because of a UGA codon in the last exon. However, TrxR1 activity was decreased despite mRNA up-regulation, which was probably due to the synthesis of aberrant TrxR1 protein without selenocysteine. Changes in selenoproteins GPx1 and TrxR1 mRNAs were observed earlier than was the incidence of oxidative stress and upregulation of other antioxidant enzyme mRNAs. Results indicated that the MeHg-induced relative selenium deficiency condition affects the major antioxidant selenoproteins GPx1 and TrxR1 through a posttranscriptional effect, resulting in the disturbance of cellular redox systems and the incidence of oxidative stress.

**1200 POSTTRANSCRIPTIONAL DEFECTS OF ANTIOXIDANT SELENOENZYMES CAUSE OXIDATIVE STRESS UNDER METHYLMERCURY EXPOSURE.**

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The critical role of oxidative stress in the pathogenesis of methylmercury (MeHg) cytotoxicity has been clarified, but the molecular mechanisms underlying MeHg-mediated oxidative stress remain to be elucidated. Here we demonstrate a posttranscriptional effect of MeHg on antioxidant selenoenzymes by using a myogenic model cell line showing apoptosis by exposure to MeHg. Quantitative real-time PCR analysis showed down-regulation of the major antioxidant selenoenzyme, glutathione peroxidase 1 (GPx1) mRNA in spite of up-regulation of other antioxidant enzyme mRNAs. This change was also observed in soleus skeletal muscles of rats treated with MeHg. MeHg-induced decrease in GPx1 mRNA was not suppressed by the co-addition with antioxidant Trolox but by the pretreatment with sodium selenite, suggesting that the decrease in GPx1 mRNA was due to MeHg-induced relative intracellular selenium deficiency. This notion was supported by the inhibition study of nonsense-mediated mRNA decay (NMD), which recognizes a UGA codon for selenoproteins on GPx1 as a nonsense codon and degrades GPx1 mRNA in selenium-deficient condition. In contrast, thioredoxin reductase 1 (TrxR1), another antioxidant selenoprotein, was likely skipped by NMD because of a UGA codon in the last exon. However, TrxR1 activity was decreased despite mRNA up-regulation, which was probably due to the synthesis of aberrant TrxR1 protein without selenocysteine. Changes in selenoproteins GPx1 and TrxR1 mRNAs were observed earlier than was the incidence of oxidative stress and upregulation of other antioxidant enzyme mRNAs. Results indicated that the MeHg-induced relative selenium deficiency condition affects the major antioxidant selenoproteins GPx1 and TrxR1 through a posttranscriptional effect, resulting in the disturbance of cellular redox systems and the incidence of oxidative stress.

**1201 TIME-DEPENDENT EFFECTS OF URANIUM ON CELLULAR OXIDATIVE STRESS MARKERS.**


Oxidative stress is a common mechanism of many heavy metals but is poorly described concerning a particular one, Uranium (U). This mechanism is regulated by transcription factors, in particular Nrf2, which induces transcription of many antioxidant enzymes namely catalase (CAT), Copper, Zinc Superoxide Dismutase (Cu, Zn SOD) or Heme Oxygenase 1. The aim of this work was to study how U induces a cellular disturbance of pro/antioxidative equilibrium and cell death at different times (1 to 24 hours) and different concentrations (10 to 1000 μM) on a Human liver hepatocellular carcinoma cell line (HepG2). Antioxidative enzymes, Nr2, caspases and cell death were evaluated at mRNA and activities levels. After 24 hours of exposure with 500 μM of U, cell death was enhanced 10 folds (p<0.001). Furthermore, activity of caspases 3 and 7 was increased 4 folds (p<0.05), suggesting a cell death by apoptosis pathway. No cell death or caspase activation were observed at lower range.

Gene expression of CAT showed first at 50 μM of U an increase (4 folds, p<0.05) after one hour, whereas a decrease after 2 (45% as compared to control, p<0.01) and 4 hours of exposure (-76% as compared to control, p<0.01) was observed. The study of another antioxidant enzymes Cu, Zn SOD underlined the same variations (p<0.05) as CAT for one and 2 hours. Accordingly, gene expression of Nr2 was similarly modified at 50 μM of U, an increase of 5 folds after 1 hour and a decrease of 80% after 2 hours of contamination (p<0.05) and 90% after 4 hours (p=0.01).

Regarding these data, contamination of U seems to induce Nr2 and antioxidant enzymes at low concentrations without leading to caspase activation. For higher U concentrations, activation of caspases and death cell were observed. Kinetics results suggest that adaptive mechanisms of antioxidative system could be set up at low concentration.

**1202 INVESTIGATION OF NOVEL ROLES FOR HEME OXYGENASE IN RESISTANCE TO CADMIUM-INDUCED OXIDATIVE STRESS.**

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Cadmium (Cd) is a toxic metal capable of causing severe oxidative stress. Cd-resistant cells, when exposed to a variety of chemicals capable of inducing oxidative stress, exhibit significantly lower occurrences of apoptosis compared to non-resist-
ant cells, suggesting that Cd-resistance is at least in part an adaptation to oxidative stress. To begin investigating the role of hiruligin-hirulin recycling in adaption to Cd-induced oxidative stress, we exposed the human HepG2 Tet-On (Clontech Laboratories, Inc) cell line continuously during culture to 1 or 5 μM of Cd for a period of 12 weeks. Several papers routinely dosed HepG2 cells with Cd concentrations ranging from 5 to 120 μM and have proposed a 24 h LC50 of ~24μM (Shimoda et al 2001, Tox Sci) and an IC50 of ~14 μM as determined by the MTT assay (Lawal and Ellis, 2010, J. Toxicol Sci). Based on these studies, we dosed the control and Cd-adapted HepG2 cell lines with 1, 5, 10 and 25 μM of Cd for 4 hours before measuring gene expression. qPCR was used to determine the expression level of metallothionein (MT-1b) and heme oxygenase 1 (HO-1), the rate limiting enzyme in the catabolism of heme to bilirubin, in response to the additional Cd treatments. Interestingly, although overall expression of MT-1b and HO-1 was significantly increased in both the 1 μM and 5 μM HepG2 cell lines at all dose compared to control HepG2 cells, all three cell lines had an inverted U-shaped dose response curve with maximum induction of gene expression at 1 μM and suppression at the higher concentrations. A similar dose-response curve was assessed using a regular HepG2 cell line and the same U-shaped dose response curve was obtained. New dose-response experiments with the control, 1 μM and 5 μM HepG2 Tet-On cell lines were performed with Cd concentrations ranging from 20 nM to 1 μM and yielded classic dose-response curves. These data suggest that HepG2 cells may be even more sensitive to Cd toxicity than previously determined. Current efforts are underway to create a HO-1 stable transfected cell line to assess the role of HO-1 in Cd adaptation. [R00E50170404]

1203 THE BODY WEIGHTS AND FLUORIDE CONCENTRATIONS IN THE URINE OF ICGN MICE AND ICR MICE AFTER SUBACUTE ADMINISTRATION VIA DRINKING WATER.

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The contaminations of ground water by fluoride (F) have been reported in China and India. Many inhabitants who drink well water with high concentrations of F are suffering from endemic fluorosis. F is excluded from the kidney. Greater accumulation of F occurs in ICR-derived glomerulonephritis mice (ICGN) which have impaired renal function and affects them more seriously in our previous study. The xenobiotics of F in animals with impaired kidney function is not elucidated. The F concentrations in the urines of ICGN and ICR mice were determined in this study. F was administered to ICGN mice at 0, 50, 100 and 150 ppm and ICR mice at 0, 100 and 150 ppm in drinking water for four weeks. When a mouse died, the data of the day of death or nearest to the day of death was assigned. The body weight of each mouse were monitored daily. The urine of each mouse was sampled once a week by using a metabolic cage. F in the urine were determined by a flow injection apparatus with a F-selective electrode as the detector. All ICGN mice exposed to 100 and 150 ppm died during the observation period. The mean body weight in the 100 and 150 ppm ICGN mice was significantly lower than those in the 0 and 50 ppm. For each ICGN group, there were no significant differences in the urine F concentration in the between the beginning and the end of the observation. For ICR mice, no mice died, and the mean body weight were not significantly different among the groups. The mean urine F concentration at the end was significantly higher than that at the beginning in the 150 ppm group. F in the drinking water at 100 ppm and more was lethal for the ICGN mice. Fluoride in the urine at the end was higher compared to the beginning in ICR mice but not in the ICGN mice, suggesting the impairment of the exception of F in the ICGN mice.

1204 INCREASED KIDNEY TUBULAR DAMAGE MARKERS IN RATS EXPOSED SUBCHRONICALLY TO FLUORIDE.

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Fluoride (F−) is a frequent contaminant in drinking water. Some in vivo and in vitro studies employing tissue samples are employed to assess the early injury caused by toxic agents. The aim of this study was to determine the kidney injury induced by the F− exposure at environmental relevant concentrations. Male rats recently weaned, were exposed through drinking water at two concentrations of F− (15 and 50 mg/L) for 40 days. At the end of the study, blood and urine samples were collected, and urinary injury biomarkers (β-2-Microglobulin, clusterin, Kim-1, cystatin C, osteopontin) were measured using Lumexin xMAP technology. Mean urine F− levels were 0.88, 5.68 and 18.17 μg/g creatinine for the control and exposed low and high F− groups, respectively. The rats exposed to F− showed an increase of the median Kim-1 excretion in urine 2.39 (0.63-5.29), 3.39 (2.35-7.83) and 4.86 (0.66-14.83) ng/24h for groups exposed to 0, 15 and 50 μg/L, respectively. Moreover, the urine level of clusterin was also increased as follows: 192.6 (111.8-304.6), 238.6 (161.1-368.3) and 269.6 (206.6-632.1) ng/24h for groups exposed to 0, 15 and 50 μg/L, respectively. These results show for the first time that F− exposure at environmentally relevant concentrations is able to increase some novel especially sensitive and specific markers of tubular injury, suggesting that F− could be a risk factor in the promotion or progression of renal diseases and have likewise an enhanced sensitivity to nephrotoxins like some antibiotics. Supported by Conacyt 152416.

1205 IDENTIFICATION OF PRENATAL KIDNEY INJURY BY THE DETECTION OF EARLY BIOMARKERS IN AMNIOTIC FLUID.

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Over recent years, nephropathies have been rising in infants and newborns in Mexico, and worldwide. Prenatal exposure to cadmium (Cd2+)−, a nephrotoxic agent ubiquitous in the environment, could be related to some of these cases as it has been demonstrated that it can be freely diffused through placenta and accumulate in the kidney. Amniotic fluid, mainly formed of fetal urine, contains proteins whose concentrations change depending on the developmental stage of the fetal kidney. Some of these proteins could be used as biomarkers of congenital renal pathologies. However, amniotic fluid as a potential matrix to diagnose kidney injury has yet not been considered. Pregnant Wistar rats were exposed to Cd2+ (gavage, 0.6 mg/kg/day) and gentamicin (subcutaneous injection, 75 mg/kg/day), both well-known nephrotoxins, during gestational days (GD) 8-20. On GD20, rats were sacrificed and samples of the fetuses’ plasma and amniotic fluid were obtained. Creatinine was quantified using Jaffe’s reaction. Cd2+ and gentamicin treatments significantly raised plasma creatinimic levels in fetuses (0.76±0.03 vs. 0.92±0.05 respectively, vs. 0.62±0.02 mg/dL, P<0.001 (mean±S.E.). However, no difference was observed in amniotic fluid. Cystatin C, β2-microglobulin (β2-MG) and Kidney Injury Molecule-1 (Kim-1) levels on amniotic fluid were determined with kits based on immunofluorescence. We observed a decrease of β2-MG levels in the dams exposed to gentamicin, yet it was not statistically significant. Cystatin C levels remained unchanged whereas Kim-1 showed a non-statistically significant rise on its levels when the dams were exposed to Cd2+, but gentamicin induced a significant increase (22.28±4.73 vs. 6.29±1.74 pg/mL, P<0.01). These results suggest that Cd2+ is possibly affecting the glomeruli and, to a lesser extent, the proximal tubule whereas gentamicin seems to be damaging both glomeruli and proximal tubules in fetuses. The rise of Kim-1 levels on amniotic fluid points out its potential use to diagnose fetal kidney injury on proximal tubule before birth.

1206 HYALINE DROPLET NEPHRAPHONY INCREASED DIFFERENT RENAL BIOMARKERS DEPENDING ON COMPOUND AND TIME.

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Hyaline droplet nephropathy (HDN) is a male rat specific lesion induced when a compound or metabolite binds to alpha-2-microglobulin. The objective of this project was to investigate the impact of HDN on a set of renal biomarkers (BM) approved for inclusion in male rat toxicity studies. Rats were dosed (po) 7 days to 1) determine if HDN altered renal BMs using vehicle, and 3 HDN positive agents: 1000 mg/kg/day 2-propanol, 100 mg/kg/day potassium bromate or 300 mg/kg/day d-limonene (dL) with necropsy on day 8 (n = 5/group), 2) determine sensitivity of HDN BMs to different induction protocols, necropsy on day 8 (n = 5/group), 3) determine reversibility of HDN and renal BMs using vehicle or 300 mg/kg/day dL with necropsy on day 8, 15, 29, 57 and 85 (n = 6/group). Rats treated with 2-propanol had HDN = 1.7 (average severity score using 1-4 scale) without increased renal BM levels. Potassium bromate treated rats had HDN = 3, and possibly increased clusterin (54%), ΔT 300 mg/kg/day treated rats had HDN = 4.0 and increased GSTβ1 (759%), GSTβ1 (182%) and albumin (539%). All three compounds induced HDN with different BM patterns. DL induced a dose dependent increase in HDN
Drug-induced kidney injury (DIKI) is a serious adverse drug reaction that causes attenuation in drug development (Bedfors et al., 2010). New biomarkers may enable early detection of DIKI in non-rodent models. Our aim was to determine if a panel of human DIKI biomarkers (Rules Based Medicine) can detect kidney injury mediated by the β-lactam antibiotic imipenem in cynomolgus monkeys, and to compare with conventional plasma and urinary factors. Monkeys (2-3M/3F) were studied for their potential to deliver mechanistically related information in renal toxicity have been reported. However several questions remain unanswered and there is still the need for more predictive, mechanistically based biomarkers. To investigate some of these questions we used the well described nephrotoxin, Vancomycin, using several different biomarker types. 1. Qualified and exploratory miRNA-155 upregulation in response to kidney damage was established by not observing any change in the kidneys of rats treated with a well-established hepatotoxic galactosamine at 24 h following 1.1g/kg, subcutaneously. Current work is focused on identifying the target genes of miRNA-155 and characterizing the phenotype of miRNA-155 knockout mice towards kidney damage. In summary, we have identified that miRNA-155 is significantly upregulated following kidney damage and is strongly depended on the time of exposure as well as the health status of the kidney. With massive renal mass loss a significant reduction of urinary proteins was observed. Additionally, a panel of transcriptional biomarkers, specific for different molecular pathways, was identified and used for further mechanistic analysis. We also could show that it is possible to profile urine derived miRNAs and to identify potential markers, which could be used as real-time biomarkers in renal toxicity studies. A combined testing strategy based on urinary protein and transcriptional biomarkers is optimal to achieve optimal mechanistic understanding. Urinary miRNAs needs further investigations to discover the potential of these small molecules as toxicity biomarkers.

MicroRNAs are endogenous non-coding RNA molecules that are involved in post-transcriptional gene silencing. The aim of this study was to identify microRNAs (miRNAs) that not only serve as sensitive markers of kidney injury but also modulate tissue repair. We performed global miRNA expression analysis using Taqman® microRNA Low Density Array (TLDA) for 349 miRNAs on rat kidney samples following 30 minutes renal bilateral ischemia reperfusion injury (IRI) over time at 24, 72 and 120 hours. Using a cut off Ct value of 35, we obtained miRNA-155 among 16 genes that were upregulated > 2-fold as compared to sham. Real time quantitative PCR analyses confirmed that the cortex showed a 6-fold elevation of miRNA-155 at 24 h, which is the peak of injury (serum creatinine (SCr) - 1.9 mg/dL, blood urea nitrogen (BUN) - 51 mg/dL). Medulla showed no appreciable change at 24 h, which increased to nearly 5-fold by 72 h and 20-fold by 120 h. Similarly in rats treated with gentamicin (0, 200, or 300 mg/kg, subcutaneously for 3 days), miRNA-155 increased 6-fold in the cortex whereas medulla had only a moderate 3-fold change at 24 h at both dose groups with kidney injury (SCr - 0.6 mg/dL, BUN - 21 mg/dL). The specificity of miRNA-155 upregulation in response to kidney damage was established by not observing any change in the kidneys of rats treated with a well-established hepatotoxic galactosamine at 24 h following 1.1g/kg, subcutaneously. Current work is focused on identifying the target genes of miRNA-155 and characterizing the phenotype of miRNA-155 knockouts towards kidney damage. In summary, we have identified that miRNA-155 is significantly upregulated following kidney damage and could potentially play a critical role in the pathogenesis of kidney injury and tissue repair process.
1211 THE POSSIBLE ROLE OF Annexin A5 IN CISPLATIN-INDUCED NEPHROTOXICITY.


Annexin A5 belongs to a large family of calcium-binding and phospholipid-binding proteins and may act as an endogenous regulator of various pathophysiological processes. Increasing evidence points that annexin A5 is related to cytotoxicity and can be used as a biomarker for certain diseases such as Alzheimer’s disease and heart failure, but the precise function of this protein has yet to be elucidated. This study proposes Annexin A5 as a potential biomarker for cisplatin-induced nephrotoxicity and examines the function of Annexin A5 in apoptosis of kidney cells. Real-time PCR and western blot analysis together with immunofluorescence assay all showed that the expression level of annexin A5 increased significantly by the short term incubation with cisplatin at relatively low level in both human and rat kidney epithelial cells. In the aspect of function, Annexin A5 knockdown led to increased cell viability compared to the control group and reduced VDAC level. VDAC is shown to partly mediate cisplatin-induced apoptosis via the formation of VDAC oligomer pore at mitochondrial outer membrane through which apoptogenic proteins are released into the cytosol. These results implicate that annexin A5 can act as a mediator of apoptosis via regulation of VDAC.

1212 MECHANISTIC ASPECTS OF 4-AMINO-2-CHLOROPHENOL NEPHROTOXICITY IN VITRO.

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Chloroanilines are commonly used as chemical intermediates in the manufacture of numerous agricultural chemicals, dyes, pharmaceuticals and industrial compounds. Some aminochlorophenol metabolites induce nephrotoxicity in vivo and/or in vitro. Previous studies have shown that 4-amino-2-chlorophenol (4A2CP, 1.0 mM) induced nephrotoxicity in isolated rat renal cortical cells (IRCC) during a sixty minute exposure. The purpose of this study was to examine the different biological pathways (CYP, FMO, and oxidation-reduction) of activation of 4A2CP using a rat renal in vivo model. IRCC were obtained from male Fisher 344 rats and incubated in Krebs-Henseleit buffer. IRCC (4 x 106 cells/ml; 3 ml) were incubated with dimethyl sulfoxide (DMSO) or 4A2CP (1.0 mM) with shaking for sixty minutes. In some experiments, IRCC were pretreated with an FMO inhibitor [N-oxycloamine (0.2μM) or metimazole (1.0μM)], CYP inhibitor [isoniazid (1.0μM), ketoconazole (1.0μM), or metryzamol (1.0μM)], or an antioxidant [ascorbate (1.0 mM), glutathione (1.0 mM), N-acetyl-L-cysteine (NAC, 2.0 mM), DPPD (0.05 mM), deferoxamine (0.1 mM), or α-tocopherol (1.0μM or 2.0μM)]. Cytoxicity was determined by measuring the release of lactate dehydrogenase (LDH). FMO or CYP inhibitors didn’t decrease 4A2CP cytoxicity. Ascorbate, glutathione and NAC provided protection from 4A2CP toxicity, while other antioxidants did not. These results suggest that the mechanism by which 4A2CP induces toxicity does not involve activation by FMO’s, CYP2E1, 2B or 3A4. Antioxidant protection suggests that a toxic metabolite is created via an auto-oxidation process and/or oxidative stress plays a role in 4A2CP nephrotoxicity. (Supported by NIH Grant 5P20RR016477 to the West Virginia IDEaN for Biomedical Research Excellence)

1213 COADMINISTRATION OF CHLORAMPHENICOL AND MULTIVITAMIN HEMATIC COMPLEX INDUCES OXIDATIVE STRESS IN RAT KIDNEY.

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Possible synergistic effects of coadministration of chloramphenicol and multivitamin haematinic complex via free radical generation were investigated using biomarkers of oxidative stress. Lipid peroxidation and hydrogen peroxide was assessed. The levels of non-enzymic antioxidants (reduced glutathione (GSH) and enzymic antioxidants (superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST)) in the activities of gamma glutamyl transferase (GGT) and glucose-6-phosphatase were also assessed 10 days post administration. Tissues from the kidney were sectioned for necropsy. Oral administration of Chloramphenicol (28 mg/kg body weight) and multivitamin hematinc complex (5ml/kg body weight) for 10 days induced significant increase in lipid peroxidation indices and hydrogen peroxide levels. The level of GSH and the activities of SOD, CAT, and GST were also significantly increased in the treated rats. Also, the activity of GGT and glucose-6-phosphatase was significantly reduced in animals treated with chloramphenicol plus multivitamin hematinc complex and multivitamin hematinc complex alone. However, there was significant increase in oxidative stress in rats treated with combination of Chloramphenicol and multivitamin hematinc complex compared with either chloramphenicol or multivitamin hematinc complex treated group. Necropsy revealed severe generalized tubular necrosis, cellular infiltration and presence of protein casts in tubular lumen of treated rats. Together, the effect of Chloramphenicol on enzymic and non-enzymic antioxidants components is related to the action of chloramphenicol presumably, via free radical mechanism and that co administration of Chloramphenicol with multivitamin hematinc complex can potentiate its toxic action.

1214 OVEREXPRESSION OF MITOCHONDRIAL GLUTATHIONE TRANSPORTERS IN RENAL PROXIMAL TUBULAR CELLS FROM CONTROL AND DIABETIC RATS.

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Many chronic pathological states, such as diabetic nephropathy, exhibit oxidative stress. Although oxidative stress clearly exists in renal proximal tubular (PT) cells from diabetic rats, there are compensatory increases in total renal cellular and mitochondrial glutathione (mtGSH). Despite this, renal PT cells from diabetic rats are more susceptible to chemically induced injury. We hypothesized that overexpression of mtGSH transporters in renal PT cells from diabetic rats will reverse the oxidative stress and diminish susceptibility by producing larger, sustained increases in mtGSH content. cDNA encoding either the dicarboxylate (DIC, Slc25a10) or 2-oxoglutarate carrier (OGC, Slc25a11) were cloned into pcDNA 3.1/V5-HisTOPO vector. On day 3 of primary culture, renal PT cells from either control or streptozotocin-induced diabetic Sprague-Dawley rats were transfected with purified DIC or OGC plasmids using Lipofectamine. Total RNA from non-transfected or transfected cells was isolated on day 6 of culture and mRNA levels of DIC and OGC were quantified by real-time PCR. In renal PT cells from control rats, transfection with either DIC or OGC cDNA produced 7,800- or 5,000-fold increases in DIC or OGC mRNA, respectively. In renal PT cells from diabetic rats, transfection with either DIC or OGC cDNA produced 600- and 1900-fold increases in DIC or OGC mRNA, respectively. Transfected and non-transfected PT cells were pre-incubated with or without 5 mM GSH prior to assessment of cytoxicity from exposure to either oxirans or a mitochondrial toxicant (antimycin A (AA)). In the absence of GSH pre-incubation, overexpression of either carrier had little effect on cytoxicity, as judged by LDH release or MTT fluorescence. In contrast, with GSH pre-incubation, carrier overexpression provided significant protection, but only from AA. These results suggest that mtGSH carrier overexpression may be a viable approach to improving redox status of renal cells in chronic disease states.

1215 MULTIDRUG RESISTANCE-ASSOCIATED PROTEINS AND THEIR ROLE IN THE REINAL ELIMINATION OF MERCURIC IONS.

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Inorganic mercury (Hg(II)) accumulates and exerts its toxic effects in proximal tubular cells. Mercuric ions are taken up at the basolateral membrane of these cells via the organic anion transporter 1, and at the luminal membrane by transport systems such as system b0,+ and Mrp2. The mechanisms responsible for the renal elimination of mercuric ions are not well-understood. We hypothesize that the multidrug resistance-associated protein (Mrp2) plays a significant role in the export of mercuric ions from proximal tubular cells. Mrp2–/– or FVB (control) mice were exposed (i.p.) to 0.5 μmol/kg HgCl2, containing [38H]Hg, and 24 h later were treated (i.p.) with either saline or 2,3-dimercaptopyrrolo-l-sulfonate (DMPS). Mice were sacrificed 48 h after initial injections. The renal and hepatic burden of Hg was greater in Mrp2–/– mice than in control mice. DMPS reduced significantly the renal burden of Hg, with a greater effect in the FVB mice. Focal and urinary excretion of Hg was greater in FVB mice than in Mrp2–/– mice. Transport studies using inside-out membrane vesicles expressing Mrp2 suggest that Mrp2 does not mediate the export of mercuric species. PCR analyses of kidneys show that expression of gamma-glutamylcysteine synthetase and metallothionein are greater in Mrp2–/– than FVB mice. Surprisingly, initial studies using Ellman’s assay suggest that levels of glutathione in kidneys of Mrp2–/– mice were similar to those of FVB mice. The results of the current study confirm that Mrp2 is a mechanism for the export of mercuric species.
In the renal proximal tubule, P-glycoprotein (P-gp) is an extrusion transporter located on the apical membrane which affects the excretion of a broad range of substrates, including digoxin (DIG). In view of the increasing incidence of diabetes, it was of interest to evaluate the affects of growth media glucose level on the P-gp activity of cultured kidney cells. LLC-PK1 cells were grown to confluence in media containing 5 or 17.5 mM glucose for cytotoxicity and transport studies. The LC50 of DIG was found to be significantly lower for cells grown in 17.5 mM glucose (11.2 ± 0.6 μM) compared to cells grown in 5 mM (15.8 ± 1.0 μM), p < 0.05. The transport activity of P-gp was evaluated by assaying both transepithelial movement and intracellular accumulation of 3H-DIG by cells grown on membrane filters. The basolateral (B) to apical (A) transport of DIG (B→A) was significantly less in cells grown in medium with 17.5 mM glucose (832 ± 75 fmol DIG/tg DNA/2 hr) compared to 5 mM (1268 ± 41 fmol DIG/tg DNA/2 hr). In contrast, the apical to basolateral (A→B) transport of 3H-DIG was not affected by glucose concentration. Intracellular accumulation (C) of DIG applied to the basolateral side (B) was found to be significantly higher in cells grown in 17.5 mM glucose (227 ± 24 fmol DIG/tg DNA/2 hr) compared to those in 5 mM glucose (153 ± 14 fmol DIG/tg DNA/2 hr). Taken together, the significant decrease in B→A transport of DIG and increase in B→C accumulation for cells grown in 17.5 mM glucose suggests decreased extrusion of DIG from the apical side. The B→A transport of DIG in the presence of the P-gp inhibitor quinidine was reduced by 50% in cells grown in either 17.5 mM or 5 mM glucose, while B→C accumulation was increased 20-50%. In conclusion, the activity of P-gp in LLC-PK1 cells is reduced in the cells grown in presence of the P-gp inhibitor quinidine was reduced by 50% in cells grown in either 17.5 mM glucose, and this phenomenon can explain the observed increased cytotoxicity to DIG.

1219 BROMATE INCREASES 3-BROMOTYROSINE CONCENTRATION IN MALE RAT KIDNEYS.

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Fibronogen (Fg), a soluble 340 KDa hexameric blood glycoprotein composed of three pairs of polypeptide chains (α, β and γ) has been recognized as an important regulator of wound healing however, there is very limited knowledge about the functional significance of Fg signaling in kidney epithelial cells. The objective of this study was to investigate the mechanism of Fg in modulating kidney tissue repair. We observed that Fg protein levels in the kidney as well as its urinary excretion are significantly increased in mice following 25 min bilateral ischemia reperfusion (I/R)-induced injury or 20 mg/kg (ip) Cisplatin-induced nephrotoxicity. We hypothesized that Fg binds to membrane protein caveolin-1 (Cav-1), a major constituent of caveolae (known as signaling hub of the cell), and triggers epithelial cell proliferation and survival. Caveolin-1 activity was significantly increased at 24 and 48 h following I/R injury as measured by p38-membrane immunoblotting. Immunoprecipitation results confirmed the binding of Fg with Cav-1 in the kidney at 48 h following I/R injury. Furthermore, we observed a dose dependent increase in survival and proliferation of human proximal tubular epithelial cells (HK-2) in response to Fg (0.5, 1 or 2 mg/ml) as measured by MTT and BrDU assay respectively. Fg stimulated cell proliferation was abrogated following addition of an inhibitor of Cav-1 (methyl-β-cyclodextrin, 1 mM) thereby suggesting that Fg binding to Cav-1 is necessary to induce cell proliferation. In summary, our results suggest that upregulated fibrogen in the kidney binds to Cav-1 and modulates tissue repair by inducing proliferation of tubular epithelial cells.

1220 PRIMARY CULTURES OF MOUSE RENAL PROXIMAL TUBULE EPITHELIAL CELLS AS A MODEL FOR STUDYING ACETAMINOPHEN (APAP)-INDUCED NEPHROTOXICITY.

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The kidney is the major extrahepatic organ for APAP toxicity. Recent studies in our laboratory show that mice lacking the membrane transporter breast cancer resistance protein (Bcrp; Abcg2) are more susceptible to APAP-induced nephrotoxicity compared to wildtype. However, the mechanism by which the absence of this transporter leads to more nephrotoxicity by APAP remains unknown. Currently, there are no well-established in vitro models for studying mechanisms of APAP toxicity in the mouse kidney. The purpose of this study was to establish primary cultures of mouse renal proximal tubule epithelial cells to study the role of drug transporters in APAP-induced nephrotoxicity. For this purpose, renal proximal tubules from C57Bl/6 mice were isolated and grown in culture for 1 week to form a monolayer identical to that of the intact, functional epithelium. The utility of these cultures is demonstrated by the fact that there are no changes in the expression pat-
1221 DIGLYCOLIC ACID, THE NEPHROTOXIC METABOLITE OF DIETHYLENE GLYCOL, INDUCES NECROSIS VIA INTRACELLULAR ACCUMULATION AND METABOLIC DISRUPTION.

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Diethylene glycol (DEG) is an organic solvent used in antifreeze blends, brake fluid, and the production of various polymers allowing the risk of consumer exposure. The hallmark of DEG poisoning is acute renal failure caused by cortical tubular degeneration and proximal tubule necrosis. DEG is metabolized to two primary metabolites, 2-hydroxyethoxyacetic acid (2-HEAA) and diglycolic acid (DGA). In human proximal tubule (HPT) cells in culture, DGA, but not 2-HEAA, produces a time dependent decrease in adenosine triphosphate (ATP) that precedes an increase in lactate dehydrogenase (LDH) release indicating DGA-induced necrosis. Studies were therefore designed to assess the mechanism by which DGA might produce cytotoxicity. The chemical structure of DGA is strikingly similar to various Krebs Cycle intermediates particularly succinate, which are internalized and metabolized by renal proximal tubule cells. Accumulation of such dicarboxylates is mediated by sodium-dicarboxylate (NaDC) transporters such as NaDC-1, which is primarily apical in the proximal tubule cell. Incubation of HPT cells with Na-p-amylcinnamoyl)anthranilic acid (ACA), a potent NaDC-1 inhibitor, decreased the degree of DGA-induced cell death by approximately 50%. No inhibition of DGA-induced toxicity was observed in the presence of a specific NaDC-3 or organic anion transport (OAT) inhibitor, suggesting a role for NaDC-1 in the intracellular uptake of DGA. In HPT cells, increasing concentrations of DGA increased total cellular oxidation producing indicating that DGA disrupts cellular redox status. These results indicate that after internalization, DGA likely induces proximal tubule cell dysfunction by specific mitochondrial-mediated processes, which lead to decreased energy production and ultimately cellular necrosis.

1222 CADMIUM-INDUCED PROTEINURIA IS PREVENTED BY LOSARTAN AND N-ACETYL-CYSTEINE TREATMENT IN A MURINE EXPERIMENTAL MODEL.

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Cadmium (Cd) is a heavy metal that has become an environmental and public health problem due to its constant release by industrial activity. Chronic exposure to low levels of Cd produces an urinary waste of low molecular weight proteins (LWP), which is considered the critical effect of exposure to Cd. In normal physiological conditions, LWP can be filtered through the glomerulus, but they are reabsorbed by endocytosis in the proximal tubule (PT), preventing urinary loss of amino acids. In this study, we investigated the effect of Losartan (LOS) and N-acetylcysteine (NAC), on the proteinuria of Cd-exposed rats. LOS is an angiotensin II type (AT1) receptor antagonist, and NAC is an antioxidant. Female Wistar rats (180-220g) were used. Animals were divided into 6 groups (5 rats/dose): Cd (3 mg/kg/day), LOS (10 mg/kg/day), NAC (125 mg/kg/day), Cd-LOS and Cd-NAC. All treatments were administered daily by gavage for 8 weeks. At the end of the 8th week, rats were placed in metabolic cages (1 rat per cage) for 16 h to collect urine samples and blood samples were obtained by cardiac puncture. Total protein (Bradford assay), microalbuminuria (HemoCue albumin) and N-acetyl-p-b-D-glucosaminidase (NAG, colorimetric assay) were determined in urine. Creatinine concentration in the serum samples was measured by Jaffé method. Excretion of total protein and NAG levels did not show significant differences among treatments; however, Cd treatment significantly increased (P=0.012) microalbuminuria. This effect was reversed by coexposure to LOS or NAC showing significant differences. Since we did not observe changes in serum creatinine, the increase in microalbuminuria could be considered as exclusively due to an injury in TP. These findings demonstrate that Cd-induced proteinuria involved signaling pathways related with oxidative stress (ROS) and/or regulation of angiotensin II and AT1 receptor.

1223 HIGH-CONTENT IMAGING STUDY REVEALS METABOLITES OF DB289 INDUCE PRIMARY KIDNEY CELL INJURY.

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Human African trypanosomiasis (HAT; sleeping sickness), is transmitted by tsetse flies carrying the parasite Trypanosoma brucei and caused ~48,000 deaths in 2008. Pafuramide maleate (DB289) was developed as an oral therapy for stage I HAT. In Phase 3 clinical trials, DB289 demonstrated an 89% cure rate, but development was halted following an expanded Phase 1 safety trial in which DB289 caused liver toxicity and severe, delayed-onset renal toxicity in a subset of healthy volunteers. DB289 is a prodruk metabolized through cytochrome P450 4F enzymes (CYP4F) into the active metabolite 2,5-Bis-(4-aminodiphenyl) furan (Furamidine, DB75), which has been shown to accumulate in tissues to millimolar concentrations; this accumulation was hypothesized to play a role in the toxicity although the underlying mechanism is unclear. To better understand the mechanisms of the organ toxicities, high-content imaging (HCl) technology was used to examine primary human renal proximal tubule epithelial cells. Cellular mechanistic and morphological endpoints were measured by HCl including cell count, nuclear size, apoptosis (cytochrome-C release), mitochondrial function (MitoTracker), endoplasmic reticulum (ER) stress (CHOP/GADD153), oxidative stress (DHE, MnSOD/SOD), and DNA damage (phospho-H2AX). The cellular endpoints were evaluated following 4, 24, and 72 hour exposures to DB289, DB75, and 3 other intermediate metabolites (DB290, DB755, and DB810) in a 9-point dose response up to 100 uM. Perturbations in endpoints tested were evident at doses >10 uM with DB75 and to a lesser extent with DB289 only demonstrated ER stress and at the highest concentration (100 uM). Taken together these data suggest DB289 is not the underlying causative toxin, rather it is the metabolites of DB289 that induces renal cell injury.

1224 TOXICOPROTEOMIC ANALYSIS OF NEPHROTOXICITY INDUCED BY ARISTOLACTIC ACID.

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Advancements of proteomics approaches and their application to toxicological studies have led to the development of a new discipline, toxicoproteomics. Use of dietary supplements containing aristolochic acid (AA) can result in severe nephrotoxicity in humans. In addition, AA is a potent carcinogen, inducing urothelial cancers in humans and kidney tumors in rats. To understand the molecular mechanisms underlying AA-induced nephrotoxicity and identify injury biomarkers, a quantitative proteomic analysis was conducted on kidneys from AA-treated rats. Animal treatment was conducted according to the protocol approved by the NCTR Institutional Animal Care and Use Committee. Six-week-old Big Blue rats were treated with different doses of AA or with 0.9% sodium chloride as the control. After treatment, six rats from each treatment group were sacrificed. The kidneys were isolated, frozen quickly in liquid nitrogen, and stored at -80 °C. Kidney tissues from the control and the highest AA dose treatment (10 mg/kg body weight) were processed for proteomic analysis using trypsin-catalyzed 13C/15N stable isotope labeling and two-dimensional liquid chromatography coupled online with tandem mass spectrometry (LC-MS/MS). Approximately 3000 proteins were quantified and more than 150 proteins were consistently over expressed or under expressed across all the AA-treated samples. Pathway analysis indicated that significant changes include proteins involving xenobiotic metabolism, DNA damage, cell proliferation and neoplasm, etc. Intriguingly, several FDA-QC-prequalified preclinical kidney injury biomarkers were identified in this study. The quantitative proteomic study identified proteins related to potential toxicity and carcinogenicity of AA and proteins that could serve as biomarkers of renal damage inflicted by AA exposure and potentially other agents that cause similar renal damage. The views presented do not necessarily reflect those of the US Food and Drug Administration.
NextGen transcriptome sequencing (RNA-Seq) is a platform for generating millions of short sequence reads aligned to a reference genome for accurate and thorough description of gene expression. In this study, liver RNA was extracted from male F344 rats orally exposed to 1 ppm aflatoxin B1 (AFB1) for 90 days vs controls (Ctl) (n=4/group), before development of histopathological lesions or tumors. Our hypothesis was RNA-Seq would reveal differences in low copy and novel gene expression related to AFB1’s potent carcinogenic activity. Illumina Ilga RNA-Seq sequencing produced 29-37 million reads/sample using 100bp paired-ends. Principal component analysis showed concordance of genomic coverage among group samples and AFB1 and Ctl treatments were well separated. Reads were aligned to the Rv4 build with Tophat and differential expression was assessed by both Cufflinks and DESeq algorithms. DESeq analysis showed >500 differentially expressed genes and 3 ncRNAs at >2 fold, false discovery rate<0.05. Cufflinks identified ~295 genes with significantly expressed splicing variants. AFB1-altered transcripts filled 35 canonical pathways for signaling of AhR, Nrf2, GSH, xenobiotic, cell cycle, extracellular matrix and cell differentiation networks at p<0.05. Some >10 fold, over-expressed transcripts included Ddit4l, Cdh13, Nrcam, Il17rd and others. Also, novel, unannotated, hepatic AFB1-responsive transcripts (HAT's) increased 10 to 25-fold above Ctl were found, located on chromosomes 1q55 and 15q11. PCR-cloning of Chr1 transcript revealed >1 possible open reading frame and several exons, and functional analysis as related to AFB1 carcinogenesis is underway. We conclude that RNA-Seq represents an unbiased platform for comprehensive and sensitive measurement of the rat transcriptome and is capable of providing new insights into the biology of AFB1-mediated gene expression leading to hepatocellular carcinoma.

**1225 RNA-SEQ PROFILING REVEALS NOVEL GENE EXPRESSION PATTERN AFTER SUBCHRONIC AFLATOXIN B1 (AFBI) IN RATS.**


Hepatobiliary injury associated with drug treatment, particularly biliary duct hyperplasia (BDH), has been difficult to clearly identify in short duration (4 day) rodent toxicology studies. Although measurement of circulating levels of bilirubin and GGT are routinely used to diagnose hepatobiliary injury, these markers have been shown to lack sensitivity and specificity, and historically only histological assessment can be relied upon for verification of BDH. However in short duration studies declaration of BDH is challenging as the lesions are often subtle and it is difficult to predict if BDH would manifest in longer duration studies. In order to address these shortcomings, efforts have been undertaken by a number of groups to develop gene expression based diagnostic and predictive markers (signatures) of bile duct hyperplasia. Using previously established gene array based BDH signatures as a starting point we used a data mining approach to compile a set of 19 genes likely to be informative based on their frequencies and weightings across all the examined signatures. Using these 19 genes as a starting point, along with a training set of liver RNAs from rats treated with compounds that were positive or negative for BDH induction in 4 day rat studies, a new six gene quantitative PCR (qPCR) based signature was developed. The signature was generated using a machine learning algorithm based approach which yielded a logistic regression model that utilizes quadratic penalization of the six genes. The signature was subsequently tested with liver RNA from additional BDH positive and negative compound treatments in standard 4 day rat toxicology studies. This new qPCR based BDH gene signature has an overall performance of 63% sensitivity and 97% specificity and, when combined with histopathology, improves our ability to correctly identify adverse hepatobiliary injury in screening studies.

**1228 DEVELOPMENT OF A QUANTITATIVE PCR BASED GENE SIGNATURE FOR IDENTIFYING BILE DUCT HYPERPLASIA IN SHORT-DURATION RAT STUDIES.**

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In vitro assays have had limited success in predicting systemic toxicity arising from a variety of mechanisms. A major reason for this may be the limited endpoints that are typically measured in in vitro assays (e.g., cell viability, proliferation rate). However, given that cells in culture can maintain a differentiated, specialized phenotype, it is likely that their responses are far richer than what we can evaluate. Whole genome micro array analysis provides an all-in-one system, where one in vitro system may potentially be used to provide mechanistic insight, which in turn can be the basis for predicting toxicity potential. A proof of principle study was performed for the 10 hepatotoxicants, Aetaminophen, β Naphthoflavone, Chlorpromazine, Clofibrate, DEHP, DNP, Methyprylon, Sodium Valproate, Phenobarbital and WY14643 (which included 2 structurally similar compounds and structurally dissimilar compounds that have similar molecular targets) using primary rat hepatocytes. Gene expression microarray analysis was performed on the 10 chemicals, 24
h post exposure (at two different dosages) and the 1000 most significantly changed genes were used for further analysis. Both whole genome and pathway based clustering analysis resulted in the phosphatases clustering together, while the other 8 components which were not structurally similar, clustered based on their mode of action. The peroxisome proliferators, Clofibrate and WW14643 and the phosphatases clustered together, Acetaminophen and Sodium Valproate grouped together and the endocrine inducers, Phenoxybutyl, Methyprylene, Chlorpromazine and β Naphththalene clustered together. These results indicate that transcription profiling using an in vitro assay may offer pertinent biological data to support predictions of in vivo hepatotoxicity potential.

1230 GLOBAL GENE EXPRESSION CHANGES IN HUMAN EMBRYONIC LUNG FIBROBLASTS INDUCED BY ORGANIC EXTRACTS FROM RESPIRABLE AIR PARTICLES.

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To obtain insight into the biological mechanisms of action of the extractable organic matter (EOM) from ambient air particles, human embryonic lung fibroblasts (HEL12469) were treated with organic extracts from PM2.5 collected in four localities of the Czech Republic which differed in the extent and sources of air pollution. We assess changes in the genome-wide expression profiles compared to DMSO treated controls. Cells were incubated with subtoxic EOM concentrations of 10 - 60 μg EOM/ml for 24 h and gene expression changes were analyzed using human whole genome microarrays (Illumina). Dose-dependent increases in the number of deregulated transcripts as well as dose-response relationships in the levels of individual transcripts were observed. The transcriptomic data did not differ substantially between the localities, suggesting that the air pollution originating mainly from various sources may have similar biological effects. This was further confirmed by the analysis of deregulated pathways and by identification of the most contributing gene modules. The number of significantly deregulated KEGG pathways varied depending on the locality, between 12 to 29. The metabolism of xenobiotics by cytochrome P450 exhibited the strongest upregulation in all 4 localities and CYP1B1 had a major contribution to the upregulation of this pathway. Other important deregulated pathways were ABC transporters (toxin excretion activity), the Wnt and TGF-beta signaling pathways (associated particularly with tumor promotion and progression), steroid hormone biosynthesis (involved in the endocrine-disrupting activity of chemicals), and glycerolipid metabolism (pathways involving the lipids with a glycolic backbone including lipid signaling molecules). These results suggested a prominent role of activated aryl hydrocarbon receptor-dependent gene expression. Supported by the Grant Agency of the CR (503/11/0142).

1231 CONCENTRATION-RESPONSE ANALYSIS OF DIFFERENTIAL GENE EXPRESSION IN THE ZEBRAFISH EMBRYOTOXICITY TEST FOLLOWING FLUSILAZOLE EXPOSURE.

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The zebrafish embryotoxicity test (ZET) may be a useful alternative model in predictive toxicology. Currently, morphological assessment of the embryo is the main readout for this assay. However, implementation of transcriptomics may reveal more subtle effects at the level of gene expression, which may increase sensitivity and predictability of the test. As effects on morphology and gene expression are dependent on compound concentration, this may influence developmental toxicity prediction. In this study, we tested a concentration-response of flusilazole in the ZET Exposure was initiated shortly after fertilization. At 24 h post fertilization, microarray analysis revealed a number of processes regulated in a concentration-dependent way. We identified development related processes, retinol metabolism and transcription, as well as processes corresponding to the antifungal mechanism of action, steroid biosynthesis and fatty acid metabolism, to be regulated. Retinol metabolism and transcription were already significantly regulated at concentrations that were not inducing morphological effects. Differential expression of genes related to steroid biosynthesis and fatty acid metabolism showed a concentration-reponse similar to morphological response. An increase in concentration was also positively associated with a maximum magnitude of expression for individual genes. Our study shows that transcriptomics analysis in the ZET is a more sensitive readout of compound-induced effects than morphological assessment. Moreover, specific knowledge on mechanisms of action becomes available for study. However, the interpretation of differential gene expression in terms of predicting adverse morphological effects requires further study.

1232 INVESTIGATION OF TROGLITAZONE-INDUCED HEPATO AND CARDIO TOXICITY IN NORMAL AND HIGH-FAT DIET-INDUCED DIABETIC MOUSE MODELS.

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Two major toxicological reasons for post marketing drug withdrawal are hepato and cardio toxicity. Members of the thiazolidinedione family of Peroxosamine Fillericceptor Gamma (PPARY) agonists, Trogilitazone (TRO) and Rosiglitazone have been withdrawn from the market due to incidences of hepato and cardio toxicity. The mechanism of TRO induced toxicity has yet to be fully elucidated. Using both a normal and a high fat diet induced diabetic mouse models we have investigated the toxicity of TRO using transcriptomic analysis of liver and heart. Pathway analysis of these data has shown modulated pathways involved in a process related to hepatic steatosis, cholestasis, fibrosis and sarcromere remodelling. The potential cardiotoxicity of TRO, through pulmonary oedema leading to sarcomere remodelling was observed in the normal mouse model. TRO induced hepatotoxicity is difficult to model in normal animal models and in this study it was observed only in the high fat diet diabetic model. The increased expression of PAPPY in fatty liver compared to the levels found in normal liver, may account for the difficulty in investigating TRO hepatotoxicity in a normal animal. In fatty liver, expression of PAPPY is increased and administration of TRO further elevates expression levels in turn activating a number of downstream targets that are associated with the formation of hepatic steatosis. These findings correlate with clinical observations of hepatic steatosis in both healthy volunteers and patients and evidence of hepatic steatosis that was observed in liver biopsy samples taken from patients presenting with TRO induced hepatotoxicity. Using Next Generation Sequencing the role of microRNAs in TRO induced toxicity is being investigated.

1233 PREDICTION MODEL OF POTENTIAL HEPATO CARCINOGENICITY OF RAT HEPATO CARCINOGENS USING A LARGE-SCALE TOXICOCGENOMICS DATABASE.

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The present study was performed to develop a robust gene-based prediction model for early assessment of potential hepatocarcinogenicity of chemicals in rats by using our toxicogenomics database, TG-GATES (Genomics-Assisted Toxicity Evaluation System). The positive training set consisted of high- or middle-dose groups that received 6 different non-genotoxic hepatocarcinogens during a 28-day period. The negative training set consisted of high- or middle-dose groups of 54 non-carcinogens. Support vector machine combined with filter-type gene selection algorithm was used for modeling. Consequently, our best classifier yielded prediction accuracies for hepatocarcinogenicity of 99% sensitivity and 97% specificity in the training data set. Pathway analysis of feature genes revealed that the mitogen-activated protein kinase p38- and phosphatidylinositol-3-kinase-centered interacome and the v-myec myelocytomatosis viral oncogene homolog-centered interacome were the two most significant networks. The usefulness and robustness of our predictor were further confirmed in an independent validation data set obtained from the public database. Interestingly, similar positive predictions were obtained in several genotoxic hepatocarcinogens as well as non-genotoxic hepatocarcinogens. These results indicate that the expression profiles of our newly selected candidate biomarker genes might be common characteristics in the early stage of carcinogenesis for both genotoxic and non-genotoxic hepatocarcinogens in the rat liver. Our toxicogenomic model might be useful for the prospective screening of hepatocarcinogenicity of compounds and prioritization of compounds for carcinogenicity testing.
The toxicity of polybrominated diphenyl ethers (PBDEs), flame retardant components, was characterized in offspring from Wistar Han dams exposed by gavage to a PBDE mixture (DE71) starting at gestation day 6 (GD 6) and continuing to weaning on postnatal day 21 (PND 21). Offspring from the dams were directly dosed by gavage at the same dose as their dam beginning on PND 12, continuing through weaning on PND 21, and for an additional 13 weeks post-weaning. Prior to weaning animals were dosed daily and after weaning animals were dosed 5 days/week. Liver samples were collected at PND 22 (both sexes) and week 13 (males) for liver gene expression analysis (Affymetrix Rat Genome 230 2.0 Array). We applied analysis of variance (ANOVA) incorporating shrinkage variance components to identify differentially expressed genes. One-way layouts were used to find dose- and sex-specific effects, while two-way layouts were used to explore interactions between age and dose (for males) and sex and dose (PND22). PBDE treatment induced 1,066 liver gene transcript changes in females and 1,200 transcriptional changes in males at PND 22 (false discovery rate (FDR) < 0.01), but only 263 liver transcriptional changes at 13 weeks in male rats (FDR <0.05). No significant differences in dose response were found between male and female pups, but we identified 447 age-dependent changes in gene expression response to PBDEs (FDR < 0.05). Transcript changes at PND 22 coded for proteins in xenobiotic, sterol and cholesterol and triglycerides metabolism including PPAR dependent peroxisomal and mitochondrial fatty acid oxidation genes; significant upregulation of CYP1A1 and TCDD response element and glutathione metabolism. The cyanide-insensitive mitochondrial oxygen consumption was significantly decreased in treated animals compared to controls. Treatment-related increases in hepatic KEGG pathways were observed in this study. Gene ontologies and pathways identified by microarray analyses to generate a working hypothesis related to the mechanism of toxicity. Taken together, the overall results from this study indicated that the liver toxicity of SSR101010 is not likely related to the pharmacologic action of the compound.

Liver damage resulting from acetaminophen (APAP) overdose is a serious human health problem. However, the precise molecular mechanism of toxicity remains unclear. Basic helix-loop-helix (bHLH) proteins are dimeric transcription factors connected as a network through protein-protein interaction (PPI) to coordinately regulate the expression of a large number of genes. In this study, we analyzed the expression of all hepatic bHLH genes in groups of mice pretreated with either APAP (400mg/kg ip) or vehicle and then challenged 48 h later with APAP (600mg/kg/ip) or vehicle. We also built a conceptual PPI network model to mimic the interaction among all known 112 bHLH proteins. The results show that different clusters of genes were differentially expressed in a time-dependent manner by the different APAP treatments. Each of the clusters contained between 7 and 17 genes. These clusters were then subjected to PPI network modeling based on our conceptual bHLH PPI network model, and the resulting subnetworks were analyzed topologically. This analysis showed that the connectivity among bHLH proteins in all networks was compact and well organized, with ID2, ID3, MAX and MYC serving as important nodes for connectivity. Changes in the expression of ID1, ID2, ID3 and Hes6 genes were prominent in all APAP treatment groups, while MYC gene expression was only found to change in mice pretreated and challenged with APAP. In conclusion, the bHLH sub-networks generated in this study could serve as signaling assembly units in response to APAP hepatotoxicity. Our findings also provide an initial and systematic guide to further investigate the role of bHLH proteins during APAP liver toxicity. Supported by NIH DK809557.
1238 TRANSCRIPTOME ANALYSIS FOLLOWING LOW-DOSE BISPHENOL A EXPOSURE REVEALS EFFECTS ON SIGNALING PATHWAYS IMPLICATED IN NERVOUS SYSTEM DEVELOPMENT AND FUNCTION.

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Bisphenol A (BPA) is a suspected endocrine disruptor that presumably elicits its effects through binding to estrogen receptors (ERs) or other targets such as G-protein coupled estrogen receptor (GPER) or estrogen related receptor gamma (ERRγ). There is concern that BPA at low exposure levels could impact the developing nervous system. Global gene expression analysis was used to investigate the molecular levels of low-dose BPA exposure with the goals of identifying differentially regulated genes indicative of developing organ systems and determining whether ER or ERRγ activation mediates these effects. We exposed 8 hours post fertilization (hpf) zebrafish embryos to 0.1% DMSO control, 0.1 μM BPA, 0.1 μM estradiol (E2; a classical estrogen receptor ligand), or 0.1 μM GSK4716 (GSK; a synthetic ERRγ ligand). At 24 hpf, pools of 40 embryos (N=3) were collected and RNA was processed for hybridization to a Nimblegen zebrafish 12-plex 135K microarray. A total of 983 genes (BPA 343, GSK 531, E2 375) were statistically significant, although the gene expression magnitude changes resulting from these low-dose exposures were modest (≤2 fold changes). Ingenuity Pathway Analysis revealed the top affected gene network for both BPA and GSK was G-protein coupled receptor signaling. For BPA, the top 2 functions affected were cell signaling and nervous system development, particularly synaptic transmission. Among the significantly regulated genes were 2 serotonin receptors (HTR6 and HTR1D) and a neuronal-related factor. Supported by NIEHS T32ES07060, R21ES019870, F30 ES008210, and an EPA STAR Graduate Fellowship to KSS. Note: the EPA has not officially endorsed this abstract.

1239 PROTEOMIC PROFILES OF ZEBRAFISH LIVER CELL LINE FOLLOWING THE ADMINISTRATIONS OF CADMIUM ION.

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In this study, a zebrafish liver cell line - ZFL was used as a model to investigate the toxicity mechanism of heavy metal-cadmium on hepatocytes. Reactive oxygen species (ROS), antioxidant levels, related enzyme activity and gene expression were detected. The intracellular level of ROS was however decreased by cadmium ion administrations. Furthermore, a proteomic approach was used to study the proteins differentially expression profile related to sub-lethal cadmium toxicity. A total of 77 differentially expressed proteins were detected by two-dimensional gel electrophoresis (2-DÉ) after cadmium exposure: 43 of them were identified by MALDI-TOF-MS. The proteins that responded to cadmium toxicity in ZFL cells were found relate to stress response, transporters, regulation of transcription, redox homeostasis, or some signaling pathways, with half of these proteins having metal ion binding capabilities, indicating that the toxicity mechanism is more closely related to interfere biological processes in ZFL cells.

1240 PROTEOMIC PROFILES AND GENE EXPRESSION STUDIES OF FISH LIVER CELL LINES FOLLOWING THE ADMINISTRATIONS OF COPPER ION.

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We report here the use of a proteomic approach to identify copper-affected proteins in fish liver cell lines following the administrations of copper ions. A copper sensitive liver cell-line from zebrafish, ZFL and a less copper sensitive cell-line from tilapia liver cell-line from zebrafish, ZFL and a less copper sensitive cell-line from tilapia liver of Hepa-T1 cell were used in this investigation. The mechanism of copper toxicity to the both cell-lines was highly conserved with the copper-affected proteins found to be involved in lipid metabolism, cell proliferation, cytoskeletons, etc. However, copper caused higher levels of expression of proteins in Hepa-T1 than ZFL related to anti-oxidant effects and inhibition of reactive oxygen species, as well as copper transportation in mitochondria. As a result, copper ions caused more stress effects to ZFL cell than to Hepa-T1 cell and result from protein profiling were also studied by using real-time quantitative polymerase chain reactions to examine the mRNA levels of these corresponding genes. We also started to study and compare the copper transporters (ATP7A, ATP7B, ATOX1, CTRL) involved in copper removal and tolerance using Hepa-T1 and ZFL cell models. By comparing the regulation of ATP7A in tilapia and zebrafish, it was found that the regulation of this gene in tilapia was higher than that of zebrafish in vitro and in vivo. As it is known that ATP7A plays an important role in copper efflux, it is proposed that copper tolerance of tilapia might be due to a higher fold induction of ATP7A to remove the excessive copper ions entered into the system.

1241 EFFECTS OF BENZYL ISOTHIOCYANATE ON METHYLATION AND EXPRESSION OF p21 TUMOR SUPPRESSOR GENE IN JURKAT LEUKEMIA CELLS.

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The anticancer effects of Benzy l isothiocyanate (BITC), isolated from cruciferous vegetables, have been studied extensively but the epigenetic molecular mechanisms of BITC as a potent epigenetic therapeutic agent have not been reported. In the present study, we investigated the effects of BITC on the methylation and expression of p21 tumor suppressor gene in Jurkat leukemic cell lines to evaluate its epigenetic therapeutic potential.

Jurkat cells were treated for six days with various concentrations of BITC. To estimate global DNA methylation patterns by BITC, we performed DNA methylation analyses of Sat2 and LINE-1 repeated elements, which have been shown to be highly correlated with global methylation. BITC treatment decreased global DNA methylation levels in Jurkat cells in a dose-dependent manner. It also reduced expression of the enzymes involved in DNA methylation, DNA methyltransferases (DNMT) 3a and 3b, determined by quantitative mRNA expression assay. Alterations in methylation and expression levels of p21 after treatment of Jurkat cells with BITC were evaluated by quantitative real-time methylation specific PCR and reverse transcription-PCR (RT-PCR). The treatment with BITC resulted in demethylation of promoter of p21 and led to the re-expression of the mRNA of the gene. Finally, p21 expression in Jurkat cells caused dose- and time-dependent apoptosis detected by flow cytometry. Taken together, BITC induces promoter hypomethylation of p21 via down-regulation of DNA methyltransferases 3a and 3b, resulting in up-regulation of their expression and induction of apoptotic cell death in Jurkat cells. Our study provides new insight into the epigenetic mechanism of action of BITC that may contribute to the chemoprevention of leukemia and may have important implications for epigenetic therapy.

1242 CHEMOPREVENTIVE ACTIVITY OF TRIBUTYRIN IS ASSOCIATED WITH INDUCTION OF THE P53 APOPTOTIC SIGNALING PATHWAY IN EXPERIMENTAL HEPATOCARCINOGENESIS.

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The reversibility of epigenetic alterations has been explored in order to develop novel preventive and therapeutic approach for cancer control. Recent interest in histone deacetylase (HDAC) inhibitors has expanded from the field of clinical cancer research to cancer chemoprevention. Previously, it has been demonstrated that tributy r in (TB), a butyric acid pro-drug, has chemo-preventive effect on rat hepatocarcinogenesis. The goal of the study was to determine molecular mechanisms of this chemo-preventive effect of TB. With the exception of 5 rats that were not submitted for any experimental treatment (controls), 5 rats were treated with TB (200mg/100g body weight) and 5 rats were treated with maltdextrin (MD; 300mg/100g body weight; isocaloric to TB group) daily for 8 consecutive weeks. Two weeks after treatment initiation, rats from TB and MD groups underwent "resistant hepatocyte" model of hepatocarcinogenesis. Treatment with TB resulted in lower HDAC activity, increased expression of histone acetyltransferase 1 (HAT1), and an increase of histone H3 lysine 9 and 18 and histone H4 lysine 16 acetylation as compared to MD group. In addition to the increase of histone acetylation, TB caused an increase in the acetylation of p53 protein in the nucleus. These changes were accompanied by the activation of p53 signaling pathway, particularly by the up-regulation of pro-apoptotic genes, and consequent increase of apoptosis in the liver of TB-treated rats. The results of the present study indicate that chemo-preventive activity of TB may be related to the increase of histone and p53 acetylation, which could lead to the induction of the p53 apoptotic pathway.
Environmental or occupational exposure to trichloroethylene (TCE) has been linked to immune hypersensitivity including autoimmune disease in humans. In a model used to study TCE immunotoxicity MRL+/+ mice chronically exposed to TCE developed a T cell-mediated liver disease similar to human idiopathic autoimmune hepatitis. TCE-induced disease in mice was accompanied by alterations in CD4+ T cells including increased cytokine (IFN-γ) production after short-term (4 week) and chronic (32 week) xeposure. In contrast, several experiments involving sub-chronic TCE exposure in mice showed cytokine suppression by the CD4+ T cells. The current study confirmed the CD4+ T cell decrease in cytokine production after a 12-week TCE exposure. A mechanistic evaluation suggested that the CD4+ cell suppression involved DNA methylation. Several significant TCE-induced alterations in events controlled by epigenetic processes were identified including increased expression of DNA methyl transferases and changes in the expression of retroviral genes largely controlled by DNA methylation. These results show for the first time that the immune toxicity of an environmental pollutant such TCE involves a biomodal effect regulated by epigenetic alterations.

### 1244 EPIGENETIC CHANGES IN P21 EXPRESSION IN RAT KIDNEY AFTER EXPOSURE TO BROMATE.

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The fetal basis of adult disease (FEBAD) theorizes that embryonic challenges initiate pathologies in adult life through epigenetic modification of gene expression. We tested the hypothesis that metals, such as cadmium (Cd), alter epigenetic pathways, by measuring total histone protein, histone H3 lysine mono-methylation (H3K27me1), and cell viability, in differentiated and undifferentiated mouse embryonic stem (mES) cells. Stem cells were exposed to Cd for 1-h (plus 23-h recovery period) and 24-h, protocols. The data demonstrates that inhibitory concentrations (50% IC50) for 24-h exposures were significantly lower in undifferentiated cells when compared to differentiated cultures. Both undifferentiated and differentiated mES cells recovered from 1-h Cd exposure, significantly increasing IC50s when compared to 24-h exposures. In addition, 24-h exposure to Cd in undifferentiated mES cells lowered total histone protein at a faster rate than cell proliferation and H3K27me1, suggesting that Cd preferentially affects the former. Alternatively, results of 24-h Cd exposure in differentiated cells was reversed, where cell proliferation and H3K27me1 dropped at a faster rate than total histone protein. Interestingly, H3K27me1 levels were significantly decreased in differentiated cells due to Cd exposure when compared to respective controls or undifferentiated mES cells. The data suggests that Cd targets histone protein production early in stem cell development and H3K27me1 in later stages. Histone H3 is a core histone protein in chromatin while H3K27me1 is associated with transcriptional activation. Thus, if low dose acute exposure to Cd selectively suppresses total histone protein in undifferentiated mES cells then it is capable of disrupting chromatin structure, an effect not seen in differentially matured cells. Additionally, Cd exposure suppresses H3K27me1 levels in differentiated cells, thereby affecting transcriptional activation in the absence of mutual alterations.

### 1247 TRANSPLACENTAL EXPOSURE TO LOW-DOSE ARSENIC IN MICE ALTERS DNA METHYLATION AND EXPRESSION OF CELL CYCLE GENES.

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Exposure to arsenic has been linked to diabetes, cardiovascular diseases, and several types of cancer. Recent epidemiological data have shown that early-life exposure to arsenic may have a significant impact on human health. The EPA has set the action level for the amount of arsenic in drinking water at 10ppb, although many in rural Maine may be exposed to higher concentrations in their well water. Validation of a mouse transplacental model provides a method to uncover toxic mechanisms. Epigenetic studies have shown that exposure to toxicants during early development can modify genome expression patterns that may impact susceptibility to disease in later life. To examine consequences of early life exposure, female C57BL/6j mice were exposed to sodium arsenite (0, 10, 50, 500 ppb) in drinking water 4 days prior to parturition over 2 to 3 months was associated with decreased repetitive element methylation using blood sample. However, the toxicological evidence is lacking. The objective of this study is to investigate the effect of partuculate matter (PM) on DNA methylation in lung cells of healthy Sprague Dawley (SD) rats. We hypothesized that chronic exposure to particulate air pollution might also modify DNA methylation and be a potential mechanism by which chronic air pollution exposure adversely affects health. To explore methylation changes in response to PM exposure, we used the methylated-CpG island recovery assay (MIRA)-assisted microarray method. Agilent 105K CpG microarray, Gene Pix 4000A Scanner, and GenePix Pro 4.0 software were used to identify methylated target genes. SD rats exposed to PM had genes (Ado, Igf2, Inha, Kcnm3, Klc3, LOC691448, Pcdh11, Pmch, Pramel1, Rasl12, predicted, RGD1307381, RGD1310111, RGD136214, predicted, Sox15, predicted, and Trim52) that have an increased level of promoter CpG island methylation relative to the controls without exposure to PM. The difference observed comparing non-exposure and exposure lung tissue samples may be suggestive of a change in DNA methylation induced by PM. Future studies are needed.

### 1248 DIFFERENTIAL EFFECTS OF CADMIUM IN UNDIFFERENTIATED AND DIFFERENTIATED MOUSE EMBRYONIC STEM CELL (mES) ON TOTAL HISTONE PROTEIN PRODUCTION AND H3 HISTONE MODIFICATION PATHWAYS.

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The fetal basis of adult disease (FEBAD) theorizes that embryonic challenges initiate pathologies in adult life through epigenetic modification of gene expression. We tested the hypothesis that bromate (BrO3-) induces epigenetic changes in renal cells. Analysis of 5-methylcytosine staining, a global marker of epigenetic changes, demonstrated that exposure of normal rat kidney cells to cytosolic doses of BrO3- (100 and 200 ppm) did not affect global DNA methylation after 72 hr. In contrast, BrO3- exposure increased p38, p35 and histone 2AX (H2AX) phosphorylation, and p21 expression, compared to control cells. Treatment of cells with inhibitors of DNA methyltransferase (5-azacytidine) and histone deacetylase (trichostatin A) had additive effects on p38 and p35 phosphorylation, but slightly decreased H2AX phosphorylation, and significantly decreased p21 expression, compared to cells exposed only to BrO3-. We assessed p21 expression under chronic conditions that did not induce cell death or DNA damage (0-10 ppm BrO3- for 28 days). Under these conditions BrO3- induced a biphasic response in p21 expression, with lower concentrations increasing expression, but higher concentrations decreasing expression. Increases in p21 expression correlated to decreases in p21 gene methylation. To verify that BrO3- has similar effect in vivo, female rats were exposed to KBrO3 in drinking water (0 to 400 mg/kg) for 28 days and the expression and methylation of renal p21 was assessed. Similar to NRK cells, BrO3- exposure increased p21 expression and altered p21 gene methylation at doses that did not increase DNA damage or cell death. Collectively, these data support the novel finding that BrO3- exposure induces epigenetic changes in p21 that correlate to alterations in its expression. We hypothesize that p21 plays a reparative role in DNA repair and differentiation.

### 1249 EFFECTS OF SUBCHRONIC EXPOSURE TO PARTICULATE MATTERS ON DNA METHYLATION OF LUNG TISSUE IN SPRAGUE DAWLEY RATS.

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Epigenetic mechanisms are a potential pathway linking environmental exposures to diseases. Recently, acute air pollution exposure has been associated with changes in DNA methylation in heavily methylated sequences with high representation throughout the human genome. Epidemiological study also indicated that exposure to particulate matter (PM) on DNA methylation in lung cells of healthy Sprague Dawley (SD) rats. We hypothesized that chronic exposure to particulate air pollution might also modify DNA methylation and be a potential mechanism by which chronic air pollution exposure adversely affects health. To explore methylation changes in response to PM exposure, we used the methylated-CpG island recovery assay (MIRA)-assisted microarray method. Agilent 105K CpG microarray, Gene Pix 4000A Scanner, and GenePix Pro 4.0 software were used to identify methylated target genes. SD rats exposed to PM had genes (Ado, Igf2, Inha, Kcnm3, Klc3, LOC691448, Pcdh11, Pmch, Pramel1, Rasl12, predicted, RGD1307381, RGD1310111, RGD136214, predicted, Sox15, predicted, and Trim52) that have an increased level of promoter CpG island methylation relative to the controls without exposure to PM. The difference observed comparing non-exposure and exposure lung tissue samples may be suggestive of a change in DNA methylation induced by PM. Future studies are needed.
Hypomethylation of mir-22 and negative regulation of its apoptosis-related target gene expression in a BPA-exposed HepG2 cell line.

Bisphenol A (BPA), an widely used environmental chemical, is encircled to human life. However, we generally do not know whether or not it can cause negative health effects. One of the representative epigenetic changes that inhibit gene expression is DNA methylation, which has been very well studied in association with cancer and development. Gene function is changed by DNA methylation; however, its genetic code does not change. Our study hypothesized that a post-transcriptional change in DNA occurs due to exposure to BPA. These changes then cause regulation of microRNA and gene expression. To identify these transitional regulations, we conducted microarray-based methylation, miRNA, and gene expression assays. For validation, also we conducted bisulfite sequencing, quantitative real-time PCR, miRNA inhibitor assay, and Western blotting. We found the expression of a miRNA was up-regulated in an miRNA array and real-time PCR. miR-22 has been reported to inhibit estrogen signaling by direct targeting of the estrogen receptor alpha mRNA. Taking notice of this point, we analyzed gene expression profiles that included its predicted targets. In the present study, we found the cause of hypomethylation of mir-22 and negative regulation of its apoptosis-related target gene expression by BPA-exposure. These results suggest that BPA can alter sequential genomic appearances in HepG2 cells, a potentially effective BPA toxicity. Also, the results of our study support that toxicology study need to integrated analysis of array-based assays for help in understanding of the molecular action of environmental toxicants.

DNA methylation and persistent organic pollutants (POPs) in systemic lupus erythematosus.

Hypomethylation (reduced 5-methylcytosine or 5-mC) has been observed in Systemic Lupus Erythematosus (SLE), and it is known that demethylating agents can cause a reversible lupus-like syndrome in humans. In addition, separate studies have demonstrated inverse correlations between exposure to certain persistent organic pollutants (POPs) and 5-mC levels. We hypothesized that the SLE in Gullah Health (SLEIGH) cohort would exhibit hypomethylation in SLE patients and that 5-mC would be influenced by exposure to POPs that are known to be present in the SLEIGHL geographic area. Whole blood and serum were collected from subjects at the same study visit, and DNA was purified from PBMCs from whole blood samples. Serum was analyzed for several species of PFAAs and PBDEs via HPLC, and 5-mC was quantified using commercial ELISA-like kits with an antibody specific for 5-mC. Total 5-mC (0.67% of DNA, range 0.3-1.4%) was detected in PBMCs of all subjects (n=90); however there were no differences in 5-mC mean levels between patients and controls. In addition, there was no evidence of hypomethylation based on antinuclear antibody (ANA) status, nor did 5-mC levels differ based on ANA status for patients or controls. We did observe decreased levels of 5-mC (20%) in female (n=80) compared to male (n=10) subjects. POPs were analyzed in 12 subjects, PBDE exposure did not correlate to total 5-mC levels for any of the species tested. Conversely, PFNA and PFDA showed positive correlations to 5-mC levels, and PFUnDA showed a trend towards a positive correlation.
1252 EFFECT OF PRENATAL EXPOSURE TO DIETHYLSTILBESTROL (DES) ON MICRORNA PROFILE IN THYMOCYTES.

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Micro RNAs (miRs) are small (19–22-nucleotide) noncoding RNAs that account for 1% of the genome and play a critical role in various cellular processes. They are post-transcriptional regulators that bind to complementary sequences on target messenger RNA transcripts. Prenatal exposure to diethylstilbestrol (DES) is known to cause altered immune functions and increased susceptibility to autoimmune disease in humans. Also, experimental studies indicated that prenatal exposure to DES causes alterations in T cell differentiation in the thymus. In the current study, therefore, we investigated the effects of prenatal exposure to DES on miR profile in thymocytes and examined their role in causing immune dysfunction in neonatal mice. Of the 610 miRs examined by performing high-throughput miR arrays with thymocytes on postnatal day 18 (GD18) of C57Bl/6 mice exposed to DES, we observed more than 60 miRs that were up- or down-regulated (-1.5-fold) when compared to vehicle treated group. Upon further analyses, we observed that DES-mediated changes in miR expression may be involved in important functions such as apoptosis, toxicity, and cancer. Some of the miRs such as miR-18a, -18b, -23a, -23b, -98, -146a, -146b, -677 were downregulated in DES-exposed fetal thymus when compared to controls. We selected miR-18b and miR-23a for further analysis as they possess binding affinity for FasL and Fas 3’UTR regions respectively. We confirmed their expression in DES-treated thymuses by performing Real-Time PCR. Our studies demonstrated significant blocking of FasL or Fas expression in the presence of miR-18b and miR-23a respectively and the effects of these were reversed in the presence of DES. Together, these data demonstrate that prenatal exposure to DES can cause alterations in thymocyte differentiation through dysregulation in miR leading to altered gene expression (Supported in part by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755).

1255 ALTERATIONS OF GENE METHYLATION IN THE LIVERS OF RATS AND MICE CHRONICALLY EXPOSED TO LOW-DOSE CADMIUM.

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Cadmium (Cd) has been classified as a human carcinogen probably associated with epigenetic changes. DNA methylation is one of several epigenetic mechanisms by which cells control gene expression. Therefore, the present study was designed to aim at genome-wide screening of the altered methylation genes in the livers of rats chronically exposed to low-dose Cd. Rats were exposed to Cd at 20 nmol/kg every other day for 4 weeks and gene methylation was analyzed at the 52nd week with methylated DNA immunoprecipitation-CpG island (CGI) microarray. Among the 1629 altered gene promoter CGIs 675 gene CGI methylation levels were up-regulated, 899 gene CGI methylation levels were down-regulated, and 55 gene CGI methylation levels partially up-regulated or down-regulated. We performed comprehensive comparative bioinformatics analysis of microarray data in Cd treated and control group. Changes in gene methylation with Cd revealed that 25 Gene Ontology terms (GO) were up-regulated and 35 GOs were down-regulated; 6 pathways were up-regulated and 22 pathways were down-regulated. Furthermore, up-regulated CGI methylation of caspase-8 gene was confirmed in Cd treated mice by quantitative real-time PCR. To link the increased CGI methylation of caspase-8 promoter to the pathological changes induced by previous exposure to chronic Cd, mice were given chronic exposure of Cd with and without methylation inhibitor (5-aza-2-deoxycytidine) for 6 weeks and at the 60th week caspase-8 promoter CGI methylation was examined. Results showed that levels of mice previously exposed to chronic Cd displayed an increased caspase-8 CGI methylation along with the decreased cell death and increased cell proliferation, which was prevented by 5-aza treatment. These results suggest that DNA methylation of caspase-8 gene promoter down-regulated its activity, leading to a reduction of hepatic apoptosis, and may be a potential cause of Cd-induced hepatic cancer.

1253 METHIONINE SUPPLEMENTATION OR RESTRICTION HAS NO EFFECTS ON LIPOPEROXIDATION BUT ITS RESTRICTION REDUCES DNA DAMAGE IN MICE LIVER.

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Methionine (Met) is an amino acid present in diet and metabolized by liver. It is required for protein synthesis and as methyl donor in the DNA methylation process, especially during pregnancy. Its metabolization product, homocysteine (HCY), might interfere with reactive oxygen species (ROS) production and consequently the lipoperoxidation induction by evaluating the thiobarbituric acid reactive substances (TBARS). These TBARS can be genotoxic disturbing the genome stability. Methionine (Met) is an amino acid present in diet and metabolized by liver. It is required for protein synthesis and as methyl donor in the DNA methylation process, especially during pregnancy. Its metabolization product, homocysteine (HCY), might interfere with reactive oxygen species (ROS) production and consequently the lipoperoxidation induction by evaluating the thiobarbituric acid reactive substances (TBARS). These TBARS can be genotoxic disturbing the genome stability. Methionine (Met) is an amino acid present in diet and metabolized by liver. It is required for protein synthesis and as methyl donor in the DNA methylation process, especially during pregnancy. Its metabolization product, homocysteine (HCY), might interfere with reactive oxygen species (ROS) production and consequently the lipoperoxidation induction by evaluating the thiobarbituric acid reactive substances (TBARS). These TBARS can be genotoxic disturbing the genome stability. Methionine (Met) is an amino acid present in diet and metabolized by liver. It is required for protein synthesis and as methyl donor in the DNA methylation process, especially during pregnancy. Its metabolization product, homocysteine (HCY), might interfere with reactive oxygen species (ROS) production and consequently the lipoperoxidation induction by evaluating the thiobarbituric acid reactive substances (TBARS). These TBARS can be genotoxic disturbing the genome stability.

1254 ARSENIC-INDUCED MALIGNANT TRANSFORMATION CONTRIBUTES TO LONG-RANGE EPIGENETIC SILENCING THROUGH DNA METHYLATION.

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Long-term exposure to arsenicals can induce malignant transformation of immortalized urothelial and prostate epithelial cells which is accompanied by widespread DNA甲基化 changes. Epigenetic dysfunction is known to play an important role in the genesis of all cancers and recent studies have discovered large regions of the epigenome that undergo long range epigenetic silencing (LRES). Our aim is to determine whether long-term arsenical exposure contributes to LRES by altering the DNA methylation profile. To this end, we compared immortalized urothelial and prostate epithelial cells to their arsenic transformed counterparts using methyl DNA immunoprecipitations coupled to human promoter microarrays. In the arsenic transformed prostate epithelial cell line, 12.5kb of DNA within the protocadherin cluster is hypermethylated. This is consistent with previous reports of LRES at the protocadherin cluster in Wilms' tumors and breast cancers. Additionally, some such as EN1 and HOXD11 are also within known regions of LRES and become hypermethylated in the malignant prostate cells. Arsenic transformed urothelial cells also display DNA hypermethylation in the HOXD cluster, which contains ~7kb of hypermethylated DNA. These data indicate that chronic arsenic exposure contributes to LRES during malignant transformation through DNA hypermethylation and suggest that silencing these gene clusters may play an important role in malignant transformation.

1256 MECPI2 EXPRESSION IN DIFFERENTIATING P19 MOUSE EMBRYOCARCINOMA AND HUMAN GLOBLASTOMA NEUROSPHERE CELLS.

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Aberant epigenetic regulation is being investigated as a possible mechanism by which stem and progenitor cells undergo neoplastic progression. Stem and progenitor cells are more sensitive targets for carcinogens and, consequently, changes in
the stem cell population would be expected to be a risk factor for carcinogenesis. An epigenetic mechanism affecting differentiation of astrocyes and neurons involves methyl DNA binding protein 2 (MeCP2), which is highly expressed in the brain and is known to mediate methylation-dependent gene silencing. We are using two models of stem cells to investigate the role of MeCP2 in differentiation: (1) P19 mouse embryocarcinoma cells, which have the potential to differentiate into neurons, cardiomyocytes, and trophoblasts and (2) human glioblastoma (GBM)–derived cancer stem cells, which display high capacity for tumor propagation in mice and the potential to differentiate into neuronal and glial cells. MeCP2 levels progressively increase in P19 cells that are induced to undergo differentiation with retinoic acid. The appearance of MeCP2 first occurs when the dendritic protein Map2e appears and progressively increases at later stages of differentiation when the synaptic protein synaptoxins is observed. In P19 and GBM cells, the major MeCP2 protein is in the cytosol at 105 kDa where in maturing neurons MeCP2 is in the nucleus with a molecular mass of 75 kDa. In GBM -derived cancer stem cells, differentiation resulted in increased levels of MeCP2 in the nucleus but no change in total levels of MeCP2 protein or MeCP2 mRNA. Furthermore, levels of two MeCP2 targets, glutaminase and inhibitor of DNA-binding 1, decreased in differentiating cells. Further studies are directed to understanding the involvement of MeCP2 in differentiation and regulation of genes involved in stem cell differentiation and tumor formation.

1257 REPEAT-DRIVEN METASTABILITY OF DNA METHYLATION IN RESPONSE TO ENVIRONMENTAL EXPOSURES.
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Select retrotransposons in the long terminal repeat (LTR) class exhibit environmental sensitivity in DNA methylation status, resulting in variable expressivity of nearby genes. In the agouti viable yellow (Avy) mouse, a recently inserted LTR, an intracisternal A particle (IAP) element, displays interindividual variation in methylation, resulting in genetically identical individuals with varying coat color and obesity phenotypes. Further, the distribution of these phenotypes is sensitive to both environmental and stochastic change. A locus such as the Avy allele with variable gene expression due to inconsistent DNA methylation without alterations in the underlying DNA sequence is termed a “metastable epiallele.” Using a computational approach within the mouse genome, we identified 12,000 IAP LTRs and filtered by subtype to yield 1,453 IAP LTRs of the most active class. Within these, 116 were phylogenetically similar to Avy and from this set, 19 loci were randomly chosen for verification, of which 6 amplified and 2 showed stochastic metastability. To validate our candidate IAP LTRs for environmental metastability, we assayed N-26 isogenic a/a mice (11 males and 15 females) exposed in utero to either bisphenol A (BPA) in the diet (50 mg/kg and 50 ug/kg or lead (Pb) in drinking water (2.1 ppm and 32 ppm) for shifts in tail DNA methylation patterns. Thus, through the combination of computational methods and experimental verification, we increase the number of known epigeneditically modifiable loci and provide evidence of their environmental liability. Since repetitive elements comprise 46% and 39% of the human and mouse genomes, respectively, and must be repressed, they are likely targets for toxicological disruption, especially during early development. The characterization of repeat driven metastable epialleles in mice, the most widely used animal model, is crucial for the discovery of parallel metastable epialleles in humans as well as the development of strategies for the prevention and treatment of human disease.

1258 AGE AND SEX DIFFERENCES IN LIVER MRNA EXPRESSION DURING THE RAT LIFE CYCLE.

Increasing evidence for epigenetic regulatory mechanisms of gene regulation has fueled interest in the role of miRNAs in toxicogenomics and biomarker discovery. While relatively immature in comparison to other genomic resources, the growing knowledge base of individual miRNAs and their putative gene targets allows for large scale inquiry into more comprehensive, genome-wide analysis of miRNA expression. Liver tissues in the F-344 rat model system were examined over the life cycle for expression of miRNAs during development and aging. Hepatic miRNA expression was characterized at 2, 5, 6, 8, 15, 21, 52, 78, and 104 weeks of age in both sexes using Agilent 8x15k rat miRNA microarrays containing multiple probes for 677 unique miRNAs. Five animals per sex and age were used for at least 90 samples. Agilent’s Feature Extraction software was used to perform initial analysis and processing of the raw data and 199 miRNAs were found to be expressed at least once per sex. In 287 of the expressed miRNAs (28%) exhibited predominant levels of expression early in the life cycle at 2 weeks of age followed by low or no expression at subsequent ages. Among these early expressed miRNAs was miR-335 which has been shown to play a role in hepatic stem cell activation during liver regeneration. Furthermore, miR-33a, which is known to target SIRT1 in humans, a key regulator of various metabolic diseases, exhibits age-specific increases in expression beginning at 52 weeks of age (t-test, p < 0.05). Sex-differences in miR-378 expression were evident at 52 and 78 weeks of age (F > M). miR-378 has been shown to directly regulate CYP2E1 in humans, a key Phase I drug metabolizing enzyme. Collectively, these results comprise one of the first large-scale characterizations of global miRNAs in the liver over the entire rat life span and sex and age-dependent effects that may impact drug metabolism and liver disease.

1259 EFFECTS OF INSULIN-LIKE GROWTH FACTOR-2 (IGF2) LOSS OF IMPRINTING IN AGING MURINE PROSTATE TISSUES.
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Loss of imprinting (LOI) is an epigenetic alteration involving loss of parental origin-specific expression at normally imprinted genes. A LOI for Igf2, a paracrine growth factor, has been implicated in the development of prostate and other cancers. In the current study, we utilized epigenetic mice model of Igf2 LOI to study the effects of LOI and chronic Igf2 overexpression in aging murine prostate. We utilized mouse model of Igf2 LOI (*142) in which three of the four CTCF target sites were imprinted. For Igf2 LOI mice, analyses in *142 mice prostates, male mice homozygous for M. castaneus alleles (H19-p57) were bred with female *142 mutant heterozygous mouse. Igf2 LOI in male mice was analyzed using Fluorescent primer extension assay at maturity and expression analyzed using QPCR. Igf2 LOI mice were aged to 6, 12 and 18 months time points. At maturity, Igf2 LOI mice had significantly higher body weights compared to imprint control mice (32.5 ± 0.53 g vs 28.6 ± 0.37 g; *P = 0.005). Reexpression of silenced Igf2 allele was seen when *142 mutation was inherited from maternal parent. In wild-type mice with no *142 mutation, Igf2 imprinting was maintained in both ventral (VP) and dorsolateral prostate (DLP). Two-fold higher Igf2 expression was seen in VP and DLP tissues of LOI mice compared to control mice prostate tissue. Histopathological changes seen in 6 and 12 month old LOI mice prostates. However, in 18m old Igf2 LOI mice, significant histopathological changes were seen like prostatic hyperplasia and undifferentiated phenotype. Igf2 LOI is also seen in aging human prostates and prostates with tumors. In the present study, we characterized the epigenetic mouse model for Igf2 LOI in prostate tissues. We show that chronic Igf2 overexpression due to LOI of Igf2 has ability to cause undifferentiated phenotype, hyperplasia and speed up the carcinoma process in murine prostate.

1260 EPGENIC EFFECTS OF ENVIRONMENTAL TOXICANTS IN MINORITY CHILDREN.

The CHAMACOS longitudinal birth cohort study investigates exposure to pesticides and other environmental pollutants and their effects on growth and neurodevelopment of children from low-income Mexican-American, farmworker families in California who were followed from birth to 9 years of age. We have found that prenatal exposures to several of these pollutants are associated with shortened gestation and decreased IQ at age 7. Epigenetic changes, particularly DNA methylation, may play a significant role in mediating the effects of environmental exposure on human health and development. Global and site-specific DNA methylation was assessed in 254 newborn- and 9-year-old CHAMACOS children by Illumina Infinium HumanMethylation450K ReadChips to simultaneously interrogate methylation at 485,577 CpG sites, and by pyrosequencing of Alu and LINE-1 repeats. We found that global DNA methylation increased with age and differ by sex but the measures were not correlated and neither showed a trend. Analysis of Illumina data presents an opportunity to assess differential parts of 24,275 genes across methylome. After adjusting for multiple testing by controlling for the False Discovery Rate, we observed that approximately 15.5% of all investigated CpG sites, representing >15,000 genes, were differentially methylated between children at birth and 9 years of age. More than 2% of CpG sites investigated in >1,900 genes, were differentially methylated between children at birth and 9 years of age. Epigenetic changes, particularly DNA methylation, may play a significant role in mediating the effects of environmental exposure on human health and development. Global and site-specific DNA methylation was assessed in 254 newborn- and 9-year-old CHAMACOS children by Illumina Infinium HumanMethylation450K ReadChips to simultaneously interrogate methylation at 485,577 CpG sites, and by pyrosequencing of Alu and LINE-1 repeats. We found that global DNA methylation increased with age and differ by sex but the measures were not correlated and neither showed a trend. Analysis of Illumina data presents an opportunity to assess differential parts of 24,275 genes across methylome. After adjusting for multiple testing by controlling for the False Discovery Rate, we observed that approximately 15.5% of all investigated CpG sites, representing >15,000 genes, were differentially methylated between children at birth and 9 years of age. More than 2% of CpG sites investigated in >1,900 genes, were differentially methylated between children at birth and 9 years of age.
1261  SITE-SPECIFIC DNA METHYLATION AND OBESITY IN MEXICAN-AMERICAN CHILDREN.


In the last 30 years there has been a sharp increase in obesity among children, and minority populations are particularly vulnerable. Although etiology of obesity is thought to be multifactorial with causes stemming from diet, environment, genetics and their interaction, no clear molecular pathways have been identified. However, there is increasing evidence that epigenetic changes, specifically differential CpG methylation, play important roles in determining body weight and metabolic status. The goals of this study are to (1) characterize associations between site-specific methylation and obesity in newborns and 9-year old children and (2) determine whether methylation of the key adipogenic gene peroxisome proliferator-activated receptor gamma (PPARγ) is associated with child obesity and adiponectin levels. Site-specific DNA methylation, interrogating 485,577 CpG sites, was assessed in 138 newborns and 9-year old CHAMACOS boys and girls by Illumina Infinium 450k BeadChips. Adiponectin and leptin levels were measured for a subset (n=105) of these 9-year old children. Preliminary results showed negative associations between child adiponectin and triglycerides (β=-0.43, P<0.01) and very low-density lipoproteins (β=-0.08, P=0.01) and systolic blood pressure (β=0.06, P=0.02), adjusting for child BMI z-score. Child leptin levels were positively associated with systolic and diastolic blood pressure (P<0.01; P=0.07). Further, we found that higher PPARγ methylation was directly associated with higher adiponectin (β=15.4, p<0.01, n=53) and negatively associated with lower leptin (β=-0.16, p<0.08, n=53), in girls. This research takes advantage of the samples and data from the ongoing longitudinal study of Mexican-American mothers and children from Salinas Valley, CA (CHAMACOS). This population is amenable to studying pathways of obesity as 53% of children are overweight or obese, improving power to examine effects of methylation on obesity and related metabolic parameters and biomarkers. Supported by NIEHS and EPA grants.

1262  EPGENIC ALTERATION IN THE TESTICULAR GENE EXPRESSION FOLLOWING IN UTERO TRICLOSAN EXPOSURE.

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Transgenerational epigenetic modification is playing an important role in regulation of testicular development and spermatogenesis. Previous study has been indicated that several genes in the testes are regulated by epigenetic mechanisms on the process of spermatogenesis. The current study investigates the direct effects of in utero triclosan exposure on the testes of F1 mice. Pregnant BL6 mice were treated with triclosan (0, 10 or 50 mg/kg/day) from 8 days before mating to gestation day (GD) 17 and male pups were sacrificed at PND 42. In F1 mice, dose-dependent increase in sex ratio was observed at dose of 10 mg/kg triclosan compared to control. However, and the ratio of anogenital distance (AGD) was significantly decreased in both male and female F1 mice. Down-regulation of acetylated H3 and H4 and the up-regulation of HDACs were observed in the testis and caudal epididymis of mice treated with triclosan. Microarray analyses were performed to compare control and triclosan treated testis transcriptomes. A total of 250 differentially expressed genes (DEGs) were identified and the major cellular functions and pathways associated with these altered transcripts were examined. The sets of regulated genes at the testes were found to be protein metabolism, intracellular protein traffic, signal transduction and cell cycle. We measured the DNA methylation status in several genes and Rrh and Cyp4f40 were hypermethylated in the promoter region. Triclosan may affect fertility via epigenetic modifications, but specific cellular pathway involved in spermatogenesis is needed to understanding molecular mechanism of triclosan-induced male infertility.

1263  MOLECULAR MECHANISMS OF SILENCING AND REACTIVATION OF LINE-1 RETROELEMENT BY BENZO(A)PYREN.

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The mammalian genome is largely constituted by repetitive elements that play important roles in global control of chromatin structure and function. Long Interspersed Nuclear Element-1 (LINE 1 or L1) is a retroelement that mobilizes within the mammalian genome via a copy and paste mechanism involving RNA intermediates and self-enclosed reverse transcriptase activity. We recently reported that the retinoblastoma (Rb) family of proteins regulates the epigenetic landscape around the L1 promoter by stabilizing histone methylation marks and recruiting protein complexes with histone modifying activities. Although the complex transcriptional control mechanisms of L1 are not yet well understood, L1 reactivation has been described in several human cancers and following exposure of human or murine cells to benzo(a)pyrene (BaP), an environmental carcinogen. Here we investigated the epigenetic mechanisms involved in L1 silencing and reactivation of mouse and human cells. Using real time PCR we found that BaP challenge of Rb family-null mouse embryonic fibroblasts (TKO MEFS) exhibited a markedly exaggerated expression of L1 transcripts. Chromatin immunoprecipitation in MCF-7 and HeLa cells using antisera against the pRB-interacting protein complexes showed recruitment of proteins involved in the formation of multiple corepressor complexes to the human L1 promoter. Live cell cycle sorting coupled to L1 mRNA quantification showed that retronetwork expression varies as a function of cell division cycle. On the basis of these data we conclude that the presence of Rb proteins is essential for maintaining L1 epigenetic silencing, and likely this process requires assembly of repressor complexes. These modifications ultimately establish the long-term silencing effect that is lost during the course of environmentally-mediated human and animal disease.

1264  AEROSOLIZATION, FATE, AND PULMONARY TOXICITY OF MESOPOROUS SILICA NANOCAGES.

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Background: Lung cancer is the leading cause of cancer-related deaths worldwide. Many toxic cancer therapeutic drugs are non-specific and damage healthy cells. Nano carriers offer the potential for targeted drug delivery. Functionalized mesoporous silica nanocages (F-MSiN) have internal pores that can store drugs and possess surface modifications to assist in unloading drugs at specific sites. Inhalation allows for direct delivery to the lungs without encountering liver metabolism, systemic dilution, or gastrointestinal proteolytic cleavage experienced in intra-venous and oral delivery. However, the effectiveness and safety of F-MSiN in an inhalation model has not been extensively studied. Objective: To assess aerosolization of F-MSiN in suspension to deliver, detect, and assess toxicity of F-MSiN to the respiratory system. Methods: F-MSiN (50 nm, polyethylene glycol—polyethyleneimine copolymer, fluorescently tagged) were dispersed in nanopure water. Aerosolization was achieved through a micro-droplet nebulizer administered through a nose-only port system to mice for 5 hours. Fluorescent microscopy, SEM, EDS, and TEM analysis of cascade impactors and electrostatic precipitator samples characterized aerosol size distribution. Animals were necropsied 1-day and 8-days post exposure; bronchoalveolar lavage fluid (BALF) was collected for fluorescent imaging, cell counts and cell differentials to assess pulmonary inflammation. Results: Aerosolized F-MSiN sizes ranged from 50 nm to 2 μm, appropriate for lung deposition. F-MSiN was found in BALF alveolar macrophages (500-900 nm) at 1 and 8 days. BALF demonstrated no influx of neutrophils or eosinophils. Conclusions: Not only can F-MSiN be effectively aerosolized using a standard nebulizer system, but the process also creates completely respirable particles that reach the entire respiratory system and likely this process requires assembly of repressor complexes. These findings suggest that inhalation delivery of F-MSiN has the potential to be used as drug carriers to directly deposit hydrophobic and even toxic drugs to their targeted sites for respiratory diseases.

1265  PHENOTYPIC ANCHORING OF SUBCHRONIC CARBON NANOTUBE AND ASBESTOS EXPOSURE TO SMALL AIRWAY EPITHELIAL CELLS: LINKING TOXICOCOGENIC AND NEOPLASTIC TRANSFORMATION RESPONSES.

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Recent studies reported that inhaled carbon nanotube (CNT) exposure results in elevated risk for rapid intestinal fibrosis and persistence within exposed tissues. To our knowledge, no study has yet evaluated long-term human health risks associated with chronic pulmonary CNT exposures compared to asbestos, a known lung carcinogen with similar shape. To address this knowledge gap, we conducted sub-chronic in vitro exposures of dispersed single-walled CNT (D-SWCNT), multi-walled CNT (D-MWCNT) and crocidolite asbestos (ASB) to human small airway epithelial cells (SAEC). Ultradeine carbon black (D-UFCB) and dispersant-only exposed cells (DISP) served as negative controls. SAEC were exposed for 25 weeks to 0.02 μg/cm² and evaluated for cancer cell phenotype. Next, mRNA samples were
subjected to whole genome microarray and rrtPCR analyses for toxicogenomic evaluation. Differential gene expression was then analyzed using Ingenuity Pathway Analysis to identify novel mechanisms promoting neoplastic transformation. Both D-SWCNT and D-MWNT-treated cells exhibited increased proliferation, invasion, anchorage-independent cell growth and angiogenesis compared to other treatments. Hierarchical cluster analysis revealed that D-SWCNT and D-MWNT cells were identified by a similar gene expression profile while ASB, D-UPCB and DSSP cells expressed dissimilar genome profiles. Both D-SWCNT and D-MWNT cells expressed significant changes in genes associated with cell death, movement, proliferation and cancer. Top ranked pathway along with western blot analyses identified several altered signaling pathways and transcription factors associated with oncogenesis. Phenotypic anchoring of toxicogenomic response to neoplastic cell transformation following in vitro subchronic nanomaterial exposure can potentially serve to identify novel mechanisms of action and provide human health risk assessment data.

1266 MAGNETITE NANOPARTICLES FUNCTIONALIZED WITH ALPHA TOCOPHYL SACCEINATE: CYTOTOXICITY AND ANTITUMOR EFFECT IN BREAST CANCER CELLS.

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BACKGROUND: Magnetite nanoparticles can be used to enhance and improve the efficiency in anticancer drug delivery. Herein we report the effect of magnetite nanoparticles functionalized with Alpha tocopheryl succinate (alpha-TOS), the most effective form of vitamin E in inducing apoptosis in cancer cells. PURPOSES: To investigate the cytotoxicity and antitumor effect magnetite nanoparticles functionalized with Alpha -TOS. MATERIALS AND METHODS: Magnetite nanoparticles were prepared by a coprecipitation method and functionalized with alpha-TOS. The particle size was analyzed by SEM. Then two different human breast cancer cell lines (MDA MB231 and T47D) were treated with the various concentrations. Its effects on cytotoxicity, cell proliferation, and apoptosis were evaluated using confocal microscopy. RESULTS: We found magnetite nanoparticles coupled to alpha-TOS is more cytototoxic and effective that alpha-TOS alone and inhibits the growth of breast cancer cell at lower doses. We also observed dramatic changes in morphology in treated cells associated to apoptosis and more cellular uptake of the nanoparticles functionalized with alpha-TOS. Conclusion: In this study we found that magnetite nanoparticles when is functionalized with alpha-TOS enhances the anti-tumor effect in breast cancer cells. We propose that addition of magnetite nanoparticles to alpha-TOS may be considered for cancer therapy.

1267 DIFFERENTIAL GENE EXPRESSION CHANGES IN TWO HUMAN HEPATOCYTE MODEL SYSTEMS TO QUANTUM DOT EXPOSURE.

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Quantum dots (Qdots) are a class of engineered nanoparticle (ENP) that hold promise in advancing cancer diagnostic and therapeutic modalities, and thus require preclinical safety assessment. The core structure of semiconductor Qdots is like (form, of an inert metal oxide engineered nanomaterial (ENM) on its distribution, biopersistence, and effects after a single systemic administration to rats, compared to polyhedral 5 to 55 nm ceria. Methods: An aqueous dispersion of citrate-stabilized ceria ENM rods (~ 10 nm diameter, 40 to 650 nm long), synthesized and characterized in-house, was infused intravenously into rats (50 mg/kg), terminated 1 hour or 30 days later. Control rats received vehicle. At termination, 6 organs were weighed and samples collected from multiple sites and blood for cerium determination by ICP-MS, oxidative stress endpoints, and histology. Results: The low-aspect-ratio ceria ENM was not acutely toxic and did not produce mortality. Its initial distribution was similar to 15 to 30 nm polyhedral ceria, whereas at 30 days less was in the liver, skeletal system, and bone marrow. Less of the dose could be accounted for, suggesting more clearance than polyhedral ceria, which are not significantly cleared up to 90 days. Spleen weight was significantly increased at 30 days. Hepatic granulomas were fewer and smaller than after 15 and 30 nm polyhedral ceria. Protein carbonyls in the hippocampus were significantly decreased at 1 h and significantly increased at 30 days. Conclusions: Other than its more limited distribution and apparent more rapid clearance from the rat, this low-aspect-ratio ceria ENM produced effects qualitatively similar to those seen with 5 to 55 nm polyhedral ceria ENM. Support: US EPA STAR Grant RD-833772.

1268 DISTRIBUTION, BIOPERSISTENCE, AND EFFECTS OF A SYSTEMICALLY-INTRODUCED LOW-ASPECT-RATIO CERIA ENGINEERED NANOMATERIAL IN RATS.

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Background: Nanoscale ceria is used as a diesel fuel additive and in chemico-mechanical planarization and is being developed as an antioxidant therapeutic agent. Objectives: To determine the influence of shape, specifically a low-aspect ratio (rod-like) form, of an inert metal oxide engineered nanomaterial (ENM) on its distribution, biopersistence, and effects after a single systemic administration to rats, compared to polyhedral 5 to 55 nm ceria. Methods: An aqueous dispersion of citrate-stabilized ceria ENM rods (~ 10 nm diameter, 40 to 650 nm long), synthesized and characterized in-house, was infused intravenously into rats (50 mg/kg), terminated 1 hour or 30 days later. Control rats received vehicle. At termination, 6 organs were weighed and samples collected from multiple sites and blood for cerium determination by ICP-MS, oxidative stress endpoints, and histology. Results: The low-aspect-ratio ceria ENM was not acutely toxic and did not produce mortality. Its initial distribution was similar to 15 to 30 nm polyhedral ceria, whereas at 30 days less was in the liver, skeletal system, and bone marrow. Less of the dose could be accounted for, suggesting more clearance than polyhedral ceria, which are not significantly cleared up to 90 days. Spleen weight was significantly increased at 30 days. Hepatic granulomas were fewer and smaller than after 15 and 30 nm polyhedral ceria. Protein carbonyls in the hippocampus were significantly decreased at 1 h and significantly increased at 30 days. Conclusions: Other than its more limited distribution and apparent more rapid clearance from the rat, this low-aspect-ratio ceria ENM produced effects qualitatively similar to those seen with 5 to 55 nm polyhedral ceria ENM. Support: US EPA STAR Grant RD-833772.

1269 SUBTOXIC TiO2-NP PREDISPOSES TO THE MITOCHONDRIAL RESPIRATORY CHAIN AND CYTOSKELETON DISRUPTION IN ALVEOLAR EPITHELIAL CELLS.

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Titanium dioxide nanoparticles (TiO2-NPs) are used in an increasing number of human products such as cosmetics, sunscreen, toothpaste and paints. The susceptibility to develop further damage after TiO2 NPs exposure has been less investigated. The exposure. However, the harmful effects associated with TiO2-NPs exposure are not completely described, but it has been demonstrated that reactive oxygen species derived from mitochondria are involved in cytotoxic effects. The aim of this work was to test if a sub-toxic TiO2-NPs (5 μg/cm2 protein) concentration was able to enhance further mitochondrial damage induced by hydrogen peroxide (H2O2, 5 μM), which is a common molecule released during inflammation, in mitochondria isolated from lung tissue. An in silico hypothesis that mitochondrial dysfunction induced by sub-toxic TiO2 NPs exposure, could impact in cytoskeleton organization and to test this hypothesis, alveolar epithelial cells were exposed to sub-toxic TiO2-NPs (5 μg/cm2) and challenged with sub-toxic H2O2 exposure. Our results showed the following parameters for TiO2-NPs characterization: a zeta potential =−10.6±μV, a hydrodynamic diameter =10.6±nm and a polydispersity index =0.317. The isolated mitochondria exposed to non-toxic TiO2 NPs and then exposed to H2O2, developed higher susceptibility to mitochondrial dysfunction function showed as the decrease in respiratory control index (decrease of 50%), mitochondrial membrane potential (70% of decrease), P/O ratio (50% of decrease). In addition, alveolar epithelial cells exposed to H2O2 but previously exposed to TiO2-NPs showed higher cytoskeleton disruption. In conclusion, the sub-toxic TiO2-NPs exposure enhances the susceptibility to cause mitochondrial dysfunction and cytoskeleton disruption induced by H2O2.
**1270** INHALATION EXPOSURE STUDY OF TITANIUM DIOXIDE NANOPIERCLES ON BLEOMYCIN-INDUCED PULMONARY INFLAMMATION IN MICE.

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Titanium dioxide nanoparticle (nTiO2) is widely used in many industrial fields. It is known that it may cause to pulmonary inflammation by inhalation. In this study, we examined the effects of inhaled nTiO2 on pulmonary inflammation in animal model. Mice were induced lung inflammation by intratracheal instillation (TTI) of bleomycin (1 mg/kg). 7 days after instillation, mice were exposed once for 4 hours by inhalation of nTiO2 (0.12, 1.2, 12 mg/m3). The change of inflammation was evaluated by cytokine analysis, histopathology and immunohistochemistry in lung tissue. mRNA expression of IL-1β, MCP-1 and fibronectin was decrease in nTiO2 exposure groups. Histopathological changes of inflammation were inflammatory cell infiltration, bronchoalveolar formation and bronchiole-alveolar hyperplasia. In nTiO2 exposure groups, these findings were dose-dependently decreased. Also, the decrease of IL-6 was observed in nTiO2 12 mg/m3 exposure group comparing to bleomycin-treated control group. These results suggest that nTiO2 may inhibit progress of inflammation by bleomycin.

**1271** THE ROLE OF CCR5 IN INFLAMMATORY RESPONSES TRIGGERED BY SINGLE-WALLED CARBON NANOTUBES.

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Due to the development of new materials and technology, the pollutants in the environment are becoming more varied and complex over time. Recently, holding toxicologists’ attention is the outbreak of unforeseen adverse health effects, as a result of rapid expansion in the application of nanoparticles. In our previous study using ICR mice, we suggested that a single intratracheal instillation of single-walled carbon nanotubes (SWCNTs) induced early lung fibrosis and subchronic tissue damage. In this study, to investigate the role of CCR5 in inflammatory responses by the inflow of SWCNTs in BAL cell composition, cell cycles, cytokines, cell phenotypes, inflammatory response-related proteins, cell surface receptors and histopathology in CCR5 knockout (KO) mice and CCR5 wild-type (WT) mice. Results showed that the distribution of neutrophils in BAL fluid was significantly decreased in KO mice. The expression of apoptosis-related proteins including caspase-3, p53, phospho-p53, p21 and cleaved PARP, TGF beta 1 and mesothelin were markedly increased in KO mice compared with WT mice. Histopathological lesions were also more frequently noted in KO mice. Moreover, the secretion of IL-13 and IL-17 with IL-6 was significantly increased in KO mice compared to WT mice, whereas that of IL-12 significantly decreased in comparison to WT mice. The distribution of B cells and CD8+ T cells was predominant in the inflammatory responses in KO mice, whereas that of T cells and CD4+ T cells was predominant in the inflammatory responses in WT mice. Furthermore, the expression of CCR4 and CCR7 was significantly increased in KO mice. Based on these results, we suggest that the absence CCR5 delays the resolution of inflammatory responses triggered by SWCNTs inflowing into the lungs and shifts Th1-type inflammatory response in the normal state to Th2-type inflammatory response.

**1272** EFFECTS OF CARBON-BASED NANOMATERIALS ON PRIMARY HUMAN IMMUNE-COMPETENT CELLS.

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Due to their novel electrical, optical, mechanical and chemical properties, carbon-based nanomaterials are currently of great interest for a variety of technological as well as biomedical applications. In this study, we investigated the interaction of three carbon-based nanomaterials with immune-competent cells namely graphene oxide (GO), a 2-D nanomaterial composed of layers of carbon atoms forming six-member rings; single-walled carbon nanotubes (SWCNT), a 1-D nanomaterial formed by the rolling of graphene sheets into hollow tubes, and 3-D hollow carbon spheres (HCS). The materials were first confirmed to be free from endotoxin contamination. We then compared the effect of these nanomaterials on cell viability by Trypan Blue exclusion using primary human monocyte-derived macrophages (HMDM). No significant loss of cell viability was seen in cells treated with SWCNTs or GO up to 100 μg/ml up to 48 h, while a dose-dependent cytotoxic effect was seen for HCS. Cell membrane damage was detected by transmission electron microscopy. We also studied the production of the pro-inflammatory cytokines interleukin (IL)-1β and IL-1α, using LPS-primed HMDM. Cytokine and time-dependent activation of IL-1β was noted for cells incubated with SWCNT and HCS, while GO only induce the secretion of IL-1β, but to a lesser degree, at highest dose (100 μg/ml). This work reveals immunotoxicity and/or immunomodulatory effects of these three carbon-based nanomaterials. Supported by the 7th Framework Programme of the European Commission (FP7-NANOMUNE-214281).

**1273** POLYAMIDOAMINE (PAMAM) DENDRIMERS INDUCE EPIGENETIC CHANGES IN A549 CELLS.


Dendrimers are promising nanomaterials that have inspired a vast range of applications in biomedical. Polyamidoamine (PAMAM) dendrimers are the most common class of dendrimers and toxicological studies of PAMAMS are mostly based on the analyses of acute cytotoxicity and cell viability. However, more subtle effects occurring at lower doses that are potentially more relevant to human exposure also need to be addressed. Importantly, whether PAMAMS could induce epigenetic changes still remains to be investigated. In the present study we exposed human alveolar epithelial cells (A549) to 4th generation PAMAM dendrimers with hydroxyl (-OH) and amino (-NH2) terminating groups and measured cell viability, DNA damage, histone modifications, and global DNA methylation. Global changes in DNA methylation were observed using the Illumina Infinium methylation array. Interestingly, PAMAM dendrimers were found to regulate DNA methylation even at low doses (0.01 μM) that do not induce a loss of cell viability, as measured by LDH release assay. Cells exposed to 1 μM of PAMAM-OH displayed more hypomethylation of genes compared to cells exposed to the lower dose (0.01 μM). However, exposing cells to 1 μM of PAMAM-NH2 resulted in more hypermethylation of genes compared to the 0.01 μM dose. Hence, specific changes occur depending on the surface charge (cationic versus neutral) of PAMAMS. Furthermore, immunohistochemistry and western blotting indicated that PAMAMS may induce histone modifications. Finally, DNA damage was observed with the comet assay after exposure to 1 μM doses of both PAMAMS. This study suggests that PAMAMS cause DNA damage and may alter gene expression in cells through epigenetic mechanisms which could, in turn, lead to alterations in cell function.

**1274** DOSE-METRICS FOR NANOPARTICLES IN IN VITRO TOXICITY TESTS.


Traditionally, safe exposure limits to chemical substances are based on mass concentration, for example, as a maximum tolerable daily intake of chemical substance Y in mg per kg body weight. For nanomaterials, characteristics other than chemical composition (e.g. size, shape) may also determine their toxic potential, implying that information on the administered mass of the nanomaterial consisting of chemical substance Y may not be a sufficient description of the dose. As a result, risk assessors are faced with the question of what dose description to use when setting exposure limits for nanomaterials. A simple dose-metric summarizing the material properties to a single number (i.e. administered total number of particles, total mass or total surface area) would be most pragmatic for risk assessment and regulatory purposes, since only one exposure limit would have to be derived for different nanomaterials consisting of chemical substance Y. However, it needs to be demonstrated that the use of such a simple dose-metric is appropriate. We tested whether the dose of spherical silver (20, 80 and 113 nm) and silica (11, 34 and 248 nm) nanoparticles could be appropriately described by one of the simplest dose-metrics administered total number of particles, mass or surface area, using a novel graphical method. Data from in vitro assays on markers of cytotoxicity, inflammation and cell cycle were used to obtain equal-response curves: continuous curves connecting concentrations of particles leading to the same response level in a test system. These curves were compared to those of...
constant total particle surface area S, constant particle mass M and constant number of particles N. This approach demonstrated the existence of a simple dose-metric for nanoparticles, although there were differences in appropriate dose-metrics between silver and silica nanoparticles, and between different toxicity endpoints. Collectively, our results imply that using a simple dose-metric may not always be the best approach when deriving safe exposure limits for nanomaterials.

1275 MULTIWALLED CARBON NANOTUBES, CYTOTOXICITY, AND OXIDATIVE STRESS IN THE LUNGS OF RATS.


As the production of multiwalled carbon nanotubes (MWCNT) increases, so does the potential for occupational human exposure. The high aspect ratio and durability of MWCNT suggests that their toxic properties may be analogous to other fiber particles, such as asbestos. Further, they can occur in many different formulations. In the current study a single acute exposure to MWCNT was tested by inhalation in rats. Three types of MWCNT were tested in the same exposure system: original (o-), purified (p-) and functionalized (f). Functionalized MWCNT were purified MWCNT that were carboxylated. Our hypothesis is that o-MWCNT would cause greater oxidative stress due to the presence of metals attached to the o-MWCNT. To test this, we exposed adult rats nose only to MWCNT aerosol for 6 hrs. Lungs were removed 18-hours post exposure. We assessed airway glutathione (GSH) levels by HPLC and cell permeability in situ in the lung using ethidium homodimer and confocal microscopy. GSH levels were significantly decreased in the o-MWCNT group, with no significant change noted in the other groups. We further challenged the dissected airways with hydrogen peroxide ex vivo to determine if MWCNT exposure differentially changed the ability of the airway to regulate GSH levels in the face of continuing oxidant stress. While hydrogen peroxide exposure depleted GSH, this was not changed by previous exposure to o-, p- or f-MWCNT. The ethidium assay indicated the greatest cytotoxicity from the o-MWCNT as indicated by numerous ethidium positive membrane permeable cells at bifurcations in both proximal and distal airways. The p-MWCNT had few positive cells at proximal bifurcations, but more in distal regions. The f-MWCNT had the least ethidium positive with no difference proximally or distally. These results indicate that the o-MWCNT causes more oxidative stress and cytotoxicity in the lung than the other MWCNT tested. Supported by RC1ES018232, P42ES04699 and UI01ES02027.

1276 MULTIWALLED CARBON NANOTUBES EXERT ASBESTOS-LIKE SUPPRESSIVE EFFECT ON NK CELLS, DECREASED CYTOTOXICITY WITH ALTERED EXPRESSION OF NK CELL-ACTIVATING RECEPTORS.

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Carbon nanotubes (CNTs) are new materials, industrial application of which is expected. However, CNTs are similar to asbestos, long and thin, and CNTs-induced malignant mesothelioma in animals has been reported. We have previously reported that asbestos exerts immune-suppressive effects, decreases in cytotoxicity of NK cells and function of Th1 cells. NK cells in culture with asbestos showed a marked decrease in expression level of NKp46, one of NK cell-activating receptors. Therefore, we examined the effect of multi-walled CNTs (MWCNTs) on cytotoxicity of NK cells, MWCNTs, kindly given by Dr. Hiranved in National Institute for Environmental Studies, were suspended in Phospho-F68 and dispersed by sonication. Human PBMNCs, CD3-CD56+ NK cells or others were cultured with IL-2 Environmental Studies, were suspended in Pluronic F68 and dispersed by sonication. Therefore, we examined the effect of multi-walled CNTs (MWCNTs) on cytotoxicity for NK cells freshly sorted and then cultured upon exposure to MWCNTs, but not to CB. Both of NK cells sorted form PBMC exposed to CNT and CB exhibited decreased cytotoxicity for NKp46-dependent targets. The annexin V+ apoptotic cells in CD14+ monocytes increased by exposure to CB, whereas MWCNTs caused both apoptosis of monocytes and NK cells. The cell growth of NK decreased upon exposure to MWCNTs, but not to CB. These results indicate that MWCNTs exert asbestos-like suppressive effect on NK cells, partly differing from effect of asbestos. These findings may raise an alarm for harm of MWCNTs exposure.

1277 SYNERGISTIC EFFECT OF NANOPARTICLE SIZE AND ALUMINUM CHEMICAL COMPOSITION IN PRIMARILY CULTURED NEURAL CELLS INDUCED BY NANO-ALUMINUM OXIDE.

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Manufactured nano-particles of aluminium oxide (nano-alumina) have been widely used in many areas. However, relatively few studies are available with regard to the neurotoxic effect of nano-alumina. The aim of this study was to determine the contribution of nanoparticle size and aluminum chemical composition to the cellular toxicity of nano-alumina. Size-effect was determined by comparing the toxicity of nano- and micro-particles of alumina, while aluminum chemical composition-effect was determined by comparing the toxicity of nano-alumina and nano-carbon with the identical particle sizes. Toxicity was evaluated by determining the ultra- structural lesions, mitochondrial damage and loss of membrane integrity in cells exposed to different particles. The results showed that nano-particles of alumina induced higher toxicity compared to micro-alumina particles, indicating a nanoparticle size-dependent toxicity induced by nano-alumina. In addition, nanoparticle of alumina induced higher toxicity than that induced by nano-carbon particles, indicating that toxicity induced by nano-particles of alumina was contributed to its unique chemical composition. In conclusion, toxicity of nano-alumina has a synergistic effect of both nanoparticle size and aluminum chemical composition.

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1278 INHALATIONAL EXPOSURE OF CADMIUM OXIDE NANOPARTICLES LEADS TO ENDOCRINE DISRUPTION IN THE REPRODUCTIVE TRACT OF FEMALE MICE.

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We have previously demonstrated that inhalation of cadmium oxide (CdO) nanoparticles (NP) leads to the translocation of Cd to the reproductive tract of pregnant mice. Follow-up studies were recently performed to determine what effects, if any, exposure has on pregnancy-related steroid hormone systems in this model as bulk Cd can act as a “metalloestrogen.” To test the hypothesis that inhaled CdO NP (geometric mean size 11.5±15 nm) can lead to endocrine disruption, timed-pregnant CD-1 mice were exposed (for 2.5 hr) either every other day to 100 μg CdO NP/m3 and euthanized on GD 17.5. In addition, mRNA expression of estrogen receptors (ER) α and β and progesterone receptors (PRs) A and B was determined in uterus, ovary, and placenta from both controls and mice exposed to 230 μg CdO NP/m3 and euthanized on GD 10.5, 14.5, and 17.5. Results demonstrate that the highest CdO NP concentration, serum estradiol (E2) levels were decreased on GD17.5 compared to both control and mice exposed to 100 μg/m3; no changes in progesterone (P) levels were observed. We have previously demonstrated that inhalation of cadmium oxide (CdO) nanoparticles (NP) leads to the translocation of Cd to the reproductive tract of pregnant mice. Follow-up studies were recently performed to determine what effects, if any, exposure has on pregnancy-related steroid hormone systems in this model as bulk Cd can act as a “metalloestrogen.” To test the hypothesis that inhaled CdO NP (geometric mean size 11.5±15 nm) can lead to endocrine disruption, timed-pregnant CD-1 mice were exposed (for 2.5 hr) either every other day to 100 μg CdO NP/m3 and euthanized on GD 17.5. In addition, mRNA expression of estrogen receptors (ER) α and β and progesterone receptors (PRs) A and B was determined in uterus, ovary, and placenta from both controls and mice exposed to 230 μg CdO NP/m3 and euthanized on GD 10.5, 14.5, and 17.5. Results demonstrate that the highest CdO NP concentration, serum estradiol (E2) levels were decreased on GD17.5 compared to both control and mice exposed to 100 μg/m3; no changes in progesterone (P) levels were observed. Inhalation of CdO NP decreased expression of both uterine PRs and ERβ on GD 14.5 and ERα on GD 17, but IRBP on GD 14.5. Exposure of pregnant mice to CdO NP also led to decreased placental expression of ERα on GD 14.5, with no significant changes to the other steroid receptors. Receptor expression in the ovary was unaffected by CdO NP exposure. These data show that Cd associated with inhaled CdO NPs has the potential to affect the molecular endocrine signaling in the reproductive system during pregnancy in both the uterus and placenta. Such changes could lead to altered fetal growth and/or direct effects including epigenetic alterations affecting the offspring later in life. Supported by ES017427, T32ES007324, and NYU Center Grant ES000260.
Mounting evidence indicates that exposure to nanoparticles (NP) is able to modify immune responses. However, cellular and molecular mechanisms of immune responses elicited by NP are poorly understood. In the current study, we evaluated site-specific pulmonary inflammation and systemic immune response in mice after pulmonary exposure to single walled carbon nanotubes (SWCNT). SWCNT exposure caused inflammation, pulmonary damage and an altered cytokine network in the lung. SWCNT-induced inflammation facilitated the recruitment of dendritic cells (DC) to the lung tissues, increasing chances of direct DC/SWCNT interactions. Local inflammatory response in vivo was accompanied by modified systemic immunity as documented by decreased proliferation of splenic T cells. To assess if DC could be responsible for modulation of systemic immunity, in SWCNT-treated mice, we evaluated the ability of SWCNT-exposed DC to alter T cell responses in vitro. Here we demonstrate that co-culturing of T cells with SWCNT-exposed DC suppressed the T cell proliferation response upon re-stimulation with freshly generated, unexposed DC. Further, exposure of DC to SWCNT did not alter DC phenotype. Exposure of DC to E. coli LPS induced phenotypical maturation of DC. When LPS-exposed DC were mixed with T cells we observed facilitated T cell proliferation. Administration of LPS + SWCNT to DC did not change LPS-induced DC phenotypical maturation. Indeed, when T cells were mixed with LPS+SWCNT treated DC we observed decreased proliferation. Combined, these findings suggest that SWCNT do not interfere with recognition of LPS by DC. We can speculate that SWCNT exposure may intervene with antigen capture/processing and/or presentation, thereby leading to compromised DC/T cell interactions. Overall, our data suggest that exposure to SWCNT modifies systemic immunity by modulating DC function.
the risks associated to the use of engineered NP in an industrial scale and recovery processes, we assessed the redox ability of these NP and their induction of cytotoxicity mediated by reactive oxygen species (ROS) in human alveolar epithelial A549 cells. The free-cell dithiothreitol (DTT) oxidation assay showed a greater oxidant activity of CIGS vs. CdS (4:1.2 and 1.2 pmol DTT/μg protein, respectively). To guarantee NP dispersion in culture medium, pretreatment with 2% of BSA was performed, confirmed by SEM and STEM. Induction of cytotoxicity was evaluated using the crystal violet and MTT reduction assays, CIGS induced more cytotoxicity than CdS in all the concentrations and time points tested. We determined intracellular ROS levels using DCF-DA, a 1.46 to 1.92-fold increase after the exposure to CIGS was observed, while CdS increased 1.2 to 1.5-fold, versus control (non-treated cells). To determine whether mitochondria is a potential target for NP we assessed changes in mitochondrial membrane potential (Δψm) where both NP caused a loss in Δψm of 28% and 35% at 6 h and 40% and 28% at 24 h after the exposure to CIGS and CdS, respectively. Antioxidant pre-treatment (Trolox) prevented the loss of Δψm and cytotoxicity induced, suggesting that the induction of ROS is the main mechanism by which these NP are cytotoxic. CdS NP showed a lower, yet steady, ability to induce ROS compared to CIGS, thus long-term effects should be further investigated considering the possible deposition of Cd, a known toxic metal. Our results support the hypothesis that intrinsic properties of NP, i.e. bandgap energy, are underlying factors that determine cellular toxicity of engineered NP.

1284 LOCALIZATION, QUANTIFICATION, AND GENERAL TOXICOLOGY OF INTRAVENOUSLY INJECTED HAFNIUM-BASED NANO Particles (NBTXR3) AFTER REPEATED X-RAY EXPOSURE TO COMPUTED T OMOGRAPHY (CT) IN THE RAT.

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NBTXR3 is developed to potentiate the effect of radiotherapy on solid tumors, by amplifying the production of reactive oxygen species generated by X-ray exposure of its hafnium core. Although NBTXR3 is intended to be injected in the tumor, spilling in the circulation may occur, leading (in rats) to steady trapping of NBTXR3 nanoparticles (NP) in the liver. Clinically, it is unknown whether patients previously treated with NBTXR3, and at risk of having liver-engaged NP, may show toxic signs when exposed to X rays later in life, for instance, during a CT scan. We modeled and maximized this clinical situation in the rat by a single bolus intravenous (IV) administration of NBTXR3, followed by 3 consecutive daily X-ray exposures by CT, focused on liver, which was also used to localize and quantify radio-opaque NP in the liver. The rats were followed for clinical signs, growth, food intake, clinical pathology, and were necropsied for histopathology of the liver and spleen, 5 or 36 days after dosing. The effects of NBTXR3 vs vehicle, CT exposure vs none, and time after exposure (36 versus 5 days) were evaluated. NBTXR3 NP were homogenously distributed in the liver (about 77% of its volume), with no changes in distribution up to 3 days of observation. A single injection of NBTXR3 followed by repeated CT exposure was devoid of effects on health, growth, food intake and clinical pathology. In particular, despite liver trapping of NP, hepatic enzyme activities were unaffected. The liver and spleen structure was normal; only brownish granular pigment deposits (possibly NP) were observed up to a slight extent in the spleen and liver. In conclusion, intravenously-injected NBTXR3 nanoparticles were homogenously and steadily distributed in the liver up to 3 days after dosing. NBTXR3 administration, followed by 3 days of once daily CT exposure, did not exert any short- or long-term toxic effects (in particular on the liver), in the rat.

1285 CYTOTOXICITY AND GENOTOXICITY OF SILVER NANO Particles IN HUMAN LUNG CELLS.

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Silver is one of the most commercialized nano-compound due to its conductive, stabilizing, catalytic and antibacterial activities. Silver nanoparticle applications in consumer products range widely from disinfecting medical devices to home appliances. Human exposure occurs in manufacturing settings, through consumer products, and potentially from nanoparticles being released into the environment. Consequently, the potential toxic effects of silver nanoparticles have become a public concern. Thus, it is essential to understand if and how nanoparticles induce damage in normal human cells. To address this issue, we investigated the cytotoxicity and genotoxicity of silver nanoparticles in human lung cells. Three types of silver nanoparticles, bare, pegylated and fluorescein isothiocyanate functionalized (FITC), were used in this study. CdS nanoparticles were more toxic to human lung cells after 24 h exposure than the other two particle types. Both bare and pegylated particles were toxic to the cells after chronic exposure. Bare and pegylated silver nanoparticles did not induce chromosome damage in human lung fibroblast cells after 24 or 120 h exposures. FITC silver nanoparticles were weakly clastogenic after a 24 h exposure but were not clastogenic after a 120 h exposure. There was no induction of DNA double strand breaks in cells exposed to silver nanoparticles for 24 h, however, after 120 h exposure, a weak induction of DNA double strand breaks are occurred. Further work will include characterize silver nanoparticle-induced chromosome instability in human lung cells. This work was supported by ARO grant W911NF-09-1-0296 (J.P.W.).

1286 KIDNEY INJURY MOLECULE-1 AS AN EARLY BIOMARKER OF RENAL INJURY FOLLOWING INHALATIONAL EXPOSURE TO CADMIUM OXIDE NANO Particles BY FEMALE MICE: COMPARISON WITH URINARY PROTEIN, CREATININE, AND GLUCOSE.

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The use of nanoparticles (NPs) is becoming pervasive in many areas of modern life. However, little is known regarding the potential toxicities of these particles. One industrially important NP is cadmium oxide (CdO) that is used as a starting material for other Cd-containing NPs that are then used in medicine, batteries, solar cells, and color pigments. While Cd is a well-known nephrotoxicant, previous animal studies have only examined the nephrotoxicity of soluble Cd salts and/or particles > 100 nm. Since NPs can behave in a manner different than their bulk-sized counterparts, the nephrotoxic potential of CdO NPs remains unknown. Therefore, a study was performed in pregnant mice examining the effects of inhaled CdO NPs on several parameters of renal toxicity, including changes in total urinary protein, creatinine, glucose, and the novel kidney injury molecule-1 (Kim-1). Kim-1 is a transmembrane glycoprotein that is normally not detectable in the kidney, but is up-regulated and shed into the urine following nephrotoxic injury. Recent studies utilizing a sub-chronic model of Cd exposure in the rat have shown that Kim-1 is a very early urinary marker of Cd-induced renal injury. In the present study, mice were exposed via inhalation to CdO NPs (11-15 nm) at either 100 or 230 μg CdO/m² through day 16.5 of gestation. At euthanasia on GD 17.5, urine was sampled from the bladder and analyzed by ELISA for Kim-1, total protein, creatinine, and glucose content. Urinary Kim-1 increased ~6-fold above control levels in pregnant mice exposed daily for 2.5 h/d to the highest CdO concentration. No significant changes were detected in any of the more traditional urinary biomarkers. Results suggest that urinary Kim-1 may be a useful biomarker for nephrotoxicity in female factory workers exposed by inhalation to CdO NPs. NIEHS ES017427.
were euthanized, bronchoalveolar lavage cells were isolated and processed for scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Results from chemotaxis assay on RAW and BAL macrophages showed that exogenous exposures to SWCNTs resulted in increased cell migration as compared to control cells. Results from TEM study demonstrated the presence of macrophages with irregular and ruffled surface in SWCNTs exposed groups as compared to their respective controls. TEM demonstrated the presence of SWCNTs in phagolysosomes, and vacuoles of bronchoalveolar macrophages. Such particles were arranged in a tubular fashion inside the phagolysosomes and some of the particles appeared to be trapped in the surfactant material inside the vacuoles. These data indicate that SWCNTs increase cell migration in vitro and activate alveolar macrophages resulting in phagocytosis and deposit within the alveolar macrophages of hamsters exposed to inhaled nanotubes.

1289 THE PROINFLAMMATORY RESPONSE OF PORCINE BRAIN MICROVESSEL ENDOTHELIAL CELLS TO METALLIC NANOPARTICLES SHOWS COMPOSITION AND SIZE DEPENDENCY.

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The purpose of the current studies was to determine if systemic exposure of various metallic nanoparticles differing in size and composition (silver (Ag-NPs), 25, 40 and 80 nm, copper oxide (Cu-NPs, 40 and 60 nm) or gold (Au-NPs, 3 and 5 nm)) can induce the release of pro-inflammatory mediators that influence the restrictive permeability characteristics of an in vitro model of the blood-brain barrier (BBB). Confluent porcine brain microvascular endothelial cells (pBMEC) monolayers (8-12 days) were treated with various metallic nanoparticles (15 μg/ml). Extracellular concentrations of pro-inflammatory mediators (IL-1β, TNFα and PGE2) were evaluated by ELISA at 0, 2, 4, 6 and 8 hr following exposure. pBMECs were cultured in standard 12-well transwell inserts and permeability was evaluated following 24-hr apical NP exposure by measuring the transport of fluorescein across the pBMEC monolayers allowing the determination of (apparent) permeability coefficients. PGE2 release following Cu-NP exposure was significantly increased (≈2-fold at 8 hr for 40 and 60 nm) when compared to control. Similar results were observed for Ag-NPs but not Au-NPs. The secretion of TNFα and IL-1β were observed for both Cu-NPs and Ag-NPs but not in response to Au-NPs exposures. The post-treatment time-profiles of TNFα and IL-1β show the IL-1β response to be more persistent when compared to TNFα over the 8-hour experiment. The permeability ratios (exposure/control) were significantly increased following exposure to Cu-NPs or Ag-NPs, but not Au-NPs (approximately 8-fold, 3-fold for Cu-NPs or Ag-NPs, respectively). Together, these data suggest that composition and size of NPs can cause significant proinflammatory response that can influence the integrity of the BBB.
There are many efforts in understanding the effects of nanoparticles on cells, however, not much is known regarding the distinct molecular mechanisms of inflammation and cellular stress using low dose concentrations. To address this gap in the literature, we utilized a novel experimental design that specifically probes the effects of a panel of commonly studied engineered nanomaterials along immunomodulatory pathways. The panel of particles selected for this study included quantum dot nanocrystals (QDs), titanium dioxide (TiO2), hydroxylated fullerenes, and silver (Ag) nanoparticles. Cell viability, antioxidant activity, select messenger RNA, and protein modulation were studied in primary human dermal fibroblasts (HDF) and NF-kB knockdown HDF. Inflammatory and non-inflammatory immune responses were measured using protein and real time PCR array analysis from HDF exposed to sub-lethal concentrations of nanoparticles. Differences in cellular response to nanoparticles in protein and antioxidant experiments were evident in NF-kB knockdown cells. All nanoparticles caused concentration-dependent (10-200 ppm), time-dependent (8 h or 24 h), and NF-kB-dependent cell death, as evidenced by membrane integrity-based viability studies. The order of NF-kB/MAPK response intensity was as follows: Ag-fullerol-QD> TiO2, ERK1/2 phosphorylation was nearly concurrent with phosphorylation of the NF-kB inhibitor, IKB, in Ag, fulleroil, and TiO2-treated cells. Phosphorylation of p38 occurred after exposure to all four nanoparticles. Heme oxygenase-1 was upregulated with Ag exposure only. Phosphorylated p38 expression decreased with NF-kB knockdown. Nanoparticles also caused modulation of genes known to be associated with inflammatory (IL-1, IL-6, IKBKB, and TLR3), immune (CD55 and IFNA1), oxidative stress (HMOX1 and FN1), and apoptotic (NLR4) responses. The methods used in the study, along with the resultant data sets, serve as a potential model for studying the complex pathway-specific biochemical responses in cell and tissue systems associated with nanoparticle exposures.

Silver nanoparticles (AgNPs) have been used to manufacture nanomaterials with new biophysical properties and functions. However, few experimental approaches have been used to assess their potential toxic or beneficial effects on human health, in association with size, concentration and biological target. The aim of this work was to evaluate the effects of the AgNPs on the rat tracheal smooth muscle. A single administration of AgNPs did not modify the smooth muscle tone. However, when the tracheal rings were pre-treated with acetylcholine (ACh), exposure to AgNPs, resulted in a contractile effect. Simultaneous administration of AgNPs and ACh led to a slight increase in smooth muscle contractility. AgNPs pretreatment followed by ACh administration, showed contraction effect induced by ACh after which muscle tone did not return to the basal level. This effect was associated with a large production of nitric oxide (NO). The contractile response to the AgNPs induced by ACh was completely blocked when the tracheal rings were incubated following the ACh but before the AgNPs administration, with 1400 W (a specific blocker of the inducible nitric oxide synthase, an enzyme that generates high levels of NO during hyper-reactivity). The contractile effect was also abolished by atropine, which suggests that AgNPs alter ACh muscarinic receptor signaling, through the activation of iNOS expression. These data also show that AgNPs modify the contractile action dependent on ACh-NO, possibly inducing hyper-reactivity of the tracheal smooth muscle.
dehydrogenase (LDH) and cytokines. Histopathology of the lungs was evaluated. Total number of cells in BAL was significantly (p<0.01) increased in animals necropsied at 0 wk post exposure in comparison with sentinels (192 and 99 x 10^3 cells/mouse, respectively). Total cell counts returned to baseline 2 wks post exposure. There was no inflammatory recruitment of neutrophils to the lungs in either group. The percentage of CNT-laden macrophages in BAL decreased from 78% at 0 wk to 65% at 2 wks post exposure, suggesting some clearance of material; however the majority of macrophages were still filled with CNT. Particles were found to be evenly distributed in the lung tissues immediately post exposure. Masson’s trichrome stain showed higher collagen deposition at 0 wk in comparison with senile animals (280 SOT 2012 ANNUAL MEETING).

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Silica nanoparticles (SiO2-NPs) are widely used in nanotechnology industry and applications in various products. Previous studies have indicated that nanoparticles can induce oxidative stress damage leading to cell death. Alveolar type II epithelial cells are known to be vulnerable to oxidative stress. However, the toxic effect of SiO2-NPs on alveolar type II epithelial cell damage remains unclear. In this study, cells are known to be vulnerable to oxidative stress. However, the toxic effect of SiO2-NPs on alveolar type II epithelial cell damage remains unclear. In this study, the aim of this study was to determine whether exposure to three different formulations of MWCNTs cause lung inflammation and cellular injury, dependent upon dose and physicochemical particle characteristics. The three formulations of MWCNTs used were the original material (o); a second form with metals partially removed (p); and a third form in which the surface was functionalized through carboxylation (f). To determine a dose response effect of exposure to MWCNTs, male Sprague Dawley rats were subjected to 0, 10, 50, or 200 μg of MWCNT via intratracheal instillation. At 1 or 21 days post-exposure, bronchoalveolar lavage fluid (BALF) was recovered for analysis. One day post-exposure there was a significant difference in the cell differential found in the BAL between sham controls and those exposed to the only highest dose of all three MWCNT types due to the influx of neutrophils into the lung airspaces. By 21 days the number of neutrophils in BAL decreased, but still remained significantly elevated above control values for only o- and p-MWCNTs, while the BAL from animals exposed to f-MWCNTs returned to sham control levels. These findings suggest modification of the surface of MWCNTs, but not partial metal removal, reduces their toxicity to the lungs.

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Nanotechnologies and products are enabling advancements in medicine, energy, and commercial products, such as electronics and cosmetics. Multivalved carbon nanotubes (MWCNTs) are of special interest due to their unusual tensile strength, thermal conductivity, and highly marketable electrical properties. At the same time, morphological similarities of MWCNTs to needle-like asbestos fibers, associated with several cancerous and non-cancerous lung pathologies, are cause for concern. To date, only a few studies have investigated MWCNTs in vivo. While workers, consumers, or the general public may potentially be exposed to nanoparticles through a number of pathways (e.g., dermal, ingestion, ocular), inhalation, at least from an occupational standpoint, is likely to be one of the most significant routes of exposure. The aim of this study was to determine whether exposure to three different formulations of MWCNTs cause lung inflammation and/or cellular injury, dependent upon dose and physicochemical particle characteristics. The three formulations of MWCNTs used were the original material (o); a second form with metals partially removed (p); and a third form in which the surface was functionalized through carboxylation (f). To determine a dose response effect of exposure to MWCNTs, male Sprague Dawley rats were subjected to 0, 10, 50, or 200 μg of MWCNT via intratracheal instillation. At 1 or 21 days post-exposure, bronchoalveolar lavage fluid (BALF) was recovered for analysis. One day post-exposure there was a significant difference in the cell differential found in the BAL between sham controls and those exposed to the only highest dose of all three MWCNT types due to the influx of neutrophils into the lung airspaces. By 21 days the number of neutrophils in BAL decreased, but still remained significantly elevated above control values for only o- and p-MWCNTs, while the BAL from animals exposed to f-MWCNTs returned to sham control levels. These findings suggest modification of the surface of MWCNTs, but not partial metal removal, reduces their toxicity to the lungs.


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Testing has begun as part of the EPA Endocrine Disruptor Screening Program (EDSP) Tier 1 battery of 11 in vitro and in vivo tests. A recognized issue with the EDSP is that the current Tier 1 screening battery is highly resource intensive in terms of cost, time, and animal usage for the large numbers of chemicals with unknown endocrine potential that need to be evaluated. The significant advances in both computational and molecular technologies have enabled a more rapid identification of markers for adverse outcome pathways since EPA began work on developing and implementing the EDSP. The EPA is proposing to evolve the EDSP by incorporating in vitro high-throughput screening (HTS) assays that can rapidly detect potential interactions of chemicals with the estrogen, androgen, thyroid hormone, and steroidogenesis (EATS) pathways. In the near term, incorporating HTS assays will focus on developing a prioritized list of chemicals for evaluation in the current Tier 1 battery. Prioritization would continue to take other factors into account, including exposure and use. A longer term goal is to evolve the Tier 1 battery by fully incorporating HTS assays in order to increase reliance on nonanimal screens for which there is confidence in their ability to predict in vivo adverse effects. Although the overall approach is reasonable, it is highly provocative and debatable for a number of reasons. On the one hand, this proposal has the potential to greatly improve the speed, cost effectiveness, and mechanistic specificity of the EDSP using fewer animals, but on the other hand, there are concerns about reliability and relevance of the HTS assays and lack of full validation (e.g., transferability between laboratory evaluation) metabolic capacity, etc. Our panel of experts will present the case for and against using this approach and will allow time for open discussion with the audience.

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Chemical safety assessment for the protection of human health is not harmonized globally. Toxicology data requirements and test guidelines that differ across geographies could result in repetition of studies and therefore waste of animals and resources. Another prominent difference is that although exposure and risk assessment approaches under certain circumstances. A roundtable session on opportunities for harmonized approaches for risk assessment can be achieved globally, considering political, economic, and cultural sources. The other prominent difference is that although exposure and risk assessment approaches under certain circumstances. A roundtable session on opportunities for harmonized approaches for risk assessment can be achieved globally, considering political, economic, and cultural sources. The other prominent difference is that although exposure and risk assessment approaches under certain circumstances. A roundtable session on opportunities for harmonized approaches for risk assessment can be achieved globally, considering political, economic, and cultural sources. The other prominent difference is that although exposure and risk assessment approaches under certain circumstances. A roundtable session on opportunities for harmonized approaches for risk assessment can be achieved globally, considering political, economic, and cultural sources. The other prominent difference is that although exposure and risk assessment approaches under certain circumstances. A roundtable session on opportunities for harmonized approaches for risk assessment can be achieved globally, considering political, economic, and cultural sources. The other prominent difference is that although exposure and risk assessment approaches under certain circumstances. A roundtable session on opportunities for harmonized approaches for risk assessment can be achieved globally, considering political, economic, and cultural sources. The other prominent difference is that although exposure and risk assessment approaches under certain circumstances. A roundtable session on opportunities for harmonized approaches for risk assessment can be achieved globally, considering political, economic, and cultural sources. The other prominent difference is that although exposure and risk assessment approaches under certain circumstances. A roundtable session on opportunities for harmonized approaches for risk assessment can be achieved globally, considering political, economic, and cultural sources. The other prominent difference is that although exposure and risk assessment approaches under certain circumstances. A roundtable session on opportunities for harmonized approaches for risk assessment can be achieved globally, considering political, economic, and cultural sources. The other prominent difference is that although exposure and risk assessment approaches under certain circumstances. A roundtable session on opportunities for harmonized approaches for risk assessment can be achieved globally, considering political, economic, and cultural sources. The other prominent difference is that although exposure and risk assessment approaches under certain circumstances. A roundtable session on opportunities for harmonized approaches for risk assessment can be achieved globally, considering political, economic, and cultural sources. The other prominent difference is that although exposure and risk assessment approaches under certain circumstances. A roundtable session on opportunities for harmonized approaches for risk assessment can be achieved globally, considering political, economic, and cultural sources.
1301 TESTING OF NANOMATERIALS FOR GENOTOXICITY—NECESSITY OR WASTE OF TIME?

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The selection or use of genotoxicity assays for testing nanomaterials remains a controversial issue. There are published studies indicating that some nanomaterials are genotoxic in vitro as well as in vivo, yet there are many unknowns and confounding factors that impact our ability to come up with the right approach. Genotoxicity tests were designed for testing chemicals and may not be suitable for the screening of particulate material. Furthermore, the classical direct interaction of a chemical or its metabolite with DNA, which our assays are optimized for, will not play a role in the majority of nanomaterials. Because we are still learning about the mechanisms by which nanomaterials can exert genotoxic effects, we can see a clear pattern evolving from acute and chronic toxicity studies. The bulk of nanomaterials that caused toxic effects seem to induce inflammatory processes that will generate oxidative stress. DNA damage triggered by inflammatory processes that is viewed quite differently in risk assessment, and the question of whether a material triggers genotoxic activity directly or through a threshold mechanism has a huge impact on public health decisions. How should this impact the selection of our assays? Does the performance of standard genotoxicity assays make sense at all? As the scientific community is facing the challenge of conducting hazard/risk assessments on nanomaterials today, guidance is desperately needed. Our panel of experts will share recent data in order to fuel discussion and seek consensus as to whether standard in vitro and in vivo genotoxicity assays should have a place in the assessment of these materials, whether additional assays should be added on, or whether we are in need of a paradigm shift for this class of materials.

1302 IRRITATION SCREENING IN VITRO: PREVALIDATION ACTIVITIES FOR THE IRE (ISOLATED RABBIT EYE) ASSAY.

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Identification of irritants is fundamental in characterizing chemical site-of-contact hazards. A comprehensive review of 4 organotypic assays for detection of eye irritation (ICCVAM, 2005) provided impetus for validation and adoption by OECD of test methods for conduct of the BCOP (Bovine Corneal Opacity) and ICE (Isolated Chicken Eye) assays as means for identification of severe irritants. One promising method, the IRE, was less well characterized as to predictive capabilities but may offer potential advantages compared to BCOP and ICE, etc., enhanced comparison with historical in vivo data in rabbit, high quality and initial viability of specimens, multiple discrete quantitative endpoints. With application of a uniform assay protocol and scoring criteria initial evaluation of 45 substances showed sensitivity of 83% (77% for solids, 100% for liquids) and specificity of 71% overall (92% solids, 38% liquids). To further assess performance of the IRE we have conducted a 2 phase study with 44 additional substances selected from FECTOC or ICCVAM validation databases. Outcomes have stimulated development of a refined model for classifying the multi-endpoint IRE results as predictive of 3 GHS categories (Cat. 1-2, and not classified). Using this model for scoring of results followed by concordance assessment with historical data in vivo data suggested: 1) generally high sensitivity and specificity with a tendency to under-predict the irritant nature of some solids, 2) excellent reproducibility by independent replicate experiments, 3) the applicability domain, although broad, may exclude substances acting via macromolecular binding and sparingly soluble solids or those solids acting in part by physical irritation, 4) histology corroborates and may refine conclusions reached by observation of corneal swelling, opacity, and fluorescein staining. This presentation will trace the history of the IRE and survey its readiness status for consideration of formal validation.

1303 INTER-LABORATORY STUDY OF THE RELIABILITY OF THE BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY: INVESTIGATIONS OF SOLID TEST SUBSTANCES.

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The Bovine Corneal Opacity and Permeability Assay (BCOP) is an ex vivo assay which may be used to assess the eye irritation potential of new chemicals and finished products. The BCOP assay has been accepted by several regulatory agencies for the identification severe and corrosive ocular irritants, replacing the rabbit eye test. According to OECD Test Guideline 437 two treatment protocols may be used: one for liquids and one for solids. Solids are tested as 20% (w/v) solutions or suspensions in deionized water. Freshly excised bovine corneas are mounted in special corneal holders and are treated with the 20% (w/v) test material dilutions for four hours at approximately 32°C. Changes in corneal opacity are measured using an opacimeter, and impairment of the corneal barrier function is determined by measuring fluorescein passage through the cornea. Histological evaluation of the treated corneas may be used to determine the degree and depth of injury at the tissue level. In this study, the reference standard solids recommended in the OECD TG 437 were tested in an inter-laboratory study. Overall, the results from the evaluation of solids were highly congruent between the two laboratories and to the historical data and for several substances histological evaluation improved the understanding of eye irritation effect. However, for chlorhexidine and dibenzoyl-L-tartaric acid there were inter-laboratory differences which were further evaluated. For chlorhexidine, differences in results were attributed to different sources of the chemical. This study demonstrates the reproducibility of the BCOP assay when evaluating solid test substances. In parallel, chlorhexidine compared the opacity scores from a newly developed opacimeter (BASF-OP2.0) to those of the standard device (OP-KIT). The comparison between the BASF-OP2.0 and OP-KIT demonstrated that the BASF-OP2.0 showed very little variability and overall corresponded very well with the OP-KIT values.

1304 CONSIDERATIONS FOR DEMONSTRATING THE INTER-LABORATORY RELIABILITY OF THE BOVINE CORNEAL OPACITY AND PERMEABILITY ASSAY (BCOP) AND CHORIOALLANTOIC MEMBRANE VASCULAR ASSAY (CAMVA).

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In vitro assays evaluating ocular irritation potential are regularly used by personal care companies. Two of these in vitro assays include the Chorioallantoic Membrane Vascular Assay (CAMVA) and the Bovine Corneal Opacity and Permeability Assay (BCOP). These assays do not require the use of live animals, provide reliable predictive data and are rapid to conduct. The BCOP uses excised bovine corneas to predict ocular irritation. The CAMVA uses the vascular network of fertilized chicken eggs as a conjunctival model to predict eye irritation. Both BCOP and CAMVA have been used for over fifteen years for product development, worker safety, and safety claims substantiation. This study presents the procedures and considerations for demonstrating the inter-laboratory reliability of the BCOP and CAMVA. It is important to have a valid assay that can be implemented consistently at several different laboratories. For Kao Brands Company, a large BCOP and CAMVA database exists that covers multiple consumer product categories such as hair shampoos, skin cleansers, and hair styling sprays (containing ethanol). Therefore, a proper review of candidate laboratories is important for seamlessly generating consistent results that can be used for assessing potential ocular irritation of new products. First, a candidate laboratory should be audited for proper facility operation and personnel training, second, the laboratory’s use of Good Laboratory Practices (GLPs) should be reviewed. Third, reference materials with known BCOP and CAMVA data (one irritant and two non-irritants for initial assessment) should be tested at each new laboratory for verification of proper assay performance.
1305 COMPARISON OF A NEWLY DEVELOPED HUMAN CORNEAL FULL-THICKNESS EYE IRRITATION TEST (EIT) AND THE BOVINE CORNEAL OPACITY AND PERMEABILITY (BCOP) ASSAY.

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Human reconstructed tissue models have been suggested for incorporation into a tiered testing strategy to replace the Draize eye irritation test (OECD TG 405). One candidate model is the EpiOcular tissue model (OCCL-200) which is an organotypic model of the human corneal epithelium. The EpiOcular-EIT, developed to discriminate between ocular irritants and non-irritants (“NI”), GHS no category, was shown to have a high level of sensitivity, specificity, and accuracy (98.1%, 72.9% and 84.8%, n=112 test articles) and its regulatory acceptance is pending. A newly developed Human Corneal Full-Thickness (hCFT) model may allow even more accurate and reliable evaluation of ocular irritants (including tissue recovery following chemical insult) due to utilization of human corneal cells and the presence of stromal and endothelial layers. Test articles (TA, n=54) from the BCOP validation study (used to validate the BCOP for the identification of corrosive and severe ocular, GHS category 1, irritants) were tested using the hCFT-EIT. For the 54 TA, BCOP had a sensitivity, specificity, and accuracy of 92.3, 57.1, and 74.1%, respectively, while performance of the hCFT-EIT was 100.0, 71.4, and 85.2%, respectively. In addition, the hCFT model was used to assess tissue recovery following exposure to increasing concentrations of surfactants and TA spanning the range of irritancy. Significant recovery at 24 and 48 hours (up to 80% and 100%, respectively) was observed for “NI” chemicals. Together, the EpiOcular-EIT and hCFT-EIT will allow industry to comply with current legislation and address multiple concerns including consumer safety, animal welfare, and testing cost.

1306 USE OF CONFOCAL MICROSCOPY TO EXAMINE ULTRA-MILD OCULAR IRRITATION IN CULTURED PORCINE CORNEAS.

M. Piehl, M. R. Carathers, G. L. DeGeorge and D. R. Cerven, MB Research Laboratories, Spinnerstown, PA.

Confocal microscopy allows for “optical histological” sectioning of a living tissue such as the cornea to determine tissue viability within the corneal epithelium without the lengthy process of traditional histology. We have developed a novel assay, PorFocal, which uses cultured waste porcine corneas from the meat industry to assay individual corneal cell death with high sensitivity due to a confocal microscopy endpoint. In PorFocal, test substances are placed directly onto living corneal tissue in culture; therefore, solubility of the test substance is irrelevant. PorFocal cultured corneas are maintained in a living state for 7 days with daily application of the test substance. This multiple-exposure screening scheme allows for quantification of extremely mild ocular cell death with additive effects over time. These additive effects are then measured by quantification of individual stained dead cells within the corneal tissue by confocal microscopy. Corneal tissue is imaged in an “optical histological” manner where a series of image “slices” are acquired at increasing depths into the corneal tissue. The images can then be digitally reconstructed to display the entire corneal tissue volume imaged. Six cultured corneas per test material were treated with Phosphate Buffered Saline (PBS, negative control), or 10-fold dilutions of Benzalkonium chloride (BAC; 0.1%, 0.01%, and 0.001%) or Sodium Dodecyl Sulfate (SDS; 0.5%, 0.05%, and 0.005%). Corneas were dosed topically with 50-µL of test substance daily for 7 days. On day 8, corneas were stained with dead cell stain and imaged using confocal microscopy. All dead cells were counted for each tissue field and statistical analysis was performed using ANOVA. Test substances dosed at 10-fold dilutions were statistically significant (p<0.001) in a dose dependent manner. These data demonstrate the potential sensitivity of the PorFocal assay.

1307 DEVELOPMENT OF THE REPLACEMENT OCULAR BATTERY TIER 1—CHORIOALLANTOIC MEMBRANE VASCULAR ASSAY.

D. R. Cerven, D. Hall and G. L. DeGeorge, MB Research Laboratories, Spinnerstown, PA.

As the initial tier of the Replacement Ocular Battery (ROBATT) – a tiered testing strategy for regulatory classification of ocular irritation without the use of live animals—the Chorioallantoic Membrane Vascular Assay (CAMVA) was used to screen the ocular irritation potential of 52 chemicals with known levels of severity ranging from non-irritant to corrosive. Changes to the vasculature of the Chorioallantoic Membrane (CAM) were evaluated and a reference value (RC50) computed for each test chemical. Individual animal data from the ECETOC database were available for 37 of the 52 chemicals. Fifteen chemicals were selected after consultation with representatives of the US EPA and US FDA. Basic US EPA classification information was available for these materials but individual animal data were not. Based on the results of the CAMVA screen, 30 chemicals with irritating potential will advance for further evaluation in the Bovine Cornea Opacity and Permeability Assay (BCOP). These chemicals are expected to be in the moderate to corrosive range of ocular irritants. Twenty-two chemicals with slight to non-irritating potential will be evaluated in the Porcine Confocal Assay (PorFocal). These chemicals are expected to be in the non-irritating to slightly irritating range. Initial comparison of the CAMVA screen results with ECETOC and FIFRA data indicated evidence of over-prediction (3 of 20) for moderate - corrosive chemicals and under-prediction (5 of 20) for slight - non-irritating chemicals. Since the ROBATT is a multi-tiered testing system, all test chemicals will be evaluated in at least two models before being classified into an appropriate regulatory corrosive category. ROBatt is a two-year research grant funded by the NIH and US FDA to develop a tiered testing strategy of alternatives to replace the need for using live rabbits in ocular irritation classifications.

1308 PROTOCOL REFINEMENT OF THE BOVINE CORNEAL OPACITY AND PERMEABILITY (BCOP) TEST FOR EYE IRRIGATION.

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Improvement of ocular irritancy prediction using a modified (shortened) 3-minute exposure in the BCOP assay was proposed for alcohols and ketones, which have been identified by ICCVAM as responsible for the most over-predictions in the BCOP. Eight alcohols and six ketones were tested using both the modified and the standard 10-minute exposure, and the data were compared with the GHS categories from the ICCVAM database. The evaluation of the 3-minute exposure data revealed that five of the 6 over-predicted alcohols showed an improved prediction, and of the 2 correctly predicted alcohols, one became an under-prediction and one remained the same. Two of the five over-predicted ketones showed an improved prediction, with the three other remaining the same. The one correctly predicted ketone remained the same. The results of the evaluation of the modified BCOP assay using the 3-minute exposures for alcohols and ketones suggest that improvements in the predictive capacity of the assay can be achieved by reducing the over-prediction of these small molecule, solvent-type chemicals, without an adverse impact upon the rate of under-prediction of similar chemicals. It is our recommendation that a) additional small molecule alcohols and ketones exhibiting solvent-like physical characteristics should be tested in the BCOP assay using the 3-minute (or shorter) and 10-minute exposures, and b) prior to any additional testing a more thorough evaluation of the supporting rabbit ocular irritation data be conducted to ascertain whether the correct standards are being used to calibrate the assay.

1309 PREDICTING EYE IRRITATION OF AGROCHEMICAL FORMULATIONS ACCORDING TO DIFFERENT CLASSIFICATION SCHEMES BY IN VITRO METHODS (BOVINE CORNEAL OPACITY AND PERMEABILITY AND EPIOCULAR EYE IRRITATION TEST).

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The bovine corneal opacity and permeability (BCOP) test has been adopted by OECD for the identification of corrosive and severe ocular irritants (GHS category 1) for single component substances and multi-component formulations. Eye irritation tests with human (EIT) reconstructed tissue models (such as EpiOcular) were capable to predict ocular non-irritants (GHS no category). Thus the ultimate replacement of the Draize rabbit eye irritation test (OECD TG 405) by a combined or tiered testing strategy could be possible. The purpose of this study was to evaluate whether the BCOP with additional corneal histology together with the EIT could be used to predict eye irritancy of agrochemical formulations according to different classification schemes including UN GHS and EPA systems. We have performed the BCOP (plus histology) and the EIT of 50 agrochemical formulations for which in vivo eye irritation data were already available (for registration purposes). Using the OECD TG guideline evaluation scheme for opacity and permeability in the BCOP was not predictive for the agrochemical formulations assessed here, while corneal histology grades and the EpiOcular tissue viabilities were useful predictors of eye irritancy potencies and could be applied for the different classification schemes.
Two organotypic assays for detecting severe ocular irritant effects of chemicals, Bovine Corneal Opacity, BCOP and the Isolated Chicken Eye, ICE, have achieved full validation status and entry into the OECD test methods framework. A similar organotypic assay for detecting severe ocular irritant effects, the Isolated Rabbit Eye assay, IRE, is also used as a screen for detecting ocular irritants, but requires an expanded dataset to more positively suggest readiness for formal validation. In order to address questions raised in the previous assessment, a preliminary study (Olson M, 2010) was conducted to assess refinements of the IRE protocol and of the prediction model. A scoring scheme for histopathological analysis was also developed. This presentation will describe the outcome of the main study using 23 chemicals drawn from the ICCVAM Recommended Test Substances List covering liquids and solids, and a range of irritancy categories, chemical classes and toxicological modes of action. The revised protocol used a 5-second application time for liquids and 10-second for solids and assessments were made of corneal swelling, opacity, fluorescein uptake, appearance of the tissues and histopathology. A prediction model following a logic tree approach, with a primary endpoint of swelling, then considering opacity and fluorescein uptake, was used to identify irritating chemicals. In the resulting concordance analysis, overall accuracy was ca 74%. All category 1 liquids were correctly identified, but there was some under-prediction of irritancy class for some solid materials (25%). One liquid was over-predicted with respect to GHS categorisation. Preliminary analysis shows that the histopathology outcome was concordant with observations made for swelling and other endpoints and that standard H&E staining was adequate to discriminate damage to both superficial and deeper tissue layers.

CDER RECOMMENDATIONS FOR OCULAR IRRITATION TESTING IN THE 21ST CENTURY.

C.D. Merrill, B. A. Hill and A. C. Jacobs, CDER US FDA, Silver Spring, MD.

CDER’s safety evaluation of drug products relies on an integrated nonclinical/clinical risk analysis process. For dermal drug products applied topically to the skin, the potential for inadvertent eye exposure requires that this include an evaluation of eye irritation potential to adequately inform consumers of the risk inherent in dermal use. Any clinical data on ocular/dermal toxicity or lack thereof will always supersede nonclinical data in this process. Generally predictions for toxicity to the eye may be predicted from toxicity to the skin for dermally applied products. However, sponsors may wish to conduct a separate evaluation to screen for serious irreversible eye effects. CDER considers the Bovine Corneal Opacity and Permeability (BCOP) assay to be adequate for assessing the potential for severe irritation and corrosivity of such drug products, provided the assay is conducted according to the ICCVAM-recommended protocol (http://ccvvm.nih.gov/docs/ocutox). (Note: Drug products intended for direct ocular instillation are specifically excluded from this discussion.) Responses in an appropriately conducted BCOP assay, including nonevansere responses, will not need to be confirmed in a subsequent in vivo rabbit test. Although previously conducted Draize tests will still be reviewed, CDER does not consider the Draize eye or skin test to be either necessary or appropriate for regulatory testing of drug/biologic products. Details concerning the BCOP protocol, including the recommended use of concurrent positive and negative controls and the optional use of an appropriately selected benchmark substance will be discussed. Important considerations in the recommended risk analysis process will also be provided.

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assess the skin sensitizer-induced Nrf2-response in keratinocytes. They indicate that
screening in this cell line may circumvent the use of more expensive and variable methods directly targeting expression of native Nrf2-genes.

**1315 IN VITRO DETECTION OF CONTACT ALLERGENS: PROTOCOL DEVELOPMENT AND SUCCESSFUL INTERNATIONAL RING STUDY USING HUMAN PERIPHERAL BLOOD MONO-CYSTE-DENDRITIC CELLS.**


Allergic contact dermatitis is a delayed T-cell mediated allergic response. Until now animal experiments (e.g. the local lymph node assay) are supplying most of the data used to assess the sensitization potential of new chemicals. However, the 7th amendment to the EU Cosmetic Directive will introduce a testing ban for cosmetic ingredients after 2013. In vitro alternative methods are thus being actively developed. Although promising results have been obtained with cell lines, their reduced functionality and inherent genetic instability led us to investigate the use of peripheral blood monocyte-derived dendritic cells (PBMDCs) for the establishment of a reliable in vitro sensitization test. To solve the issues associated with the use of primary cells, the culture and exposure conditions (cytokine concentrations, incubation time, readout, pooled vs. single donors and cytotoxicity) were re-assessed and optimized. Here we propose a reproducible and scalable protocol based on PBMDCs. In the meantime 54 substances are tested which resulted in a assay specificity and sensitivity of 78% respectively. Wider acceptance and feasibility of PBMDCs for the reliable detection of human skin sensitizers were tested in an international ring study. The excellent concordance of the data measured in the different ring study labs will be presented.

**1316 ECVM VALIDATION OF SKIN SENSITIZATION ALTERNATIVES: PROGRESS UPDATE.**


Recent progress with nonanimal models in skin sensitization toxicology has resulted in the development of mechanistically based test methods which could make a valuable contribution to the replacement of the existing animal tests as requested by the European Regulations on cosmetics and chemicals. These approaches comprise the Direct Peptide Reactivity Assay (DPRA), the Myeloid U937 Skin Sensitization Test (MUSST) and the human Cell Line Activation Test (h-CLAT). Here we propose a reproducible and scalable protocol based on PBMDCs. In the meantime 54 substances were tested which resulted in a assay specificity and sensitivity of 78% respectively. Wider acceptance and feasibility of PBMDCs for the reliable detection of human skin sensitizers were tested in an international ring study. The excellent concordance of the data measured in the different ring study labs will be presented.

**1317 VALIDATION OF A GENE LIST PREDICTIVE FOR SKIN SENSITIZATION IN HUMAN KERATINOCYTES.**

J. van der Veen1, T. Pronk1, H. van Loveren1,2 and J. Ezendam1.

In response to the changes in the EU legislation for safety testing of chemicals the demand for in vitro methods to replace current animal testing has greatly increased. This is also true for skin sensitization testing. The applicability of in vitro gene expression profiling in human keratinocytes to identify skin sensitizers is being pursued. A gene profiling study using the HaCaT keratinocyte cell line was performed and the gene expression data were used to extract a short predictive gene list. It was shown that a list of 12 genes was able to identify skin sensitizers with 96% accuracy using the classiflier algorithm of random forest, support vector machine and PAM. Pathway analysis using ToxProfiler showed the importance of the Nrf2-Keap1 and Toll-like receptor (TLR) signaling pathways. Analysis using the SVM classiflier algorithm showed a prediction accuracy of 89.76% when only sensitizers and irritants were excluded. Further analysis showed that TLR ligands were correctly identified as nonsensitizers. However, Nrf2 activating compounds were all wrongly classified as sensitizers, showing that the gene list was biased towards Nrf2 activation. It can be concluded that in the validation of a predictive gene list for skin sensitization or other toxicological endpoints, it is crucial to include compounds that activate the identified molecular pathways. By including additional parameters, such as physical-chemical characteristics of the compound, the prediction accuracy of the gene list can be improved and applied in an integrated testing strategy.

**1318 DEVELOPMENT OF A NEW IN VITRO SKIN SENSITIZATION ASSAY USING RECONSTRUCTED HUMAN EPIDERMIS (EPISENS).**


With animal welfare concerns and regulatory restrictions on animal testing, in vitro assays evaluating the skin sensitizing potential of cosmetic raw materials are being developed. These assays are focused on cell activation or protein binding properties of the chemical. However, some limitations (e.g., lipophlic substances, pre/prohaptens, mixtures) exist in these in vitro methods that need to be overcome in order to fully replace animal tests. Recently, we developed a new in vitro skin sensitization assay using reconstructed human epidermis (Epidermal Sensitization Assay; EpiSensa) based on the expression of redox-related genes. In the present study, we assessed the utility of selected marker genes using 96 well format of EpiDerm® (EPI296) to develop a high throughput compatible assay that may replicate an animal test in test material capacity and solvent flexibility. We evaluated 12 sensitizers including pre/pro-haptens (e.g., cinnamic aldehyde, isoeugenol) and 4 nonsensitizers (e.g., lactic acid, sodium lauryl sulfate), which are reported as reference chemicals for developing in vitro skin sensitization assays (Casati, et al., 2009). After EpiDerm® tissue was exposed to chemicals for 6 hours, the expression of several redox-related genes (e.g., activation transcription factors (ATF3), DNA homolog subfamily B member 4 (DNAJB4)), was quantitatively analyzed by real-time PCR. 7 selected genes were upregulated (over 3-fold) by at least 6 tested sensitizers. Among 7 selected genes, ATF3 or DNAJB4 were significantly (4- or 3-fold) upregulated by 12 or 11 tested sensitizers, respectively, while neither of them were significantly upregulated by 4 tested nonsensitizers. These data suggested that EpiSensa, based on the specific gene expression changes in 96 well format of EpiDerm® is a high throughput compatible skin sensitization assay with a broader applicability domain of raw materials including pre/pro-haptens.

**1319 A NOVEL HUMAN T-CELL PRIMING ASSAY FOR THE IDENTIFICATION OF CONTACT SENSITIZERS.**

P. R. Esser1, S. S. Schmucker2, L. Dietz1, H. Thierse1, A. Richter1 and S. F. Martin1. 1Allergy Research Group, University Freiburg Medical Center, Freiburg im Breisgau, Germany, 2Millennium Biotech GmbH, Bergisch Gladbach, Germany and 3Research Group for Immunology & Proteomics, Department of Dermatology and University Medical Center Mannheim, University of Heidelberg, Mannheim, Germany. Sponsor: M. Pallardy.

Background: The replacement of animal testing for the identification of skin and mucosa sensitizers by alternative in vitro methods is urgent. One hallmark of sensitizers is the induction of T-cell activation. Our goal was the development of a human T-cell priming assay (HTCPA) within the EU-project Sens-it-iv to identify potential contact sensitizers, to assess their potency and to improve the discrimination between sensitizers and irritants. Method: Autologous naive human T-cells are primed with autologous dendritic cells (DCs). Contact allergens are either added to the culture or DCs are directly chemically modified. Another approach is to load DC with protein-allergen conjugates. Allergen-specific restimulation is performed and T-cell proliferation and cytokine production are used as
readouts to determine allergen specific T-cell responses. In addition, primed T-cells are used in a flow cytometry-based cytotoxicity assay to identify the T-cell mediated killing of antigen loaded DCs. Result: In vitro priming of naive human T-cells with DC and contact allergens results in antigen-specific CD4+ and CD8+ T-cell responses that allow in vitro identification of contact sensitizers as determined both by intracellular cytokine staining and analysis of T-cell proliferation. In addition, the same protocol used for priming is able to generate antigen specific cytotoxic T-cells, thereby enabling T-cell mediated cytoxicity as determined by FACS analysis as a highly sensitive additional readout. Conclusion: The human T-cell priming assay is a highly specific and promising in vitro test for the identification of contact sensitizers. As part of a tiered integrated testing strategy this assay may aid risk assessment and replacement of animal testing.

1320 B CELL INCREASES AND EX VIVO IL-2 PRODUCTION AS SECONDARY ENDPOINTS FOR THE DETECTION OF SENSITIZERS IN NON-RADIOISOTOPIC LOCAL LYMPH NODE ASSAY USING FLOW CYTOMETRY.

K. Jung1, W. Jang1, B. Kim2, Y. Lee3, Y. Yum1, S. Sohn4, Y. Park1 and K. Lim1.


Non-radioisotopic local lymph node assay (LLNA) using 5-bromo-2'-deoxyuridine (BrdU) with flow cytometry (FCM) is gaining attention since it is free from the regulatory issues accompanying in vivo uses of radioisotope. 3H-thymidine in traditional LLNA (LLNAP). However, there is a concern over compromised performances in non-radioisotopic LLNA, raising needs for additional endpoints to improve the accuracy of LLNA:BrDU-FCM. With the full 22 reference substances enlisted in OECD Test Guideline No. 429, we evaluated the performance of LLNA:BrDU-FCM along with the concomitant measurements of B/T cells and ex vivo cytokine production from isolated lymphocytes to examine the utility of these markers as secondary endpoints. Mice (Balb/c, female) were topically treated with substances on both ears for 3 days and then, BrDU was intraperitoneally injected on day 5. After a day, lymph nodes were isolated and undergo FCM to determine BrDU incorporation and B/T cell subtyping with B220+ and CD3+. Ex vivo cytokine production by lymph node cells (LNCs) was measured such as IL-2, IL-4, IL-6, IL-12, IFN-γ, MCP-1, GM-CSF and TNFα. Mice treated with sensitizers showed preferential increases in B cell population and the selective production of IL-2 which matched well with the increases in BrDU incorporation. When compared with guinea pig or human data, BrDU incorporation, B cell increase and IL-2 production ex vivo can successfully detect sensitizers with the performances comparable to rLLNA, suggesting that flow cytometric analysis of B cell and IL-2 production ex vivo may be useful for improving the accuracy of LLNA:BrDU-FCM or as independent non-radioisotopic endpoints.

1321 DEVELOPMENT OF AN IN VITRO MODEL TO ASSESS THE EFFICACY OF TOPICAL ANTIOXIDANTS.

M. Kicha, L. Krawiec and K. Norman. Institute for In Vitro Sciences, Gaithersburg, MD. Sponsor: H. Raabe.

Topical antioxidants, which have the capacity to neutralize reactive oxygen species (ROS), have been shown to prevent skin damage and improve the appearance of sun-damaged skin. Accordingly, we have developed an in vitro method capable of evaluating the antioxidant performance of ingredients and final formulations which may be applied to the skin. For assessment of antioxidant potential, NHEKs (normal human epidermal keratinocytes) were subjected to UVA irradiation to oxidize oxidative stress and then protection from oxidative stress was evaluated in cells incubated with antioxidants. Cells were seeded in 96-well plates and incubated with the fluorescent ROS-detecting probe, 5 (and 6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate acetyl ester (CM-H2DCFDA), for 40 minutes. Next, the cells were exposed to a serial dilution of antioxidants/antioxidant-containing formulations for 1 hour. ROS were generated by exposing the cultures to UVA light for 50 minutes (5 Joules/cm²), and then detected by fluorescence measurements of the cells. Cytotoxicity was assessed concurrently using the neutral red uptake (NRU) assay. Using this method, we evaluated several antioxidants and antioxidant-containing formulations for their ROS-reducing capabilities, including ascorbic acid, quercetin, resveratrol, kinetin, nicotinamide, and ECGC. Based on our results, several antioxidants and antioxidant-containing formulations noticeably reduced ROS levels compared to untreated controls. The greatest reduction was observed in cells treated with ascorbic acid, which has been qualified as the positive control for the assay. Other antioxidants which did not show this reduction were likely not water soluble and/or poorly bioavailable to cells. Overall, our results indicate that this method may provide a valuable in vitro tool for assessing antioxidant performance in a biologically relevant model.

1322 RETROSPECTIVE ANALYSIS OF THE EPIDERM 3-MINUTE PREDICTION MODEL FOR ASSESSMENT OF GHS SKIN CORROSION PACKING GROUP SUB-CATEGORY 1A.


OECD has adopted several ECVAM-Validated reconstructed human skin models (EpiDerm and EPISKIN/SkinEthic) for testing skin corrosion (OECD TG 431). However, TG 431 does not satisfy international (GHS) labeling guidelines for transport of dangerous goods. GHS package labeling guidelines utilize 3 corrosion sub-categories (1A: very dangerous, 1B: medium danger and 1C: minor danger). Labeling a chemical as sub-category 1A has important consequences, including very small volume package limits for air transport, prohibition from passenger aircraft, protective storage conditions, costly containers and low market acceptance. Animal tests are still utilized for assessing the 1A label requirement. In an in vitro method that discriminates 1A from 1B/1C classes will therefore have a substantial impact on reducing animal tests for this purpose. The current poster evaluates data obtained with the EpiDerm model for ability to discriminate between GHS 1A and 1B/1C classes. Data obtained from 49 chemicals tested during the ECVAM Phase I validation study plus 17 additional previously tested chemicals were retrospectively analyzed based on the MTTC viability assay (50% viability cutoff) and the 3-minute exposure period. The combined set includes 15 1A, 25 1B/1C, and 26 non-corrosive chemicals. The 3 min prediction model is shown to produce a sensitivity of 93% (14/15) and overall specificity of 76% (39/51) for predicting sub-category 1A. Testing of additional chemicals (ECVAM Phase III validation study) indicates that data correction for direct MTTC-reducing chemicals is important. Adoption of the 3 min EpiDerm prediction model would lead to significant reduction in animal use for corrosion sub-group package labeling.

1323 OECD TG 404 ACUTE DERMAL TOXICITY TESTING STRATEGY COMBINING THE USE OF THE EPISKIN VALIDATED TEST METHODS.

J. Cotovio1, N. Alépée1, M. Grandisier1, N. Seyler2, F. Soler2 and J. Meunier1.


The ability to produce irreversible or reversible alterations to the skin at the site of contact is part of the guidance based facts used for the identification of corrosive and skin irritant chemicals. During the skin corrosion validation study of the EpiSkin test method, some in vitro corrosives were identified as non corrosives in vitro. Since under classification of chemicals may be due to non specific reduction of MTTC, interference corrections were performed on 5 chemicals detected as direct MTTC-reducing chemicals is important. Adoption of the 3 min EpiDerm prediction model would lead to significant reduction in animal use for corrosion sub-group package labeling.

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1324 OECD TG 404 ACUTE DERMAL TOXICITY TESTING STRATEGY USING THE SKINETHIC RHE VALIDATED TEST METHODS.
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Testing strategies based on alternatives methods were integrated in the OECD test guidelines TG404 for predicting dermal toxicity. The validated test methods using the SkinEthic reconstructed human epidermis (RHE) were independently validated for dermal skin corrosion and skin irritation.

The aim of the present study was to develop a testing strategy combining both SkinEthic RHE skin corrosion and skin irritation test methods to support the ongoing revision of the OECD TG404 and TG431. For such purpose, more than 60 chemicals (from the ECVAM validation studies) were evaluated in both skin corrosion and skin irritation methods. A stepwise testing strategy (Top down and Bottom-Up) for the prediction of skin irritation and skin corrosion was proposed using the validated SkinEthic RHE test methods to support the ongoing revision of the OECD test guidelines TG404 and TG431. Thus among the unquestionable corrosive substances of the set, none was predicted as “non irritant” while some over prediction was observed. Among 13 chemicals identified by NICEATM/ICCVAM as having a high tendency for misclassification, 11 were correctly predicted as skin corrosives while 2 others in vitro non corrosive were finally predicted as irritating. Thus, when applying the OECD TG404 testing strategy to substances identified as potentially false negative corrosives, all these substances were correctly identified.

In conclusion, integrating testing strategies is not a strict procedure. When the determination of corrosives/irritants cannot be achieved using a weight-of-the-evidence analysis, a preferred sequential testing strategy (skin irritation / corrosion), which includes the performance of accepted in vitro SkinEthic RHE tests should be considered.

1325 EVALUATION OF IRR-IS®, AN EPISKIN™-BASED MODEL FOR QUANTIFYING CHEMICAL IRRITATION POTENCY.
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Irritation potency classification assays using in vitro 3D reconstructed epithelium models and viability measurement (MTT test) have been developed and validated by ECVAM. The ability of these methods to quantify skin irritation potency in terms of classification according to the EU GSH rules is satisfactory. Validated methods only predict two classes including irritants (R38) and non classified substances. Nevertheless, quantification of the skin irritation potential is of high importance not only for transport labeling and industry workers but also for the toxicological risk evaluation and as such should address the question of intermediate irritation levels in line with new and future regulatory demands. ImmunoSearch developed IRR-IS®, a new method based on the quantitative analysis of specific biomarkers expressed in 3D reconstructed epithelium (EpiSkin™). The aim was to provide a tool designed to provide possible ways to encompass these classification limitations and to help risk assessment approach and possible keys to get closer to potency assessment. The selection of tuned set of biomarkers was done by analyzing expression profiles in 3D reconstructed epidermis with several reference irritants. Test chemicals were topically applied neat for 30 min then washed and the tissues further incubated for 6 hrs. Tissues were teased, total RNA purified with Trizol and expression of genes measured by quantitative PCR after reverse transcription. We selected 25 biomarkers and developed an specific algorithm based on analysis of gene expression magnitude. A reference set of 45 coded irritant chemicals from the public domain was provided by L’Oréal and tested blind by ImmunoSearch. Sensitivity >90%, concordance >70% and accuracy >80% were comparable to the validated EpiSkin model performance. These preliminary good results need to be applied to larger sets in order to refine the algorithm.

1326 ASIAN RECONSTRUCTED EPIDERMIS MODEL ASSESSMENT FOR THE IN VITRO PREDICTION OF SKIN IRRITATION ACCORDING TO THE ECVAM-VALIDATED PROTOCOL.
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The European Center for the Validation of Alternative Methods (ECVAM) Scientific Advisory Committee validated in 2007 the reconstructed human epidermis EpiSkin model (SkinEthic, France) in vitro test-method as a full replacement method for the Draize acute skin irritation test (validated reference model: VRM). This method has included both in the EU Test Method B46 and the OECD Draft Test Guideline for skin irritation testing. Based on the EpiSkin™ technology, we have developed and standardized an in vitro epidermis model using human keratinocytes of Asian origin. Using the 20 reference chemicals recommended by ESAC (ECVAM Scientific Advisory Committee, 2007), we assessed the performance of this Asian EpiSkin model for skin irritation prediction using the ECVAM validated protocol. With 90% sensitivity, 80% specificity and 85% overall accuracy (viability assessment, MTT assay), the model showed good within-laboratory reproducibility and satisfactory predictive capacities similar to the VRM. In order to optimizing the evaluation, the release of IL-1 alpha after post-incubation was also measured as a complementary endpoint. When combining both cell viability and IL-1 alpha measurements, the sensitivity and accuracy were improved (100% and 90% respectively). These results suggest that the reconstructed Asian EpiSkin model can be used to predict skin irritation by using the 15 min/42 hours validated test method, in accordance with the ECVAM performance standards.

1327 COMPARISON OF DIFFERENT SKIN MODELS TO THE 3T3 NRU PT FOR CHEMICALLY INDUCED PHOTOTOXICITY.
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The 3T3 neutral red uptake assay for phototoxicity (3T3 NRU PT) is currently the only in vitro assay approved for the screening of potentially phototoxic compounds. However, the fact that the model uses a mouse fibroblast monolayer instead of human-derived cells or cell lines raises questions as to its predictivity for human exposure to phototoxins in the environment and from medicinal and cosmetic products. While the OECD guideline 432 are based on the 3T3 neutral red test validated by the ZEBET group and the intra-laboratory validation, it is stated that other cells can be used with the same test procedure, if equivalency is demonstrated (paragraph 9). These studies report a side-by-side comparison of the 3T3 results generated in our laboratory to those obtained from three human skin-derived cell-based systems. We compare the HaCaT keratinocyte cell line, primary adult human epidermal keratinocytes (HEKa) and a 3D human skin model using the same panel of chemicals to determine if a human-based system is as predictive as the 3T3 test model. The chemicals tested are a subset of those used to validate the 3T3 system. The 3T3 negative chemicals (Hexachlorophene, SDS and L-histidine) show similar negativity in the three human-derived skin systems. Of the 3T3 positives, Chlorpromazine and Norfloxacain were determined to be equal in responsiveness in the monolayer human cell lines to the 3T3 system, while the 3D human skin model was less sensitive by 30 to 100 fold in effective chemical concentration, but had photo-irritation factors (PIF) within the ‘probable phototoxin’ range defined in the 3T3 assay. All EC50 concentration values for the 3D skin model are much higher than the monolayer cell systems, but are comparable in PIF range values to those of the 3T3 system. Amiodarone, a weak positive in the 3T3 system, is also very weakly positive for the HaCaT cells and 3D skin model, but fails to be positive for the HEKa. Necessary modifications to the 3T3 protocol for the specific culture conditions of each alternative model with their respective positive and negative aspects are described.

1328 ISOFLAVONE-INDUCED RECOVERY OF BODY WEIGHT FOLLOWING EXPOSURE TO GAMMA RADIATION.
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Radioprotectors are chemical compounds that when administered prior to irradiation provide protection from ionizing radiation-induced toxicity. In a clinical setting, radiation is administered at sublethal doses to destroy tumor cells. A frequent side effect of sublethal irradiation in mammals is a reduction of body weight. Moreover, weight loss has been demonstrated to be a prognostic indicator of reduced survival rates. Therefore, the amelioration of radiation-induced weight loss is an important objective when using radiation therapy. In the present study, we characterized the effects of genistein on gamma radiation-induced weight loss in CD2F1 male mice. Mice were divided into four groups: (1) vehicle + sham irradiation, (2) vehicle + 7-Gy irradiation, (3) vehicle + sham irradiation, (4) genistein + 7-Gy irradiation. A single subcutaneous injection of genistein (200 mg/kg) or vehicle was subcutaneously administered 24 hr before either sham irradiation or a sublethal dose of cobalt-60 gamma irradiation (7 Gy at 0.6 Gy/min). Animals were weighed daily for 30 days after irradiation. Separate groups of mice were evaluated for hematological endpoints over 30 days. Mice treated with vehicle or genistein that were sham-irradiated exhibited normal weight gain over the 30 days of the experiment. In contrast, a significant reduction in body weight for both groups of irradiated animals was observed. Beginning on day 3 after irradiation, the genistein-induced reduction in body weight reached maximum levels by day 3,
Chambers to the vehicle-treated irradiated mice. These results demonstrate that genistein can platelets were significantly elevated beginning on day 10 postirradiation compared to the vehicle-treated irradiated mice. These results demonstrate that genistein can mitigate weight loss in mice receiving a sublethal dose of gamma radiation.

1329 NOVEL PYRIDINIUM OXIMES AS REACTIVATORS OF ORGANOPHOSPHATE (OP)-INHIBITED ACETYLCHOLINESTERASE (ACHE) IN THE CENTRAL NERVOUS SYSTEM.

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Exposure to OPs, including nerve agents, results in the inhibition of AChE and overstimulation of the nervous system. Currently approved therapies include atropine and an oxime, e.g., 2-PAM, to reactivate OP inhibited AChE. A major limitation of the approved oximes is their limited ability to cross the blood-brain barrier (BBB) and reactivate AChE. A series of novel pyridinium oximes has been synthesized that incorporate moieties that increase BBB penetration and reactivation of AChE. Oximes were screened in vitro for their ability to reactivate AChE inhibited by a nerve agent surrogate, phthalimidyl isopropyl methylphosphonate (PIMP), a sarin surrogate, or nitrophenyl ethyl methylphosphonate (NEMP), a VX surrogate, which phosphorylate AChE with the same moiety as sarin or VX, respectively. Rat brain homogenate was incubated with a concentration of PIMP (175nM) or NEMP (100μM) that yielded about 80% AChE inhibition, followed by an oxime (0.1mM) and AChE activity measured. Reactivation of AChE in vitro varied among oximes but was similar for each of the two surrogates; PIMP 14%-79%, and NEMP 23%-76%. Oxime lipophilicities (ω-octanol/water partition coefficients), 0.009 to 2.244, were greater than for 2-PAM (0.006). Oximes that demonstrated AChE reactivation greater than 40% in vitro were selected for testing in vivo in rats. A high sublethal dose of a stable sarin surrogate, nitrophenyl isopropyl methylphosphonate (NIMP) (0.325mg/kg) or NEMP (0.4mg/kg) was administered, yielding about 80% brain AChE inhibition, followed by an im injection (0.1mmol/kg) of a novel oxime or 2-PAM at the time of peak brain AChE inhibition (1hour). Twelve of 24 novel oximes tested yielded 10-35% brain AChE reactivation and attenuated OP induced seizures, indicating their ability to cross the BBB and reactivate OP inhibited brain AChE and demonstrating their potential as therapeutics. Supported by Defense Threat Reduction Agency: 1.E0056_08_AHB_C.

1330 NEURAL PROTECTION IN THE CENTRAL NERVOUS SYSTEM AGAINST NERVE AGENT SURROGATES USING NOVEL PYRIDINIUM OXIMES.

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Organophosphates (OPs), such as nerve agents, excessively stimulate the cholinergic system via inhibition of acetylcholinesterase (AChE); consequently, the central nervous system is vulnerable to neural damage that may have detrimental long-term effects. This study utilized highly relevant sarin and VX surrogates to further characterize the impact of the nervous system is vulnerable to neural damage that may have detrimental long-term effects. This study utilized highly relevant sarin and VX surrogates to test the efficacy of novel pyridinium oxime reactivators, created to incorporate moieties which increase blood-brain barrier penetration. Levels of glial fibrillary acidic protein (GFAP), detected using immunohistochemistry, were measured in the brain as an indicator of neural damage. Adult rats were treated ip with high sub-lethal doses of sarin or VX surrogates, nitrophenyl isopropyl methylphosphonate (NIMP) (0.325mg/kg) or nitrophenyl ethyl methylphosphonate (NEMP) (0.4mg/kg), respectively, followed at 1 hour by im administration of oxime (0.1mmol/kg). Rats were monitored for seizure activity, and kainic acid (KA; 10mg/kg) was used as a positive control. Levels of GFAP were elevated with KA, as well as with NIMP and NEMP treatments alone, all yielding significantly (p<0.05) higher levels than controls. Treatment with oxime in two different formulations (bromide vs. mesylate salt) 1 hour post surrogates attenuated seizures and reduced GFAP levels over NEMP or NIMP treatments to levels near those of control animals (p=0.05) in both the piriform cortex and the hilus region of the dentate gyrus of the hippocampus. Additionally, c-fos activity, brain monoamine levels, and markers for oxidative stress (isoprostanoids) are being evaluated to further characterize the impact of the surrogates on the brain. These results highlight the efficacy of these oximes and the potential of this novel chemistry to protect the brain from neural damage induced by OPs. Supported by Defense Threat Reduction Agency: 1.E0056_08_AHB_C.

1331 DISTINCT ALKYLATION SIGNATURES OF NITROGEN MUSTARD AND α-HALO CINNAMALDEHYDES ON THE ACTIVE SITE OF THIOREDOXIN REDUCTASE.

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Thioredoxin reductase (TrxR), a key regulator of cellular proliferation and redox homeostasis, is a selenocysteine-containing flavoprotein that catalyzes the NADPH-dependent reduction of oxidized thioredoxin. We found that two lung toxicants, nitrogen mustard (HN2) and α-bromo-cinnamaldehyde (αBrCa), are potent inhibitors of TrxR in A549 human lung epithelial cells. Inhibition of TrxR activity is concentration- and time-dependent. Using purified rat liver TrxR, we demonstrated that only the reduced enzyme is irreversibly inhibited by these alkylating agents. TrxR also catalyzes quinone redox cycling, a process that generates reactive oxygen species. Both HN2 and αBrCa were found to inhibit redox cycling. LC-MS analysis showed that HN2 modified both selenocysteine and cysteine in the C-terminal redox motif of TrxR. In contrast, αBrCa only modified the cysteine residue in the redox center of the flavoprotein. These data are supported by our findings that recombinant mutant TrxR, in which selenocysteine was replaced by cysteine, was markedly less sensitive to inhibition by HN2, but not αBrCa. Although both HN2 and αBrCa are soft electrophiles, they appear to modify TrxR by distinct mechanisms. We postulate that this is due to differences in the reaction mechanism of αBrCa, which is mediated by addition-type alkylations (Michael reactions), vs HN2, which catalyzes 1,2-addition of an α-halo alkyl radical. Taken together, these data suggest that HN2 and αBrCa target TrxR and that this may be an important mechanism mediating oxidative stress and tissue injury in the lung. Supported by NIH grants AR050753, ES004738, CA135624, GM034210 and ES05022.

1332 REMOVAL OF TOXIC EFFECTS OF SOMAN AT THE HUMAN RESPIRATORY MUSCLE BY A NONREACTIVATING COMPOUND.


Rational: The most toxic action of the nerve agent soman is inhibition of acetylcholinesterase (AChE) finally leading to death due to respiratory failure. While effects of cholinergic overstimulation at muscarinic acetylcholine receptors may be antagonised with atropine restoration of function of nicotinic acetylcholine receptors (NAR), especially at respiratory muscles, needs alternative approaches. As reactivation with oximes is generally insufficient in soman poisoning, the non-reactivating bispyridinium compound MB 327 (1,1-(propane-1,3-diyl)bis(4-tert-butylypyridinium) iodide, Turner et al. ToxLett 206, 105-111, 2011) (1) was investigated on its ability to restore function of soman poisoned human intercostal muscle. Furthermore, it was tested whether such an effect could be mediated by interaction at the orthosteric binding site of NAR. Experimental procedures: In human intercostals muscle strips force production upon indirect field stimulation was determined. After neuromuscular block by soman (0.1mM), MB 327 (100 and 200 μM) was applied (in absence of soman) and force generation recorded for 30 min. After the experiments, muscle AChE activity was determined radiochemically. Furthermore, binding properties of MB 327 in radioligand binding experiments with NAR (Torpedo californica) using 3H epi- barbital occupying the orthosteric binding site was investigated. Results: MB 327 was able to restore soman induced neuromuscular block to some 45 % of normal while muscle AChE remained inhibited almost completely (4% residual activity). MB 327 did not directly interact with 3H epibatidine binding sites of Torpedo NAR. Faint indications for an allosteric interaction were observed. Conclusion: In guinea pig MB 327 was able to improve survival after tabun poisoning (1) without reactivating AChE. Our result show that, MB 327 may also show the hilus effects in human models of the orthosteric binding sites of NAR could identified, further research is necessary to characterise possible interactions of non reactivating compounds with NAR.
Pharmacology and Toxicology, München, Germany and Deutsche Sporthochschule, sure.
neuroprotectants for their ability to provide protection against nerve agent expo-
sions in the weeks after GB-exposure. Future studies will use this model to assess acquisition of spatial memory performance and developed spontaneous recurrent seizures within 1 month of exposure. In addition, rats exposed to 3.0 LC₅₀ had impaired treatment with the standard therapy at toxic signs onset. LC₅₀ resulted in onset of toxic signs followed by seizure at 11 and 12 min, re-
proliferation and endothelial tube formation. We used intact mouse embryoid bod-
ies (EB) as a well established in vitro model.

Methods: EBs were exposed at different time points during their differentiation, fixed and immunostained (PECAM-1, Ki-67, activated caspase 3). The migration behaviour of isolated murine embryonic cells from desintegrated EBs was assessed in a Boyden chamber.

Results: Sulfur mustard and chlorambucil treatment increased significantly the number of apoptotic cells in EBs whereas proliferation remained unchanged. This effect was more prominent in early stages of EB development. Additionally, mouse endothelial progenitor cells showed impaired migration after sulfur mustard and chlorambucil treatment.

Conclusion: The previously well established EB model seems to be suitable to investigate the effects of alkylating agents on endothelial cells with respect to cell death, proliferation and migration. Thus, the presented results may be a contribution to understand impaired wound healing after sulfur mustard or chlorambucil exposure. Future research of our group will focus on the testing of candidate drugs to speed up wound healing.

Whole body exposure to sarin (GB) results in spontaneous recurrent seizures and functional impairment in male rats.

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Current therapies for nerve agent exposure increase survival but offer inadequate protection. Since the route of exposure to volatile agents such as GB is primarily via inhalation, we developed a realistic GB-exposure model in male rats to evaluate therapeutics. In Exp. 1, we determined the dose response effects of a 60-min exposure to 1.25 - 2 LC₅₀ GB, using endpoints of toxic signs and seizure onset. Rats had continuous recording of EEG activity including while in the exposure chamber. Dose response effects were observed in that the higher the agent concentration, the shorter the latency to toxic signs, and the greater number of rats that seized. All rats exposed to 1.25, 1.5 or 2.0 LC₅₀ displayed seizures; mortality increased with the higher doses with none surviving 2 LC₅₀. In Exp. 2, we administered the standard therapy of atropine sulfate and an oxime at onset of toxic signs to rats in the exposure chamber and the anticonvulsant diazepam 30 min after seizure onset. Treatment given at toxic signs onset prevented seizures in rats exposed to 1.25 LC₅₀ and reduced seizures in rats exposed to 3.0 LC₅₀. In Exp. 3, we determined the dose response effects of a 60-min exposure to 3.0 LC₅₀ resulted in onset of toxic signs followed by seizure at 11 and 12 min, respectively, after exposure. Treatment with the standard therapy at toxic signs onset resulted in all rats displaying seizure (12/12) and surviving to 24 hr; two died within 1 month of exposure. In addition, rats exposed to 3.0 LC₅₀ had impaired acquisition of spatial memory performance and developed spontaneous recurrent seizures in the weeks after GB-exposure. Future studies will use this model to assess neuroprotectors for their ability to provide protection against nerve agent exposure.

Endothelial tube formation in vitro is disrupted after exposure to sulfur or nitrogen mustards.

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Introduction: Sulfur and nitrogen mustards are DNA alkylating agents. Skin contact will cause a delayed vesication and inflammation. The resulting wounds are characterized by slow wound healing. Aim: As endothelial precursor cells have a pivotal role in this process, in rats exposed to 1.5 or 2.0 LC₅₀ (30 min exposure), 3.0 LC₅₀ resulted in onset of toxic signs followed by seizure at 11 and 12 min, respec-
tively, after exposure. Treatment with the standard therapy at toxic signs onset resulted in all rats displaying seizure (12/12) and surviving to 24 hr; two died within 1 month of exposure. In addition, rats exposed to 3.0 LC₅₀ had impaired acquisition of spatial memory performance and developed spontaneous recurrent seizures in the weeks after GB-exposure. Future studies will use this model to assess neuroprotectors for their ability to provide protection against nerve agent exposure.

Effects of selected organoseleniums and stilbenes upon the toxicity of 2-chloroethylsulphide in a skin cell model.

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2-chloroethylsulphide (CEES) is a monofunctional alkylating agent which possesses vesicant activity and exhibits a toxicity profile similar to that of sulfur must-
tard. We previously reported that the organoselenium compound ebolen (EB-1) showed antioxidant activity and reduced CEES toxicity in the A-431 skin cell model. In the present work, three analogs of EB-1, designated as EB-2, EB-3 or EB-4, and two structurally unrelated antioxidants of the stilbene class, namely resveratrol (RES) and pterostilbene (PTS), were tested for the ability to reduce CEES toxicity in vitro. DNA fragmentation analysis revealed a concentration-de-
dependent CEES toxicity after 24 h, with CEES concentrations at or above 1000 μM producing robust patterns of fragmentation. The MTT (3-(4,5-dimethylthiazol-2-
y)-2,5-diphenyltetrazolium bromide) assay was used to determine the effect of each test compound upon cellular viability in the presence or absence of CEES (1000 μM). All incubations were performed for 24 h. In the absence of CEES, the test compounds were found to be free of toxicity at concentrations of 15, 30 and 60 μM. When cells were co-incubated with CEES and test compound, no protection was observed for RES or PTS; however, organoselenium compounds reduced cell death. Among these compounds, EB-4 was the most potent at improving cell viability, while EB-3 was the least. These data indicate that stilbene antioxidants are less efficacious than organoselenium compounds at reducing the toxicity of CEES. Future studies will be required to determine whether or not the efficacy of the organoseleniums characterized in this work is related to an antioxidant activity or to an alternate mechanism.

Cytokine regulation by MK2 in keratinocytes exposed to sulfur mustard.


Cutaneous exposure to the chemical warfare agent sulfur mustard (SM; bis[2-
chloroethylsulphide] leads to epidermal damage. Underlying cell populations such as pro-inflammatory cytokine secretion contribute to this damage. Activation of the p38 mitogen activated protein kinase (MAPK) precedes cytokine secretion following SM exposure in normal human epidermal keratinocytes (NHEK). This study evaluated the necessity of p38-regulated MAPK activated kinase 2 (MK2) during this process. The cigarette/chromium kinase regulates cytokines at the transcriptional and translational level. To evaluate MK2 activation by SM, NHEK cells exposed to 200 μM SM for 5, 15, 30 or 480 min were examined for MK2 activation via phos-
phorylation using Western blot analysis. Corresponding control samples were left unexposed. Increased MK2 phosphorylation was observed beginning at 15 min and was sustained through 8 h. This activation was dependent on p38 MAPK activity as determined by studies using the p38 inhibitor SB203580. To determine SM-in-
duced cytokine changes at the mRNA level, qPCR analysis was performed 8 h after exposure. Beadly cytokine analysis was used to quantify cytokine secretion into

Adjunct treatment with carmiphene edisylate dose-dependently attenuates the behavioral deficits associated with soman-induced seizure activity.


Carmiphene edisylate (CED) is a muscarinic, nicotinic and N-methyl-D-aspartate receptor antagonist with anticonvulsant properties. Our laboratory has recently shown that rats exposed to soman (GD) and treated with a combination of CED and diazepam (DZP) have shorter seizure durations and reduced neuropathology compared to rats treated with DZP only even when treatment is delayed 30 min after seizure onset. In this study, we evaluated whether the addition of CED to the standard treatment regimen for nerve agent exposure (atropine sulfate [ATR], an oxime and DZP) would diminish/prevent the behavioral consequences associated with GD-induced seizures. Male Sprague-Dawley rats, implanted with telemetry devices to record electroencephalographic data, were administered either saline or 1.2 LD₅₀ GD (132 μg/kg, sc) followed by treatment with ATR (2 mg/kg, im) and the oxime Hi-6 (93.6 mg/kg, im) at 1 min post-exposure. Seizure activity was allowed to continue for 30 min before rats were treated with either sterile water or CED (20 or 100 mg/kg, sc) in addition to DZP (10 mg/kg, sc). Control (i.e., no GD) rats were treated with DZP at 40 min after saline administration. Behavioral signs of seizure were monitored for 6 h post-exposure, and spatial memory was tested in the Morris water maze (MWM) on post-exposure days 21-24. CED dose-dependently reduced the behavioral signs of seizure and attenuated the cognitive deficits observed in the MWM following GD exposure. Thus, CED may be an ef-
ficacious adjunct treatment for nerve agent exposure. This research was supported by the Defense Threat Reduction Agency-Joint Science and Technology Office, Medical &T Division & Physical Science Division. The views expressed herein are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense or the US Government.
cell culture media 24 h after exposure. Cytokines that were evaluated were tumor necrosis alpha (TNFα), interleukin-1β (IL-1β), interleukin-6 (IL-6) and interferon-8 (IFN-8). TNFα, IL-6 and IL-8 were up-regulated at the mRNA and protein level. IL-1β secretion was also elevated despite unchanged mRNA levels. To compare the role of p38 and MK2 during this process, siRNA (4 pmol/cm²) targeting these proteins were utilized. p38 knockdown reduced SM-induced secretion of all the cytokines examined, whereas a significant reduction in SM-induced cytokine secretion was only observed with TNFα and IL-6 following MK2 knockdown. These results demonstrate potential activation of other p38 targets during SM-induced cytokine secretion. Our observations also demonstrate the potential for anti-inflammatory therapies that target MK2 in epididymal tissue exposed to SM.

1338 COMPARISON OF SEVERAL TERTIARY OXIMES ON REACTIVATION OF OP NERVE-AGENT-INHIBITED AChE IN THE CENTRAL NERVOUS SYSTEM.


Organophosphorus (OP) nerve agents irreversibly inhibit acetylcholinesterase (AChE) and lead to an excess of the cholinergic neurotransmitter acetylcholine in the synapses, causing convulsions, respiratory distress and death. The current treatment regimen includes 2-pyrrolidinone to reactivate inhibited AChE, which due to its quaternary structure does not cross the blood brain barrier to reactivate brain AChE and to mitigate CNS toxicity. We reported earlier that the tertiary oxime monoisonitrosoacetone (MINA) provided some AChE reactivation in the brain, enhanced survival and mitigated the seizure activity following nerve agent exposure. In this study, the in vivo reactivating capabilities of several new tertiary oximes, N,N-diethyl-3-(2-(hydroxyimino)acetoxy)propan-1-aminium chloride (MINA), N,N-diethyl-3-(2-(hydroxyimino)acetamido)propan-1-aminium chloride (DHAP), RS194B, JK-3-38 and SWR53A, were compared to each other and to MINA, following subcutaneous sarin exposure. Guinea pigs were challenged with a 1.0 x LD50 dose of sarin 15 min after administration of atropine methyl nitrate (2.0 mg/kg, im), followed 15 min later by a test dose of the oxime. Four to 5 doses (ranging from 12.0 to 180.0 mg/kg, im) of each oxime were tested. The animal was euthanized 45 min after oxime treatment; blood was collected and target tissues (brain regions, diaphragm, heart, skeletal muscle) were harvested. AChE activity was measured by the Ellman assay. Of these tertiary oximes, JK-3-38 enhanced the toxicity of sarin at doses above 35.5 mg/kg. RS194B provided the greatest AChE reactivation (2-5%), with the rank order of MINA > SWR53A = DHAP = JK-3-38 = DHAP > MINA > SWR53A. In the CNS tissues, MINA displayed the reactivation (5-55%), while SWR53A showed reactivation (5-20%) only at the timepoints examined, whereas a significant reduction in SM-induced cytokine secretion was only observed with TNFα and IL-6 following MK2 knockdown. These results demonstrate potential activation of other p38 targets during SM-induced cytokine secretion. Our observations also demonstrate the potential for anti-inflammatory therapies that target MK2 in epididymal tissue exposed to SM.

1339 NITROGEN MUSTARD EXPOSURE OF THE CORNEA INDUCES ERK ACTIVATION OF ADAM17 WITHIN MINUTES.

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Nitrogen mustard (NM) exposure induces microblisters in the cornea, separating the epithelial and stromal layers. This is, in part, due to ADAM17 cleavage of the hemidesmosomal component, collagen XVII, a molecule that tacks down the epithelial cell to its basement membrane. We hypothesized that NM exposure activates ERK, which activates ADAM17 via phosphorylation. This was tested by determining whether ERK and ADAM17 had a direct interaction, and whether the MEK/ERK inhibitor PD98059 would reduce it. 100 nmoles of NM were applied to air lifted organ cultures of rabbit corneas. For some corneas, NM was immediately washed off with either medium or medium plus inhibitor (i.e. 0 min exposure). For others cultures, the NM remained on the cornea for 5 or 10 mins, then washed off with medium or medium plus PD98059. Incubation in medium (+ and - inhibitor) was 10 min for all cultures. Analysis was by microscopy and western blotting. Immunofluorescence showed that unexposed corneas had little activated ERK (i.e., pERK) and no activated ADAM17 (detectible with antibody against the ectodomain of ADAM17). However, all NM-exposed corneas showed signal along the basement membrane for both activated molecules. The NM-exposed corneas washed with medium containing PD98059 showed a decrease in pERK expression at all timepoints, but phosphorylated ADAM17 was only able to be affected (decreased) when exposures were for 0 or 5 mins. A 10 min NM exposure allowed the same amount of activation whether or not inhibitor was added. An interaction between ERK and ADAM17 was tested with pull down assays. Westerns of immunoprecipitated ERK were probed with ERK, pERK and ADAM17 antibodies. After NM exposure, both pERK and activated ADAM17 were abundant. Addition of PD98059 after NM exposure reduced the quantity of ADAM17 found in the pull down assay. Thus, the ERK inhibitor PD98059 decreased the activation of ADAM17 when it was applied to corneas after less than 10 min of exposure to NM.

1340 ABRUPTION OF NITROGEN MUSTARD-INDUCED LUNG INJURY AND INFLAMMATION BY THE INDUCIBLE NITRIC OXIDE SYNTHASE (INOS) INHIBITOR AMINOGUANIDINE (AG).

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Nitrogen mustard (NM, mechloethamine hydrochloride) is a bifunctional alkylation agent known to target the lung. Evidence suggests that tissue injury involves induction of oxidative stress and excessive production of cytotoxic oxidants, including reactive nitrogen species (RNS). In these studies we analyzed the role of RNS generated via INOS in NM-induced lung injury. Male Wistar rats were sacrificed i.d., d, 3 d, 7 d, and 28 d after treatment with NM (IT, 0.125 mg/Kg). Structural changes in the airways including thickening of the septal wall were evident within 1 d of NM exposure. By 3 d a massive infiltration of mononuclear cells was noted, followed by broncholocalification of the epithelia, a key process in fibrogenesis. At 28 d post NM, diffuse lung fibrosis was evident. These structural alterations were associated with rapid and persistent (3-28 d) expression of proliferating cell nuclear antigen (PCNA) in alveolar macrophages and epithelial cells. Western blotting and immunostaining revealed that iNOS, manganese superoxide dismutase (MnSOD) and heme oxygenase-1 (HO-1), were also rapidly (within 3 d) upregulated in alveolar macrophages following NM exposure, a response which also persisted for 28 d. Treatment of rats with AG (50 mg/kg, 2x/d, 3 d) significantly reduced NM-induced structural changes and lung inflammation. In addition, AG blunted the effects of NM on expression of PCNA, as well as HO-1 and iNOS suggesting a reduction of oxidative stress. Taken together these data demonstrate that a single exposure to NM causes persistent structural and inflammatory changes in the lung culminating in fibrosis. Moreover, the early pathologic effects of NM are dependent on RNS. Supported by NIH Grants HL096426, GM034310, ES004738, CA132624, AR055073 and ES05022.

1341 THIOREDOXIN REDUCTASE MEDIATES NITROGEN MUSTARD-INDUCED ACTIVATION OF NF-KAPPA/B/STAT3 SIGNALING IN LUNG EPITHELIAL CELLS.

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Nitrogen mustard (mechloethamine, HN2) is a bifunctional alkylating agent and a potent lung toxicant. Cytotoxicity and tissue injury are due to cross-linking of both proteins and DNA in the lung. Mammalian thiorodoxin reductase (TrxR) is a key cellular disulfide reductase, important in protecting against oxidative stress and in regulating cell signaling pathways including NF-κB and Stat3. We previously reported that HN2 alkylates and cross-links both the C- and N-terminal redox centers of TrxR, leading to enzyme inactivation. In the present studies, we determined if HN2-induced modifications in TrxR mediate signal transduction induced by HN2. HN2 was found to readily activate the p50 and p65 subunits of NF-κB and Stat3. We previously reported that HN2 alkylates and cross-links both the C- and N-terminal redox centers of TrxR, leading to enzyme inactivation. In the present studies, we determined if HN2-induced modifications in TrxR mediate signal transduction induced by HN2. HN2 was found to readily activate the p50 and p65 subunits of NF-κB and Stat3. We previously reported that HN2 alkylates and cross-links both the C- and N-terminal redox centers of TrxR, leading to enzyme inactivation. In the present studies, we determined if HN2-induced modifications in TrxR mediate signal transduction induced by HN2. HN2 was found to readily activate the p50 and p65 subunits of NF-κB and Stat3. We previously reported that HN2 alkylates and cross-links both the C- and N-terminal redox centers of TrxR, leading to enzyme inactivation. In the present studies, we determined if HN2-induced modifications in TrxR mediate signal transduction induced by HN2. HN2 was found to readily activate the p50 and p65 subunits of NF-κB and Stat3. We previously reported...
**1342 THE EFFECTS OF THE PULMONARY EXPOSURE OF RATS TO SULFUR MUSTARD ON CELLS OF THE IMMUNE SYSTEM.**


Although the main reported targets for sulfur mustard (SM) toxicity are the lungs, skin, and eyes, the attack of this vesicating agent on the immune system appears to be responsible for its delayed lethal effects in humans. A nebulizer was utilized for pulmonary exposure of rats to 3000, 2250, 1750, or 500µg SM; rats were then housed under approved conditions for various times. Any rat failing certain wellness criteria was taken off study (OS), and the relevant tissues were harvested. Most of the rats that required early sacrifice were from the highest exposure groups (2250 & 3000). Of the 219 rats exposed to SM, 30 demonstrated symptoms significant enough to be terminated. SM-induced changes were studied in the nucleated and non-nucleated cellular components of circulation and bone marrow (BM). The concentration (conc), size, status of viability, and the percentage and status of CD3+ cells harvested from these aforementioned components were compared across control, vehicle, and SM-exposed animals. In OS rats, there was a time dependent increase in the conc of the RBCs during the first 1.5 m post-exposure, but no consistent change in cell size. The WBCs in the OS rats were smaller and fewer over the 4-m post-exposure period compared to the vehicle control. In the bone marrow of the OS rats exposed to 2250 and 3000µg of SM, there was nearly a 50% decrease in the number of nucleated and non-nucleated cells, but no consistent change in the size of either cell population in these rats. Although the level of CD3+ cells in the BM of the SM-exposed rats was similar to that of control rats, the average size was increased by over 1μm in the BM CD3+ component in SM-exposed rats compared to controls. The numbers of cells in the exposed rats’ BM that were Annexin V+ appeared to decrease only slightly during the 2-m post-exposure period, but size appeared to decrease as the rats lived longer. Preliminary results indicate that SM inhalation causes a direct effect on the cells of the immune system indicated by decrease in nucleated cell in the BM that could result in latent illness.

**1343 RAPID EVALUATIONS OF AQUATIC TOXICITY FOR NEW MUNITION COMPOUNDS.**


This study used the Microtox® (a registered trademark of AZUR Environmental) assay to assess the relative toxicity of new munition compounds as part of the U.S. Army’s Ordnance Environmental Program (OEP). The OEP is dedicated to finding more environmentally-sustainable replacements for components of explosives, propellants and pyrotechnics that cause environmental and/or occupational risks to health. Candidates under development include four dye compounds, 1-isopropylamino-9,10-anthracenedione (Solvent Red 169), 2-(2-quinolyl)-1,3-indandione (Solvent Yellow 33), 1,4-diamino-2,3-dihydroanthraquinone (Solvent Violet 47), and 1-[2-(hydroxyethylamino)-4-(methylamino)-9,10-anthracenedione (Disperse Blue 3), proposed for use in smoke and pyrotechnics (military applications). 2-amino-N,N-diethylyamidamine (DMAZ) and 1,2-bis-dimethylamylamine ethane (TMEDA) for potential replacements for the propellant hydrazine; and triaminoguanidinium 1-methyl-5-nitriminotetrazole (TAGMTNT), ethylendiamine dinitrate (EDDN), diethylamidine trinitrate (DETN), and N3, N6-bis(2,2,2-trinitroethyl)-1,2,4,5-tetrazines (TN6B) are potential PK replacements. Microtox® is an in vitro toxicity testing system that uses a strain of naturally-occurring luminescent bacteria, Vibrio fischeri. Results of the assay are correlated to the toxicity of aquatic organisms. Toxicity is expressed as EC50 based upon dose-dependent responses and measured at 5 min-, 15 min- and 30 min- exposure times. The Microtox aquatic toxicity of ten new munition compounds was further ranked for ecotoxicity using U.S. Fish and Wildlife Service Acute Toxicity Rating Scales. Only Solvent Violet 47 was considered ‘Moderately Toxic’; all others were either ‘Slightly Toxic’ (DMAZ, TMEDA, DETN and BTAT) or ‘Practically Nontoxic’ (Disperse Blue 3, Solvent Red 169, Solvent Yellow 33, TAGMTNT, and EDDN). The toxicity evaluation assists munition scientists in making environmental health-based decisions regarding the design and selection of new formulas and materials.

**1344 PHARMACOKINETICS OF MIDAZOLAM IN RHESUS MACAQUES.**

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Midazolam, a potent benzodiazepine anticonvulsant, has been shown in several animal studies to be highly effective in controlling nerve agent-induced seizures. Midazolam is approximately twice as potent and twice as rapid in stopping nerve agent-induced seizures compared to the current nerve agent seizure treatment, diazepam. In small animal usage has been shown in humans to be highly effective in stopping seizures at similar dose levels and routes of administration as proposed for military use. The efficacy of midazolam against nerve-agent induced seizures was previously tested in non-human primates and guinea pigs. This study is designed to determine the pharmacokinetics (PK) of midazolam in naïve non-human primates. These studies were carried out in male and female rhesus macaques (NHP) at 4 different dose levels of midazolam to determine if dose proportionality was observed over this dose range. No adverse clinical signs were observed in any NHP during quarantine, prior to the PK studies, or at any time, up to 6 hr, post-midazolam dosing. PK parameters indicate that midazolam is rapidly absorbed and distributed in NHPs following an IM dose with peak blood concentration being attained in approximately 12 to 27 minutes and a large volume of distribution (approximately 0.8 to 1.5 L). Absorption and elimination were rapid and independent of dose in the range tested (0.09 to 0.36 mg/kg) with an absorption half-life of approximately 3 to 10 min and a terminal elimination half-life of approximately 23-38 minutes. The mean observed Tmax by dose and gender ranged from 12 to 27 min. There were gender-related differences in Cmax, Tmax, and the apparent volume of distribution (Vd). Based on the AUC and Cmax values, the systemic exposure to midazolam was proportional to dose.

**1345 HISTOPATHOLOGICAL AND INFLAMMATORY CHANGES IN NITROGEN MUSTARD-INDUCED MOUSE SKIN INJURY AND POSSIBLE ROLE OF MYELOPEROXIDASE IN NEUTROPHIL-MEDIATED INFLAMMATION.**

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Sulfur mustard (SM) and nitrogen mustard (NM) are potent bifunctional alkylating chemical agents that cause skin injury and delayed vesication. Our previous studies have established a skin injury model with SM analog 2-chloroethyl ethyl sulfide (CEES) showing DNA damage, apoptosis, increased myeloperoxidase (MPO) activity, induction of inflammatory mediators like COX2, iNOS and MMP9, and micro-vesication in SKH-1 hairless mice. To develop a more relevant model for efficacy studies, we further expanded our studies with primary vesicating agent, NM, in SKH-1 hairless mice. Topical application of NM (4 mg) for 12 h onto the dorsal skin of mice caused a) an increase in the skin bi-fold thickness, b) epidermal hyperplasia, c) micro-vesication, d) an increase in MPO activity and e) blood cell extravasation into the skin. NM exposure also induced expression of inflammatory mediators such as COX2, iNOS and MMP9. Because we observed a robust increase in MPO activity following NM exposure, we sought to further define the role of MPO/neutrophil infiltration in NM-induced skin injury. To do so, we used a genetic approach. We applied NM (6 mg) onto the shaved dorsal skin of C57BL/6J wild type and B6.129X1-Mpont1L(+/−) mice that are homoyzogous null for the MPO gene. Skin bi-fold thickness was measured at 6, 9, and 12 h time points, and mice were sacrificed 12 h after initiation of NM exposure. NM caused significant increases in the skin bi-fold thickness, epidermal thickness and epidermal apoptosis in wild type (B6) and B6.129X1-Mpont1L(+/−) mice compared to MPO KO mice. Furthermore, skin histopathology showed that NM exposure caused an increase in neutrophilic infiltration and MPO activity in wild-type mice compared to MPO KO mice. Collectively, our results show that NM causes inflammation and micro-vesication in SKH-1 hairless mice similar to SM, and that MPO potentially plays an important role in NM-induced skin injury.

**1346 POTENTIAL THERAPIES FOR VESICANT-INFLECTED OCULAR INJURIES.**

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There are no effective and easily applied therapies against devastating ocular injuries caused by vesicating chemical agents such as sulfur mustard (SM), nitrogen mustard (NM) and lewisite (LEW). Hence, studies were conducted to establish accessible ocular injury models suitable for laboratory studies on the pathogenesis of eye lesions, associated mechanism/s, and efficacy studies; availability of this is considered a major impediment. Analysis of 100 nmol SM exposed rabbit corneas for 2 h (washed and cultured for 24 h), evidenced increases in epithelial thickness, ulceration and necrosis, apototic cell death, epithelial detachment and microbulbule formation, increase in the levels of angiogenic regulator VEDE and induction of COX-2 and MMP-9. Employing these biomarkers, efficacy studies were carried out in the rabbit corneas untreated or treated with agents 2 h and every 4 h thereafter,
for 24 h following 100 nmol NM exposure. The agents employed were approved prescription drugs; dexamethasone (DM, 0.1% - anti-inflammatory steroid) and doxycycline (DC, 100 nmol-antibiotic and MMP inhibitor) that have been effective in treating vesicant-induced eye injuries, and sibutramine (SB, 100 μg, non-toxic natural flavanone) found to be significantly effective in treating SM analog-induced skin injuries in our earlier studies. Treatments of DC + DM, and SB were more effective than DC or DM alone in the reduction of NM-induced epithelial thickness, microbuccad formation, apoptotic cell death, and MMP-9 levels. However, DC and SB were more effective in significantly (p<0.05) reversing NM-induced VEGF, and DC, DM and SB were effective in reversing NM-induced COX-2 levels. Together, these results show strong efficacy of these agents in reversing various attributes of NM-induced ocular injuries. Further mechanistic as well as efficacy studies in NM, SM, and LEW-induced ocular injury models would help develop mechanism-driven effective therapies against ocular injuries by vesicants.

### 1347 A MATHEMATICAL MODEL OF MEDICAL COUNTERMEASURES FOR SULFUR MUSTARD EXPOSURE.


We present a mathematical model for treatment of injuries due to inhalation, ocular, and percutaneous exposure to sulfur mustard (HD). The model has been developed to predict the efficacy of treatment regimens for a variety of proposed medical countermeasures (MCMs). The baseline model of HD exposure and resultant injury uses a series of rate equations that describe the damage of deoxyribonucleic acid (DNA) in the nuclei of epithelial stem cells and the series of reactions that result in stem cell death, related tissue damage, and the signs and symptoms of injury at the three different exposure sites. The treatment models include rate equations for six classes of medical countermeasures proposed by the US Army Medical Research Institute for Chemical Defense (USAMRICD). These models will aid in the study of the efficacies of treatment regimens through predictions of the effect of administration time, countermeasure dose, and frequency of dosing. We provide the model framework and parameters for HD injury and for one member of each of the six MCM classes: intracellular scavengers, cell cycle inhibitors, poly(ADP-ribose) polymerase (PARP) inhibitors, calcium modulators, protease inhibitors, and anti-inflammatories. Model calculations may be used to analyze consequences of exposure to HD and medical interventions based on various treatment combinations. The model may facilitate HD treatment planning and countermeasure development by providing a tool to demonstrate how these treatments work and will enable analysis of the effects of medical resource limitations in a mass casualty situation on the outcome of treatment.

### 1348 ABSORPTION CHARACTERISTICS OF VX FOLLOWING PERCUTANEOUS EXPOSURE OF HAIRLESS GUINEA PIGS.

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The chemical warfare agent (CWA) O-Ethyl-S-[2-(diisopropylamino) ethyl] methylphosphonofluoridate (VX) is a toxic, viscous, and persistent liquid. VX toxicity is caused by the inhibition of acetylcholinesterase (AChE), which reacts covalently with the CWA to produce an inhibited O-alkyl methylphosphonylated enyme complex. This complex can generate a phosphonyl moiety of VX (i.e., O-ethyl methylphosphonofluoridate (VX-G)) following a fluoride ion regeneration reaction. To better characterize VX toxicity, the dermatopharmacokinetics following percutaneous (PC) exposure has been investigated in a hairless guinea pig (HGP) model. HGP (n=4) were exposed to a single PC dose of neat VX (10.08, 17.64 μg) for a maximum of 24 hours, or until death. Tape strip (TS) samples, full-thickness skin homogenates (exposure and non-exposure site), and plasma samples were all examined to determine VX levels. Samples were analyzed with either an Agilent Technologies 1200 series LC/MS/MS or 7000A GC/MS/MS. TS samples were found to contain concentrations ranging from a high of 1.92 μg, to a low of 0.0139 μg of VX recovered. The TS sample which contained the highest amount of VX was taken from a HGP that received 17.64 μg of VX and died 5.7 hr following exposure. The sum of 10 sequential TS samples accounted for 38.3% (6.75 μg) of the total dose applied, with the maximum amount of 10.87% (1.92 μg) found in the first tape strip sampled. For the remaining 3 HGPs that were sampled at 24 hrs, the 10 tape strips accounted for 11.47, 6.79, and 4.07% of the dose applied. The amounts of VX found in the unexposed areas of skin were negligible, while the amounts reported for exposed areas and plasma followed those levels found in the TS. An assessment of these interrelated absorption processes may provide insight into VX bioavailability and subsequent toxicity in the HGP.

### 1349 TRANSIENT INHIBITION OF CONNEXIN43 EXPRESSION ATTENUATES THE ACUTE INFLAMMATORY RESPONSE IN THE SKIN OF HAIRLESS MICE EXPOSED TO THE SULFUR MUSTARD ANALOG NITROGEN MUSTARD.

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Chemical-induced skin wounds generally result in an acute immune response. This includes inflammatory cell infiltration and the production of cytokines, chemokines and growth factors that influence migration and proliferation of cells to repair the damage at wound sites. The local cell-cell communication may also play a significant regulatory role in wound repairs. This local communication requires gap-junction proteins, the connexins, to maintain homeostasis in the skin and directly influence wound repair. Mori et al (J Cell Sci 119:5193-5203, 2006) showed that a downregulation of connexin43 (Cx43) resulted in accelerated wound healing in both incisional and burn skin wounds. We used Cx43 antisense oligodeoxynucleotides (asODN) in SKH-1 mice exposed to nitrogen mustard [NM, Bis (2-chloroethyl) maleimylene] and observed the wound repair response. Samples were collected at 1, 3, 7, and 10 days after NM exposure. Animals treated with asODN had a significantly improved survival rate after 10 days when compared to all other control groups. Histological examination of tissue sections showed less acute inflammation for the asODN treated group when compared to the control groups. RT-PCR and Western blot analysis showed a reduction of Cx43 for days 1 and 3. Cx 26 and 30 were also reduced at days 1 and 3 as shown by RT-PCR. IL1B, the proinflammatory cytokine is five times less in the asODN samples compared to NM alone. IL10, the anti-inflammatory cytokine is three fold higher compared to NM alone at three days post exposure. Macrophage elastase (MMP-12) decreased at days 7 and 10 when compared to NM alone. This suggests a reduction in secondary damage and a promotion of wound repair. Taken together this data shows the potential use of Cx43 as a countermeasure to acute vesicant exposure. Supported by ES05022, ES004738, EOY09056, and NIAMS U54AR050573.

### 1350 ANTICONVULSANT EFFECTIVENESS OF SCOPOLAMINE AGAINST SOMAN-INDUCED SEIZURES IN AFRICAN GREEN MONKEYS.


Prolonged epileptic seizures are a hallmark feature of intoxication with highly toxic anticholinesterase nerve agents such as soman. Benzodiazepine drugs like diazepam or midazolam are typically used to control these seizures. However, studies in both rats and guinea pigs have shown that potent, centrally acting anticholinergic drugs such as scopolamine can also terminate such seizures. The present study was performed to determine if scopolamine could produce similar anticonvulsant effects in a nonhuman primate model of soman intoxication. Adult male African green monkeys, implanted with telemetry devices to record cortical electroencephalographic activity, were pretreated with pyridostigmine (0.02 mg/kg; IM) and 40 min later challenged with 15 μg/kg (IM) of the nerve agent soman. One min after soman exposure the animals were treated with atropine (0.4 mg/kg; IM) and the oxime 2-PAM (25.7 mg/kg; IM). One min after the start of seizure activity the animals were administered scopolamine (0.01 – 0.1 mg/kg; IM), using an up-down dosing design over successive animals. Scopolamine was highly effective in stopping soman-induced seizures under these conditions with an ED50 = 0.033 mg/kg (0.019 – 0.056 mg/kg with 95% confidence limits). Seizure control was rapid, with all epileptiform activity stopping on average 20.7 min after scopolamine treatment. A separate PK study showed that absorption of scopolamine is complete within approximately 10 min after IM administration and a dose of 0.032 mg/kg produced maximum plasma levels of 16.9 μg/ml. The results show that scopolamine exerts potent and rapid anticonvulsant action against soman-induced seizures and that it may serve as a valuable adjunct to current antidepictive treatments for nerve agent intoxication.

### 1351 A FIELD-READY ANTIMICROBIAL WOUND DRESSING FOR THE TREATMENT OF CUTANEOUS VESICANT WOUNDS.

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Burn casualties present significant challenges to the military medical system. Also, instability in countries with suspected chemical vesicant stockpiles has increased the risk of use these weapons by terrorist organizations. Susceptibility of thermal or
vesicant burns to infection and the increasing incidence of multi-drug resistant bac-
teria highlight the need for novel approaches to combat infection. EpiReadyDefense is a tissue-engineered therapeutic designed to promote healing by providing antimicrobial peptides and barrier function to wounds. To facilitate de-
velopment of this field-ready wound dressing, we have identified methods that per-
mit prolonged storage at ambient temperature while preserving the ability to deliver bioactive peptides. Clinically-tested NIKSR keratinocytes were stably transfected with a non-viral vector to express elevated levels of the broad spectrum antimicro-
bial peptide cathelicidin. Fully-stratified skin substitutes were processed to elimi-
nate viability and prolong storage. EpiReadyDefense tissues were evaluated for ap-
pearance, histology, viability, biomechanical and biochemical properties after ambient storage for up to 12 months or after ambient storage for 7 weeks fol-
lowed by storage at 40°C for 7 days. EpiReadyDefense retained epidermal architec-
ture, tensile strength, and biological properties comparable to those of freshly-prep-
tared tissues. An in vitro antimicrobial activity assay EpiReadyDefense reduced growth of S. carnosus by 82% relative to control cultures, similar to levels of inhi-
bition achieved by viable tissues. Assessment of EpiReadyDefense activity in vivo is ongoing using a murine model of cutaneous injury resulting from exposure to the sulfur mustard analog 2-chloroethyl ethyl sulfide. These studies demonstrate that EpiReadyDefense can be stored at ambient temperature for extended periods while preserving biological activity. Further development of EpiReadyDefense should provide a field-deployable skin substitute for treatment of cutaneous injuries.

1354 DEVELOPMENT OF PRELIMINARY INDOOR CLEARANCE LEVELS FOR CHEMICAL WARFARE AGENTS FOR EMERGENCY PREPAREDNESS.
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Clean up of public facilities following a Chemical Warfare Agent (CWA) terrorist attack will require Clearance Levels for inhalation exposure. Developing these val-
ues by examination of complex toxicological and exposure issues and determination of sensitive subpopulations prior to an incident involving CWAs, is likely to have broad applicability to many incidents. This should also help risk managers to plan and expedite remediation activities because clearance levels will help guide selection of remediation and sampling methods. Therefore, we reviewed the toxicology of sarin and sulfur mustard, and evaluated the potential for the wide-scale and public use of an example indoor airport facility. The Preliminary Clearance Level for agent sarin (GB) is based on a well-conducted study by Mioduszewski 2002 on young adult male rats exposed whole-body to sarin vapor (0.01 to 0.14 mg/m3) for 10, 60, or 240 minutes. Analysis of pre- and post-exposure rat pupil diameters allowed de-
termination of EC50 values for miosis (defined as post-exposure pupil diameter of 50% or less of the pre-exposure diameter in 50% of the exposed population). We used the EC50 for miosis (0.068 mg/m3) (p = 0.014) in female SD rats after 10 minutes of exposure as our point of departure and performed a benchmark dose analysis (Hill Model) of the data. The clearance value for sulfur mustard was deter-
mined from a study by Anderson where 70 human volunteers were exposed to vapor (1-16 mg/m3) for 2 to 33 minutes and mild ocular effects was the critical ef-
fact at 12 mg-min/m3. To accommodate known variation in human cholinesterase and carboxylesterase activity that may make some individuals susceptible to the ef-
effects of cholinesterase inhibitors, such as nerve agents, the intraspecies uncertainty factor was set to 30. The preliminary indoor clearance values for sarin and sulfur mustard are 3.15 x 10^-4 mg/m3 and 8.30 x 10^-4 mg/m3, respectively.

1355 COMPLEX MODEL SYSTEM FOR INVESTIGATING NEW SULFUR DONORS FOR CYANIDE ANTAGONISM.
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Conversion of cyanide to the less toxic thiocyanate in the presence of a sulfur donor is catalyzed by rhodanese. Earlier studies indicated that the approach of adminis-
tering exogenous rhodanese and sulfur donor provides significant antidotal protection against cyanide. To investigate new sulfur donors, a “Complex Model System” was set up. This complex investigation system consists of the following units: Screening (semi-quantitative method); in vitro efficacy (sulfur donor reactivity); in vivo effi-
cacy (antidotal potency ratio on mouse model); bioavailability/pharmacokinetics/ab-
sorption kinetics and toxicity estimation on mouse model. Each unit involves appli-
cations of pre-formulation/formulation techniques. Test molecules involve synthesized sulfane sulfur compounds, and commercially available selected mole-
cules. Lipophilicity is characterized by octanol-water partition coefficient. Analytical method developments (GC-MS; HPLC) are employed to measure the test sulfur donors. The rates of product formation in the presence and in the ab-
sence of rhodanese are determined spectrophotometrically, based on the colored complex formation of thiocyanate with Fe3+. Converting lipophilic molecules to the water-soluble forms needs applications of various pre-formulation techniques (solubility enhancement, cosolvent application). The selected and characterized effec-
tive sulfur donors are further studied in vivo. Formulation techniques include applications of various nano-delivery systems, such as micelles and liposomes. The in vitro and in vivo efficacies are compared to the clinically applied thiosulfate. This complex investigation method can serve as a practical tool for developing new sul-
fur donor type cyancide antidotal systems. By applying this “Complex Model System”, one from the 20 screened molecules, (IPMA86), proved to have highly su-
perior in vitro and in vivo efficacy over the clinical therapy thiosulfate.
that both the immortalized microglia and RAW 264.7 cells are susceptible to C60.

Many studies have been conducted to address the toxicity of engineered nanomaterials (NMs). However, current in vitro methods require dispensation of NMs in biological media before administration to cells or tissue models, which does not mimic realistic inhalation exposure and often yields inconclusive results. The objective of this study is to design an in vitro chamber to mimic realistic inhalation exposure by delivering well-characterized NMs dispersed in the gas phase to cells and tissues grown at the air-liquid interface. NMs were dispersed in the gas phase using Electrospray technology, and a commercially available horizontal diffusion chamber was modified using machining techniques to optimize it for NM deposition. Fresh media was provided to the basal side of cells grown on a porous membrane, and a heat plate was used to maintain the internal temperature of the system at 37°C. Initial studies were carried out to determine whether cells could be sustained in this system without NM exposure by comparing the proliferation and morphology of cells grown on the membrane support under traditional conditions (submerged in media and incubated at 5% CO2, 37°C) versus cells exposed to air flow from the Electrospray (95% air, 5% CO2) for 0.5-120 minutes. Results show that cells can be sustained successfully in the chamber for up to 1 hour at a flow rate of 250 mL/min. Additional studies were performed to analyze the gas phase generation and deposition of NMs produced by Electrospray. Results demonstrated successful generation of 60 nm Ag and Au NMs and uniform deposition in the presence of an electric field. Future studies will be carried out to assess the effect of NMs on cells or tissue models exposed at the air-liquid interface. The results of these studies allow for optimized NM delivery and toxicity endpoints for realistic in vitro inhalation toxicology investigation.

**RESULTS**

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Potential limitations exist for the application of traditional in vitro cytotoxicity assays for nanotoxicity studies. Properties such as their large surface area increase their adsorption capacity for substrates and dyes thereby increasing the possibility of interference with the assay. In addition, gold nanoparticles display optical properties that could potentially interfere with absorption or fluorescence-based assays. Therefore the goal of this study was to use the xCELLigence RTCA system which makes use of real-time, label-free measurements of cell impedance as a measure of in vitro cytotoxicity. The gold nanoparticles used in this study were colloidal particles classified into different functional groups. Control without NMs did not affect the gold nanoparticles. However, as traditional toxicity evaluations are costly and time consuming, computational methods are being used as cost effective options to identify potential toxic NPs. The present study demonstrated that the quantitative structure-activity relationship (QSAR) method can be used to predict the toxicity of an assortment of metal oxide NPs based on the results of standard toxicity evaluations. TEM images of the NPs alone and in the cells were used to define several molecular descriptors for different metal oxides. Additionally, changes in cell proliferation and caspase 3/7 activation of reactive oxygen species (ROS) were evaluated using a human keratinocyte (HaCaT) cell line as a model for dermal exposure. The results of the toxicity assays identified a LD50 value for all the NPs and indicated that zinc oxide (ZnO) was the most toxic of the NPs tested. The ZnO LD50 value was 27 μg/mL while the majorities of the other NPs, including indium, lanthanum and tungsten, had a LD50 >250 μg/mL. The combined experimental-theoretical study allows for the development of a model that links experimental data with descriptors acquired based on the computationally predicted molecular characteristics. Such a model could be applied not only to metal oxides investigated in the current work, but also to unexplored related species. This approach provides an efficient method of evaluation of various groups of nanoparticles.

**CONCLUSIONS**

Muhammad S. Hussain

Due to the increase in mass production and use in consumer products as antimicrobial agents, silver nanoparticles (Ag NPs) toxicity has been studied to determine possible cellular interactions. Studies suggest that Ag NP toxicity is directly linked to Ag ion dissociation. However, this concept has not been fully explored. The aim of this study was to evaluate whether lung epithelial cell (A549) toxicity is derived from Ag NP diameter/surface area or concentration of ion dissociation. To investigate ion dissolution, tangential flow filtration (TFF) was conducted on nanoparticle solutions. This unique filtration method retains large particles within the continuous flow path while allowing smaller material (ions) to pass through. Use of this technique enables distinct separation of the NPs and their solvated ions for quantification utilizing inductively coupled mass spectrometry (ICP-MS). Here it is shown that when placed in solution, Ag NPs (10 and 50 nm diameter, with surface area concentrations of 1-25×10^14 cm^-2) undergo dissolution and produce silver ions. NP dissolution was monitored under complex physiological solutions (cellular medium, saline fluid, and an acidic solution) in order to mimic the cell culture exposure environment. TFF was conducted and quantification was performed using ICP-MS and a time dependent increase in ion production was seen. Once the ions were separated, they were resuspended in the NPs, or the unfiltered combination of ions and NPs. The ions alone displayed no change in cell viability, while the NPs alone demonstrated mild to moderate toxicity. The
most pronounced toxic effect was observed from the unfiltered NPs with ions indicating that both NPs and ions contribute to Ag NP toxicity. This research is essential in determining the source of toxicity from the nanoparticle/ion and can be expanded to other materials.

**1361 ROLE OF CONTROLLED AGGLOMERATION OF IRON OXIDE NANO PARTICLES ON CELLULAR RESPONSE IN MOUSE C10 LUNG EPITHELIAL CELLS.**

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Nanoparticle (NP) agglomeration in typical culture media is a known confounder of in vitro studies as it affects not only the total delivered dose and kinetics of particle uptake but also the mechanism of uptake. The use of thoroughly characterized NPs of the same composition and controlled degree of agglomeration therefore allows for separation of physical (rate of particle uptake, agglomeration, sedimentation) and biological effects (oxidative stress, cytotoxicity etc.). In the present work we investigate how the size and agglomeration state of iron oxide NPs influence cell dose and cellular response in mouse lung epithelial cells. We have developed a novel two-step approach to understand the relationship between applied and delivered particle dose and the physical state of the NP. In the first step iron oxide NPs were made to agglomerate in a controlled fashion by changing the salt concentration in particle suspension followed by coating with serum to create stable agglomerates with diameter ranging from 75nm-1um. In the next step we used magnetic particle detection (MPD) developed in our lab to quantify the absolute cell associated dose (picograms per cell) of NPs. The MPD uses a time varying electromagnetic field and utilizes particle magnetism to precisely measure cell associated dose of particles. It has several advantages including high sensitivity, signal stability and minimal sample preparation. Studies using both amine- and carboxy-modified iron oxide NPs demonstrate that agglomeration state not only changes the amount of dose delivered to cells but also affects their potential to induce cytotoxicity and oxidative stress. Our combined approach provides a highly quantitative experimental framework for evaluating relationships between biocompatibility of NPs and its physical form in a controlled manner.

**1362 ROLE OF SIZE AND SURFACE COATING ON SILVER NANOPRISM TOXICITY.**

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Since silver nanoparticles (Ag NPs) have unique optical properties and tunability toward distinct plasma resonances in the visible and near infrared regions, they are excellent candidates for technologies such as photovoltaics, molecular sensors and photothermal therapies. Therefore, it is crucial to assess the potential biological impact of Ag NPs before integrating them into biological applications. This study evaluated the toxicity of five different sized Ag NPs (30, 45, 75, 100, 145 nm with a width of 10 nm) with two different surface coatings (citrate and polyvinyl phosphate (PVP)) using realistic dosing concentrations consistent with occupational setting exposure levels. Additionally, silver nanospheres (Ag NS) with citrate and PVP surface coatings and diameters of 30, 35, 50 and 55 nm were evaluated as a control. Cell morphology, Ag nanomaterial uptake and localization, generation of reactive oxygen species (ROS) and changes in gene expression were assessed using the human keratinocyte (HaCaT) cell line as a model for dermal exposure. The results showed no decrease in cellular viability, regardless of size, surface coating, or shape. However, all of the 30 and 45nm Ag NPs (citrate and PVP coated) demonstrated an increase in ROS. Furthermore, the increase was the most pronounced in several stress and toxicity genes dependent on size and coating, indicating that while not toxic, the variation in the Ag NP parameters has a distinct impact on the cellular response.

**1363 MUCILAIR™ VERSUS RAW264.7 CELLS IN NANO TOXICOL OGY.**

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Sponsor: R. Woutersen

With increasing applications of engineered nanomaterials (ENM) resulting in increased exposure, the safety must be addressed along with the design of new materials. Animal testing may not be a desired test method for thousands of new nanomaterials because of the ethical concerns. Therefore, development of predictive in vitro toxicological screening methods is essential. It was essential to investigate the applicability of human 3D airway models in the safety assessment of nanomaterials, we compared the toxicity of SiO2, and CeO2 nanoparticles on MucilAir™ (EpiThelium Sari) to RAW 264.7 macrophages. MucilAir™ inserts and RAW 264.7 cells were exposed for 24h to the nanoparticles via droplet exposure on the tissue surface and via the medium, respectively. Cytotoxicity was measured by LDH (both) and TEER (MucilAir™) or MTT (RAW 264.7). Different cytokines were analysed in culture medium as a measure for inflammation. Oxidative stress and genotoxicity were evaluated by HO-1 expression and Comet assay, respectively. In RAW 264.7 cells, SiO2 and CeO2 were cytotoxic at similar concentrations, but SiO2 only induced TNF-α, whereas CeO2 only induced HO-1 expression and % tail DNA. In MucilAir™, no significant effects were seen on all endpoints up to 10-fold higher concentrations. It seems that MucilAir™ is less sensitive (but possibly more realistic) compared to cell lines towards particle induced toxicity. In future, we will optimize experimental conditions and further assess the applicability of these 3D models by exposure via different routes and compare the results with both cell culture and in vivo inhalation data. Ultimately these models may be useful in the first tier(s) of the safety evaluation of engineered nanomaterials.

**1364 CARDIOPULMONARY EFFECTS OF ENGINEERED IRON OXIDE NANO PARTICLES.**

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Engineered nanoparticles are widely applied in fields from medicine to industrial settings. While usage of these particles can greatly enhance therapeutics, especially in medicine, little is still known about their potential to cause harm and toxicity in humans. Among engineered nanoparticles is nano-iron oxide, also found as a constituent in combustion emissions. While it is known diesel exhaust (DE) can cause detrimental health effects, little is known which constituents of DE elicit these effects or the mechanisms involved. In this study, apolipoprotein E (apoE) deficient mice were subdivided into four groups based on diet type and exposure condition. Mice were placed on either a normal or high fat diet, and exposed to either filtered air or air-liquid interface. To investigate the applicability of human 3D airway models to these endpoints, Ag nanomaterial uptake and localization, generation of reactive oxygen species (ROS) and changes in gene expression were assessed using the human keratinocyte (HaCaT) cell line as a model for dermal exposure. The results showed no decrease in cellular viability, regardless of size, surface coating, or shape. However, all of the 30 and 45nm Ag NPs (citrate and PVP coated) demonstrated an increase in ROS. Furthermore, the increase was the most pronounced in several stress and toxicity genes dependent on size and coating, indicating that while not toxic, the variation in the Ag NP parameters has a distinct impact on the cellular response.

**1365 A STRATEGY FOR OVERCOMING CHALLENGES ASSOCIATED WITH MEASURING NANOPARTICLE BIOKINETICS: TITANOMAGNETITE NANOPARTICLE BIOKINETICS IN MICE.**


When conducting in vivo studies with metal or metal oxide nanoparticles, a lingering question exists of whether nanoparticles or dissolved ions are being measured by traditional analytical methods (e.g. inductively coupled plasma mass spectrometry [ICP-MS]). These challenges are further highlighted when measuring nanoparticle clearance from tissues; which, in the case of nanoparticles, can be very long and significant nanoparticle dissolution may exist. To overcome this challenge, we have developed a strategy utilizing titanomagnemite nanoparticles (which are magnetic nanoparticles containing titanium in the lattice structure). These nanoparticles have superparamagnetic properties that disappear upon particle dissolution, which allow accurate particle quantification using...
magnetic resonance imaging (MRI) and magnetic particle detection (MPD). These nanoparticles also have an internal “tracer” (Ti), which ICPS-MS can exploit for measurement of total mass (particle plus ionic Ti) with high sensitivity. To exploit these properties, plasma-coated titanomagnetite particles (80 μg) were administered i.v to male C57BL/6J mice with jugular vein cannulae. These particles had a hydrodynamic mode diameter of 72 nm (by relative number) as determined by dynamic light scattering. Real-time imaging of MRI demonstrated rapid uptake by the liver in as little as 5 min. In tandem, MPD and ICPS-MS quantified rapid blood clearance, and at 1.5 hr, 39% of the dose was in the liver and 3.5% of the dose was in the spleen. Additional time-course, dose-dependent, and route-dependent studies are planned to further characterize nanoparticle biokinetics using this strategy. These studies, coupled with computational modeling techniques, will ultimately provide a framework to extrapolate the biological fate of nanoparticles in mice to humans.

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**1368 ROLE OF NANOPARTICLE SURFACE CHEMISTRY ON CELLULAR RECOGNITION AND UPTAKE BY MACROPHAGE SCAVENGER RECEPTOR A.**

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The internalization of engineered nanoparticles (ENPs) into cells is known to involve active transport mechanisms, but the precise biological molecules involved in these processes are poorly understood. We previously demonstrated macrophage scavenger receptor A (SR-A) is involved in the uptake of amorphous silica nanoparticles. We have extended this work to investigate the mechanism of SR-A mediated ENP recognition across a wider range of ENP types. We used flow cytometry and magnetic particle detection to quantify the rates of uptake of fluorescent polystyrene (50-100 nm), fluorescent silica (50 nm), and superparamagnetic iron oxide (33 nm) ENPs bearing different surface charge modifications. Uptake of ENPs bearing anionic surface chemistries (unmodified, carboxylated) into macrophage cells is strongly inhibited when endogenous expression of scavenger receptor A (SR-A) is silenced. Uptake of anionic ENPs in human cells normally devoid of scavenger receptors was also significantly enhanced when SR-A was expressed. In contrast, uptake of cationic (aminated) ENPs occurred in an SR-A independent manner. Across a panel of particles with varying charge our results show a strong correlation between ENP charge and SR-A dependent uptake. We also found that human serum albumin, the major component of the plasma protein corona and a reported SR-A ligand, adsorbed to anionic and amine-modified ENPs with dramatically different affinities, which further impacted differences in the rates of uptake of the ENPs. Thus, surface charge is a critical ENP characteristic that drives protein corona formation and stability, as well as SR-A mediated uptake into macrophages. This work was supported by NIEHS Grants ES016212 and U19-ES019544.

**1369 THREE HUMAN CELL TYPES RESPOND TO ENGINEERED NANOMATERIALS WITH CELL-SPECIFIC TRANSCRIPTOMIC AND PROTEOMIC EXPRESSION PATTERNS.**

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The growing use of engineered nanoparticles (NP) in commercial and medical applications raises the urgent need for tools that can predict NP properties that are toxic or biocompatible. We conducted global transcriptome and proteome analyses of three human cell types, exposed to two distinct NP types to identify patterns of expression that might predict toxicity or biocompatibility. Macrophage like (THP-1), small airway epithelial (SAE), and intestinal (Caco-2/HT29-MTX) cell lines were exposed to TiO2 nanobelts (TiO2-NB; high toxicity), and multi-walled carbon nanotubes (MWCNT; low toxicity) at low (10 μg/ml) and high (100 μg/ml) doses for 1 and 24 hr. Each cell type responded to the NPs with a unique pattern of gene and protein expressions, with no differentially expressed (p<0.05, 1.5-fold change) genes or proteins overlapping across the three cell types. In all cells, the 1 hr response was primarily independent of NP type, showing similar expression patterns in response to TiO2-NB and MWCNT. The 24 hr response was unique to each NP type. Furthermore, the most significantly (p<0.05) enriched biological processes were also different for each cell type. The 24 hr response of THP-1 cells to TiO2-NB showed regulation of processes important for inflammation and immune response while the response to MWCNT primarily showed regulation of processes governing cell cycle and DNA repair. In contrast, the 24 hr response in SAE and Caco-2/HT29-MTX cells was mostly TiO2-NB-specific, with few significant genes regulated by MWCNT. Overall, each cell type responded with unique patterns of genes, proteins and biological processes. Early responses were mostly common to both NP types, while late responses were unique, mostly specific for TiO2-NB. This work is supported by NIEHS grants: RC2ES018724-01S1 (to AH) NIEHS 1RC2ES018776-01S1 (to GO).
**1370 DISPOSITION OF AMPHIPHILIC POLYMER-COATED CD/SE/ZnS QUANTUM DOTS AND CADMIUM IN THE SPLEEN OF GCLM- HETEROZYGOUS, AND WILD-TYPE MICE.**


Recent advances in nanotechnology have permitted the use of semi-conductor quantum dots (QDs) in a wide variety of novel applications. QDs are amenable to surface modifications that allow a high degree of target specificity for intracellular labeling and tracking studies for use in areas such as drug discovery, intracellular reporting, and molecular trafficking. However, the potential toxicity of these particles when used for in vivo human applications is of concern because their core constituents are generally composed of metals such as cadmium, tellurium, or selenium that can be released upon particle degradation. We have synthesized CdSe/ZnS core/shell QDs with a tri-n-ocetylphosphine oxide, poly(malic anhydride-alt-1-tetradecene) (TOPO-PMAT) coating. Although designed to be stable, these particles may release Cd. The well-characterized toxicity of Cd is modulated, in part, by glutathione (GSH). To further our understanding of the role GSH may play in cadmium toxicity associated with in vivo QD exposure, GCLM-null (a model of GSH depletion), GCLM-heterozygous and wild-type mice were administered a single intravenous dose of QDs at 6 μg Cd equivalents/kg body weight. Mice were sacrificed at 10, 30, 60, 180 and 480 minutes. At all time points, the amount of Cd in the spleen, as assessed by ICP-MS, was higher in male GCLM-null mice as compared to male GCLM wild-type or heterozygous mice. For female mice, this only occurred at 480 minutes. Furthermore, fluorescent microscopic analysis indicated a more rapid degradation of the QDs in the spleens of female GCLM-null mice. PBPK modeling also indicated a dramatic difference among the genotypes in the disposition of QDs and Cd. Collectively, these data suggest that mice with low GSH are limited in their ability to bind and export GSH-Cd complexes, thereby accumulating the metal in metallothionein-Cd complexes. Supported by NIH grants P30ES07033, RO1ES016189, T32ES07032, and U19ES019545.

**1371 MWNTS INDUCE AN OXIDATIVE STRESS RESPONSE IN IMMORTALIZED MURINE HEPATOCYTES.**

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Supported, and an RO1 Grant (ES016178) from the National Institute of Environmental Health Sciences.

Recent advances in nanotechnology have permitted the use of semi-conductor quantum dots (QDs) in a wide variety of novel applications. QDs are amenable to surface modifications that allow a high degree of target specificity for intracellular labeling and tracking studies for use in areas such as drug discovery, intracellular reporting, and molecular trafficking. However, the potential toxicity of these particles when used for in vivo human applications is of concern because their core constituents are generally composed of metals such as cadmium, tellurium, or selenium that can be released upon particle degradation. We have synthesized CdSe/ZnS core/shell QDs with a tri-n-ocetylphosphine oxide, poly(malic anhydride-alt-1-tetradecene) (TOPO-PMAT) coating. Although designed to be stable, these particles may release Cd. The well-characterized toxicity of Cd is modulated, in part, by glutathione (GSH). To further our understanding of the role GSH may play in cadmium toxicity associated with in vivo QD exposure, GCLM-null (a model of GSH depletion), GCLM-heterozygous and wild-type mice were administered a single intravenous dose of QDs at 6 μg Cd equivalents/kg body weight. Mice were sacrificed at 10, 30, 60, 180 and 480 minutes. At all time points, the amount of Cd in the spleen, as assessed by ICP-MS, was higher in male GCLM-null mice as compared to male GCLM wild-type or heterozygous mice. For female mice, this only occurred at 480 minutes. Furthermore, fluorescent microscopic analysis indicated a more rapid degradation of the QDs in the spleens of female GCLM-null mice. PBPK modeling also indicated a dramatic difference among the genotypes in the disposition of QDs and Cd. Collectively, these data suggest that mice with low GSH are limited in their ability to bind and export GSH-Cd complexes, thereby accumulating the metal in metallothionein-Cd complexes. Supported by NIH grants P30ES07033, RO1ES016189, T32ES07032, and U19ES019545.

**1372 CARBON MULTIWALLED NANOBRUBES AND NS574 COMPARATIVE TOXICOLOGICAL ANALYSIS OF NANOFLAKES ON HUMAN SKIN TISSUE.**

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Engineered QDs have unique physicochemical properties resulting from a combination of their crystalline metalloid core structure/composition and quantum-size confinement. They are comprised of a metalloid crystalline core (Cd, Zn, In) and a “shell”, which can then be further functionalized for a specific application. The unique physical and chemical properties of QDs impact their ability to interact with biological systems. Though several toxicological studies have attributed undesirable toxicity responses to QDs there has been little effort to correlate the physicochemical characteristics of a QD to a biological response. QD-induced toxicity may be potentially due to the metallic core, the shell or the functionalization that in turn influence its physicochemical properties (size, charge, photostability, oxidation). Utilizing an in vitro 3-dimensional organotypic human skin tissue we propose to better understand the biocompatibility of QDs on dermal exposure. Reconstructed human epidermal equivalents can be used to develop highly-relevant bio-kinetic platforms for the risk assessment of a multitude of new ENMs. Such a platform integrates the reliable, rapid and high-throughput methodology of in vitro cell based assays with the accuracy and relevance of in vivo platforms making them excellent surrogate models. In present study we performed a comparative analysis to determine size, charge and composition effects of QDs on tissue responses. The QDs used were hydrophilic, hydrophobic, anisoelectric QDs with and without a cadmium telluride (CdTe) coating. Although designed to be stable, these particles may release Cd. The well-characterized toxicity of Cd is modulated, in part, by glutathione (GSH). To further our understanding of the role GSH may play in cadmium toxicity associated with in vivo QD exposure, GCLM-null (a model of GSH depletion), GCLM-heterozygous and wild-type mice were administered a single intravenous dose of QDs at 6 μg Cd equivalents/kg body weight. Mice were sacrificed at 10, 30, 60, 180 and 480 minutes. At all time points, the amount of Cd in the spleen, as assessed by ICP-MS, was higher in male GCLM-null mice as compared to male GCLM wild-type or heterozygous mice. For female mice, this only occurred at 480 minutes. Furthermore, fluorescent microscopic analysis indicated a more rapid degradation of the QDs in the spleens of female GCLM-null mice. PBPK modeling also indicated a dramatic difference among the genotypes in the disposition of QDs and Cd. Collectively, these data suggest that mice with low GSH are limited in their ability to bind and export GSH-Cd complexes, thereby accumulating the metal in metallothionein-Cd complexes. Supported by NIH grants ES10586, ES19267 and NS65167.

**1373 FYN KINASE PLAYS A ROLE IN MANGANESE NANOPARTICLE-INDUCED NEUROINFLAMMATION.**


The advent of nanotechnology has led to an exponential increase in the utilization of man-made nanoparticles but their potential health hazards are not well characterized. Recently, we demonstrated that Mn nanoparticle exposure induces oxidative stress and apoptotic cell death in dopaminergic neuronal cells. In this study, we examined whether Mn-nanoparticles induce microglial neuroinflammationary responses and investigated potential cell signaling mechanisms associated with Mn-nanoparticle-induced neuroinflammationary events. 50μg/mL Mn nanoparticle exposure to BV2 microglial cells over 24hrs induced a dramatic increase in cytokine release, iNOS activation and ROS generation. Interestingly, the extent of production of some cytokines and ROS levels was more than that induced by LPS treatment, which is typically used as a positive control. Mn nanoparticles activated a key oxidative stress sensitive kinase Fyn as determined by phospho- tyrosine-Fyn 530 immunohistochemistry in both BV2 microglial and primary microglial cells. Next, we examined whether Fyn activation plays any role in the Mn-nanoparticle induced neuroinflammationary response by using Fyn kinase inhibitors rosmarinic and caffeic acids. Treatment with rosmarinic acid or caffeic acids (50μM each) significantly blocked Mn-nanoparticle induced iNOS activation, TNFα and IL-6, and ROS production in microglial cells. Caffeic acid was more potent than rosmarinic acid in attenuating the neuroinflammationary response. Co-treatment with rosmarinic or caffeic acid almost completely blocked Mn-nanoparticle induced Fyn activation. Collectively, our results suggest that Mn nanoparticles induce neuroinflammationary response in microglial cells and that Fyn kinase plays an important role in the neuroinflammationary process. (supported by NIH grants ES10586, ES19267 and NS65167).

**1374 COMPARATIVE TOXICOLOGICAL ANALYSIS OF QUANTUM DOTS ON HUMAN SKIN TISSUE.**

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3T3-L1 cells were treated with 50 μg/mL carbon multiwalled nanotubes and nanoscale titanium dioxide to determine the impact of nanoscale materials on 3T3-L1 differentiation. Post confluence, differentiation was induced and protein and RNA were collected at Day 10. qRT-PCR arrays were run to determine relative mRNA expression for key adipogenic genes: adiponectin, CEBPα, PPARγ, GLUT4, and lipoprotein lipase. Gene expression data showed a significant reduction in adipogenic gene expression, with a 63.4% decrease in mRNA expression for 50 μg/mL carbon multiwalled nanotubes and 62.4% decrease in mRNA expression for 50 μg/mL nanoscale titanium dioxide as compared to negative control. Additionally, there was a significant difference for lipoprotein lipase, with a 10% increase in relative mRNA expression for 50 μg/mL carbon multiwalled nanotubes and a reduction to 81.3% for 50 μg/mL nanoscale titanium dioxide as compared to negative control. Western blot analysis of adiponectin showed a reduction of protein for 50 μg/mL carbon multiwalled nanotubes to 68.4% of negative control and 81% of negative control for 50 μg/mL nanoscale titanium dioxide. Adiponectin is secreted exclusively by adipose tissue which inversely correlates with body fat percentage. Adiponectin plays a role in suppression of metabolic derangements which can lead to type II diabetes, obesity, and is a risk factor for metabolic syndrome. Overexpression of lipoprotein lipase is also linked to insulin resistance and type II diabetes. The alteration of adipogenic proteins like adiponectin and lipoprotein lipase by nanoscale materials may contribute to the array of metabolic disorders affecting modern populations. Research supported by NIH DR08666.
small dots and 16 ± 5 nm large dots. In this study, QDs were topically applied to skin tissue at concentrations of 0.5 μg/ml - 20 μg/ml to assess penetration, cellular viability, cytotoxicity and inflammatory responses. Our results implicate that the size, charge and composition properties of QDs impact the ability of these nanomaterials to penetrate skin tissue and influence cytotoxic and inflammatory responses.

1375 COPPER NANOPARTICLES INDUCE TOXIC EFFECTS ON HUMAN ALVEOLAR TYPE-II CELLS (A549) AFTER AIR-DÉLIVERY OF NANOPARTICLES.

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Copper nanoparticles (Cu NPs) find many industrial applications and their extensive use has produced concerns that they may pose risks of adverse effects. The purpose of this study was to assess cytotoxicity and oxidative stress associated with air-delivery of Cu NPs to human lung cells using a dynamic in vitro exposure system (DIVES). We generated NP aerosols and deposited the NPs directly onto lung epithelial cells grown in an air-liquid interface (ALI) to better mimic exposure conditions of lung cells in vivo. The delivery of Cu NPs (12 nm) to the cells was measured using ICP-MS. The concentration of dissolved NPs in basolateral media was also determined. To determine the cytotoxicity and ROS generation of Cu NPs, A549 cells were exposed to Cu NP aerosols sequentially (4 h exposure, 2 h recovery in an incubator, 4 h exposure). After exposure, cells were post-incubated for various times (4, 8, 12, and 24 h) in an incubator. Cell viability was measured by Alamar Blue assay and intracellular ROS was measured using carboxy-H2DCFDA. The concentration of Cu NPs was 1.3 ± 0.05 μg Cu/ml (4.7 cm3) and a substantial amount of Cu was released to the basolateral medium (3.8 ± 0.2 μg) during air-delivery of Cu NPs. Viability for cells exposed to Cu NPs was reduced to 73% at 4 h post-incubation compared to cells maintained in an incubator. Intracellular pro-oxidant levels after Cu NP exposure were increased at 4 and 8 h post-incubation (133% and 170% of control, respectively). ROS levels were resolved to baseline levels at 24 h post-exposure indicating that Cu NP-induced ROS generation did not exceed the ability of the cell to neutralize ROS. Cu NPs generate cytotoxicity and intracellular ROS after air-delivery to human alveolar cells in this DIVES. We suggest that the DIVES has great potential for screening NP toxicity in a manner that represents cellular responses of the pulmonary epithelium in vivo. (Supported by NIEHS P30 ES05605)

1376 NANOPARTICLE TOXICOLOGY: INVESTIGATING OXIDATIVE STRESS-RELATED BIOLOGICAL ENDPOINTS TO SUPPORT MODE OF ACTION.

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Nanoparticles (NPs) have unique properties; their utility has been noted in enhancing medical imaging and delivering therapeutics. However, the safety profile and potential mode(s) of action by which they may induce genotoxicity are not fully understood. Hence, we investigated the mode of action (MoA) of NPs; we are interested in developing a systems biology framework for evaluating direct and indirect genotoxicity. To address this, we examined key biomarkers of exposure using nanosize amorphous silica (15 nm; Levasil® 200 and 55 nm; Levasil® 50) NPs. Such markers included those involved in inflammation, oxidative stress, and cytotoxicity. Physical characterization of NPs was carried out by transmission electron microscopy. This step is critical to considering potential induction of cellular responses that can lead to DNA damage. We then performed the Comet assay (CA) in liver cells and the micronucleus (MN) test in circulating reticulocytes after 3 consecutives IVC injections to male Wistar rats (n=4-8) at 48, 24 and 4h before sacrifice. The CA was carried out under alkaline electrophoresis conditions. There was a statistically significant increase (1.6-fold) in DNA damage observed with the Levasil® 200 (50mg/kg), but not with the Levasil® 50 (25mg/kg). Blood samples were scored for MN using flow cytometry. The 15nm silica NPs increased the number of MN from 0.12±0.03% in the vehicle group to 0.22±0.08%, but the 55nm NPs did not cause an increase.

We examined the induction of IL-6 and TNF-α plasma levels by ELISA and detected upregulation of both markers with the 15nm and 50nm NPs. We assayed oxidative stress and antioxidant capacity by measuring hepatic glutathione levels (GSH/GSSG), but did not detect a significant change in levels. Finally, the transcriptional expression of genes involved in the antioxidant pathway, including Hsp-1 and Gclc, were upregulated only with small NP exposure. Our results indicate that the NPs induce inflammatory and anti-oxidant responses, suggesting that the genotoxicity detected may occur through an indirect MoA.

1377 EVALUATION OF DERMAL AND EYE IRRITATION AND SKIN SENSITIZATION DUE TO CARBON NANOTUBES AND FULLERENES.

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Skin and eyes have the highest risk of exposure to nanomaterials, because deposition of nanomaterials to the surficial organs has the potential to be a major route of exposure during the manufacturing, use, and disposal of nanomaterials. Two products composed of single-walled carbon nanotubes (SWCNTs), two products composed of multi-walled carbon nanotubes (MWCNTs), and fullerene C60 nanoparticles were tested regarding acute dermal and acute eye irritation using rabbits, and skin sensitization using guinea pigs. The concentrations of the SWCNTs, MWCNTs, and fullerene in the test substances were the maximum allowable for administration. The two products of SWCNTs and one of the products of MWCNTs were not irritants to the skin or eyes. The other product of MWCNTs caused very slight erythema at 24 hours, but not at 72 hours, after patch removal in the dermal irritation experiments and conjunctival redness and blood vessel hyperemia at 1 hour, but not at 24 hours, in eye irritation experiments. Although the fullerene were not irritants to the skin, the fullerene caused conjunctival redness and blood vessel hyperemia at 1 hour, but not at 24 hours, in eye irritation experiments. The SWCNTs, MWCNTs, and fullerene did not exhibit skin-sensitization effects. These findings showed that one product of MWCNTs was a very weak acute irritant to the skin and eyes and the fullerene were a very weak acute irritant to the eyes. Our knowledge of the toxicological effects of nanomaterials is still limited. Further information is needed to clarify the potential for irritation and sensitization given the complex nature of SWCNTs, MWCNTs, and fullerene.

1378 ASSESSING THE PHYSICOCHEMICAL AND BIOLOGICAL RESPONSES OF GOLD-BASED NANOMATERIALS IN EXPOSURE MEDIA.

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ABSTRACT: Nanoparticles are an important subset of nanomaterials that have been widely used in many products due to their unique physical and chemical characteristics. Currently, biocompatible ligands are being used to extend the range of nanoparticle applications. In this context, natural phytochelatin (PC) cysteine-rich peptides found in certain plants, present extremely high metal uptake, tolerance, and specificity. These advantages led to the development of a new class of gold-organic doped nanoparticles with PC peptides, that have the potential to serve as chelating and stabilizing ligands. The methodology for synthesizing these nanomaterials with PC peptides is presented, along with the TEM, UV-visible absorption and dynamic light scattering data used to characterize the peptide-based nanomaterials in aqueous suspension (exposure media). In addition, a zebrafish post-fertilization embryo-larval test was used to assess the toxicity of nanoparticle suspensions. Tenfold serial dilutions (0-200 ppm) for each nanoparticle were prepared in particle-free exposure media. At the 0.7±1.5 nm particle core size, virtually no aggregates observed by TEM differed from the average diameter calculated by dynamic light scattering in the serial dilutions. The average diameter of nanoparticles in suspension at higher concentrations remained constant in exposure media over the course of experiments, while, at lower concentrations the average diameter was increased. Zebrafish embryo assays showed uptake of nanoparticles and dose-dependent, mortality and, malformations that correlated with these parameters.

1379 UPTAKE AND INTRACELLULAR LOCALIZATION OF AMPHIPHILIC POLYMER-COATED CdSe/ZnS QUANTUM DOTS IN CULTURED HUMAN AND MOUSE CELLS.

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Developments in nanotechnology have resulted in a diversity of engineered nanomaterials with many useful properties. However, concerns exist regarding their potential deleterious effects in biologic systems. Quantum dots (QDs) are nanoscale
1380 A RAPID FLUOROMETRIC SCREEN FOR THE CATEGORIZATION OF NANOMATERIAL oxidative CAPACITY.
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A rapid, flexible, and inexpensive test to categorize the oxidative capacity of nanomaterials and small molecules was developed from the extension of existing fluorometric oxygen radical absorbance methods. Increasing quantities of engineered "nano-enabled" products are entering the environment more rapidly than our ability to perform informed risk assessments. Oxidative injury is a particular area of concern for nanomaterials and many techniques fail to identify the physicochemical properties responsible for a material's redox behavior. In this study, small molecules and nanomaterials were challenged with known concentrations of antioxidants (AO), trolox or melatonin, to observe how the test material changed the AO's ability to protect a fluorophore, fluorescein and/or melatonin, from 2,2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH), a hydroxyl radical producing azo-initiator. This method was tested against small molecules with known oxidative capacities including: EDTA (weak AO), alpha-lipoic acid (AO), ranitidine HCl (non-oxidant), ammonium persulfate (strong oxidant), and potassium permanganate (oxidant). Additionally, the following nanomaterials were tested and acted as follows: C60 as a weak AO, hydroxylated C60 (C60(OH)24) as an AO, custom gold-coated glutathione-conjugated nanoparticles as an AO, and erbium(III)oxide antiparticle as a non-oxidant. Strategically testing families of nanomaterials will allow us to glean potential nanomaterial structure activity relationships that could empower toxin/therapeutic identification and design.

1381 NEONATAL EXPOSURE TO LIPOPOLYSACCHARIDE ENHANCES VULNERABILITY OF DOPAMINERGIC DENDRITES IN THE SUBSTANTIA NIGRA TO ROTENONE IN LATER LIFE.

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Brain inflammation in early life has been proposed to play important roles in the development of neurodegenerative disorders in adult life. To test this hypothesis, we used a neonatal rat model of lipopolysaccharide (LPS) exposure (1 mg/kg, intracerebral injection in P5 neonatal rats) to produce brain inflammation. By P70, when LPS-induced behavioral deficits were spontaneously recovered, animals were challenged with rotenone, a commonly used pesticide, through subcutaneous minipump infusion at a dose of 1.25 mg/kg per day for 14 days. Our results show that rotenone treatment resulted in motor and behavioral impairments, including changes in locomotor activity, rearing activity, stereotypic behavior, vibrissa-elicited forelimb-placing test, movement initiation test, pole test, tappered/ledged beam walking test, and body weight loss, in rats with the neonatal exposure to LPS, but not in those without the neonatal LPS exposure. Neonatal LPS exposure reduced the density of dendrites, as identified by the decreased MAP2 immunostaining, in the substantia nigra of P98 rat brain. Rotenone treatment af-

1382 COMPARISON OF THE EFFECTS OF CARBON NANOtube AND ASES Exposure ON THE IMMUNE SYSTEM OF Fathead Minnows.

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Carbon nanotubes (CNT) are widely used in various military, commercial and public sector products. The unique nanostructure results in fiber-type characteristics in terms of their elongated shape, dimensions and high aspect ratio. The needle-like fiber shape of CNT has been compared to that of asbestos, raising concerns about potential toxicity. The objective of this project was to compare the immunological effects of CNT exposure to that of asbestose exposure in fathead minnows (FHM) (Pimephales promelas). FHM were exposed to 0.125mg/L, 0.25mg/L or 0.5mg/L CNT (MWN'T and SWNT) or asbestos (crocidolite and chrysotile for MWN'T and SWNT respectively) in water that was renewed daily. At days 0, 7, and 14, FHM were sacrificed and mucus swabs, blood, and anterior kidneys were collected. Plasma serum was separated from blood cells and used in ELISA assays. Lymphocytes were purified from both blood cells and anterior kidneys by density gradient centrifugation. Lymphocytes were then probed for phagocytic activity using a phagocytosis assay kit. Inflammassome activation was measured by ELISA for IL-1β, one of the first cytokines processed by the inflammasome. Antibody production was measured by ELISA for IgM. Exposure to CNT resulted in decreased in phagocytic activity, mucus antibody production and plasma serum antibody production compared to untreated controls. Asbestos exposure also decreased mucus antibody production but not plasma serum antibody production. Curiously, exposure to chrysotile asbestos resulted in a decrease in induced phagocytosis but not in un-induced phagocytosis at day 14. Inflammassome activation, as measured by IL-1β ELISA, was mostly undetectable; likely indicating that IL-1β processing occurs prior to day 7. Together, these data suggest that like asbestos exposure, exposure to CNT results in an immune response. However, the data demonstrate that further characterization of the systemic immune response to CNT is needed to assess the severity and toxicological impact of CNT.

1383 IN VITRO TOXICITY OF AMORPHOUS SILICA NANOparticles IN HUMAN COlon CARCINOMA CELLS.

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The use of nanostructured silica (SiO2) particles is no longer restricted to biomedical and (bio-) technological fields but rather finding applications in products of the food industry. Thus, our studies on the toxicological relevance of SiO2 nanoparticles focused on cytotoxic effects, the modulation of the cellular redox status and the impact on DNA-integrity in human colon carcinoma cells (HT29) as well as in human lung carcinoma cells (A549). The results indicate that these SiO2 nanoparticles stimulate the proliferation of HT29 cells, depending on the incubation time and the particle size. The cytotoxicity of the investigated SiO2 nanoparticles was found to depend on the concentration, size and on the FCS content of the culture medium. Furthermore, SiO2 seem to interfere with glutathione biosynthesis. The results indicate further, that effects of SiO2 NPs are not mediated by oxidative stress but by interference with cellular signaling pathways.

1384 EFFECTS OF SURFACE CHEMISTRY ON PROTEIN CORONA FORMATION ON SILICA NANOparticles.

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Protein adsorption and the formation of the protein corona on functionalized nanoparticles (NP) play a significant role in NP uptake and toxicity. To better understand the effects of surface chemistry on corona formation, the composition of

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the protein corona formed from the adsorption of proteins in culture media onto silica nanoparticles (NPs) having different surface chemistries was evaluated. silica NPs having native, amine- or carboxy-modified surfaces were synthesized using microemulsion techniques and modified using silane-based chemistries. Following purification, nanoparticles were incubated in mammalian growth media containing serum for 1, 4, 24 and 48 hours. Mass spectrometry was used to identify proteins adsorbed to NP surfaces at these time points. The surface chemistry of the nanoparticles was found to play a significant role in the composition of the protein corona at both early and later times. Differences in agglomeration kinetics and toxicity of the nanoparticles were also observed. Changes in effective diameter and agglomeration kinetics were studied using dynamic light scattering techniques. Toxicity was evaluated by measuring changes in membrane integrity and mitochondria health of two mammalian cell lines, mouse macrophages (RAW264.7) and mouse lung epithelial cells (C10) in response to variable levels of NP exposure. Carboxylated nanoparticles had slower agglomeration kinetics, formed smaller aggregates, and were found to have less impact on cell health. Significant differences in protein corona composition were observed between the COOH-NPs and particles of other surface chemistries. These results indicate that in vitro models of NP toxicity, intended to predict effects in vivo, must take NP transformation resulting from protein adsorption into account.

Mutations in the alpha-synuclein gene have been associated with autosomal dominant forms of Parkinson's disease (PD). Transgenic mice that over-express the human alpha-synuclein gene (primarily the point mutations A53T and A30P), developed neurological impairments similar to those of PD. Previous studies in our laboratory have shown that oxidative-stress-mediated activation of the tyrosine kinase, c-Abl, results in an increase in the phosphorylation of parkin, an important E3 ubiquitin ligase that assists in the clearance of proteins destined for proteasomal degradation. Here, we show that treatment with iron-oxide nanoparticles or methamphetamine results in an increase in the phosphorylation of c-Abl, observed via the measurement of degradation. We hypothesize that the particle structure increases the cellular uptake via the so-called Trojan horse-type mechanism. Comparisons were therefore made between the CuO and Ag nanoparticles, respectively, and ions from these metals. The cellular concentration of Cu and Ag was analyzed using atomic absorption spectrophotometry (AAS) and particle internalization was further studied using Transmission Electron Microscopy (TEM) and Laser Scanning Confocal Microscopy (LSCM). The results showed much higher uptake of the nanoparticles when compared to the ions. TEM images confirmed the cellular uptake and LSCM indicated that the Ag nanoparticles could be internalized into the cell nuclei. No DNA damage from the Ag nanoparticles was however observed using the comet assay. Increased cell death was observed already after a 4-hour exposure to 5 and 10 μg/ml of CuO nanoparticles in BEAS-2B, but not following exposure to Cu ions (from CuCl₂). In contrast, silver ions from a silver salt (AgNO₃) led to cell death, likely via extracellular mechanisms. Taken together, the cellular uptake of Cu and Ag was higher when cells were exposed to particles than when exposed to ions, thus supporting the Trojan horse mechanism. Extracellular mechanisms seem however to be of importance for toxicity of Ag ions.

Nickel (Ni) and nickel oxide (NiO) nanoparticles (NPs) are already commercially available and utilized by the medical and chemical industries for a number of pharmaceutical and engineering applications. The physical nature of NPs and their reactive surface properties may affect their ability to induce dermal toxicity thus causing adverse skin reactions. Although Ni is widely known to cause a number of skin ailments, e.g., hypersensitivity, contact dermatitis and skin cancer, the effects of nanosized Ni/NiO NP in comparison to particles of larger size are unknown. We hypothesize that Ni NPs are more toxic to the skin than larger Ni particles and this toxicity occurs via the metal’s ability to initiate oxidative stress, thereby inducing redox-sensitive transcription factors thus affecting/leading to inflammation. Because of the skin’s susceptibility to UV radiation, it is also important to evaluate the combined effect of UV-B and Ni/NiO NP co-exposures. To test the hypothesis, the effects of Ni/NiO particles were studied using murine epidermal cells (JB6 P⁺) and BALB/C mice. In vitro, Ni/NiO nanoparticles resulted in activation of AP-1 and NF-kβ as well as the induction of hypoxia inducible factor-1α (HIF-1α) which is involved in upregulating MMP-2 and MMP-9. Additionally, co-exposure of JB6 P⁺ cells to UVB and Ni/NiO particles resulted in significantly accelerated cell damage and death, accumulation of oxidative stress markers (3-HNE and protein carbonyls), antioxidant decrease (GSH), and release of pro-inflammatory cytokines. In

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The increased use of nanoparticles raises concern about their toxic properties. We showed recently that CuO nanoparticles were the most toxic among different metal oxide nanoparticles investigated. Furthermore, Ag nanoparticles belong to those most used today in various applications. Thus, in this study we investigated the effect of CuO nanoparticles 55% and 65% and fully oxidized. Our exposure system consists of a Palas spark generator, an aging system, and a high temperature calcining furnace delivering equal concentrations of 1, 0.5, and 0.25mg/m³. Animals were euthanized 24 hrs post exposure and the lung lavaged for cell counts, differentials, and protein as indicators of inflammation and acute pulmonary injury. In animals exposed to fully oxidized Ni particules (CMD74nm, MMD108nm) there was no observed difference in the control and fully oxidized groups at any concentration. When exposed to Ni at 0.25mg/m³ no effect was observed amongst any of the 3 particle types. At 0.50mg/m³ a significant difference in inflammatory response was seen between the fresh (CMD53nm MMD48nm) and aged (CMD115nm, MMD142nm) particles with % PMNs of 13±3.7 (mean±SEM) and 1.5± 1.0, respectively. Exposures at 1.0mg/m³ produced higher PMNs in both groups 31±1.8 and 33±1.1 respectively. In addition the higher concentrations of nickel nanoparticles produced lung injury as seen by increases in protein leakage in lavage fluid of mice in the fresh and aged but not the fully oxidized particle groups. The data suggest that adverse health effects from acute exposure to nanoparticles are not only due to their size and concentration but also to their surface chemistry (e.g., oxidation state).

**1385** ALPHA-SYNUCLEIN MEDIATED ACTIVATION OF C-ABL AND DOPAMINE DEPLETION IN DOPAMINERGIC NEURONAL CELLS TREATED WITH IRON-OXIDE NANOPARTICLES OR METHAMPHETAMINE.

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Recent evidence suggests that particle surface chemistry due to oxidation state has a significant impact on cellular responses. Exposure to nanoparticles from various sources is increasing and of growing concern. The active surface properties may affect their ability to induce dermal toxicity thus causing adverse skin reactions. Although Ni is widely known to cause a number of skin ailments, e.g., hypersensitivity, contact dermatitis and skin cancer, the effects of nanosized Ni/NiO NP in comparison to particles of larger size are unknown. We hypothesize that Ni NPs are more toxic to the skin than larger Ni particles and this toxicity occurs via the metal’s ability to initiate oxidative stress, thereby inducing redox-sensitive transcription factors thus affecting/leading to inflammation. Because of the skin’s susceptibility to UV radiation, it is also important to evaluate the combined effect of UV-B and Ni/NiO NP co-exposures. To test the hypothesis, the effects of Ni/NiO particles were studied using murine epidermal cells (JB6 P⁺) and BALB/C mice. In vitro, Ni/NiO nanoparticles resulted in activation of AP-1 and NF-kβ as well as the induction of hypoxia inducible factor-1α (HIF-1α) which is involved in upregulating MMP-2 and MMP-9. Additionally, co-exposure of JB6 P⁺ cells to UVB and Ni/NiO particles resulted in significantly accelerated cell damage and death, accumulation of oxidative stress markers (3-HNE and protein carbonyls), antioxidant decrease (GSH), and release of pro-inflammatory cytokines. In...
vivo, the ability of Ni/NIO to induce contact hypersensitivity was evaluated. Exposure to nanosized particles induced greater guttate papules and increased levels of streptomycin were observed from larger Ni/NiO PM. Altogether, these data indicate that co-exposure of dermal cells in vitro to UVB and Ni/NiO NPs was associated with greater induction of oxidative stress, antioxidant depletion and release of HIF-1α and inflammatory mediators as compared to those treated with NP alone.

Unintended release of AgNPs to the environment or from medical devices, e.g., wound dressings and catheters, may result in systemic distribution of AgNPs, including transfer across the placenta. We previously evaluated the distribution of iv-injected 50-nm AgNPs in pregnant mice on gestation day (GD) 10. The objective of this study was to evaluate the distribution of 10-nm AgNPs in pregnant mice and embryos on GDs 10 and 16, and 50-nm AgNPs on GD16. AgNPs (av. diam. 10 or 50 nm) were injected iv in pregnant CD-1 mice on GDs 7, 8, and 9 at doses of 0 (citrate buffer) or 66 μg Ag/mouse. For comparison, pregnant mice were similarly treated with an equivalent dose of soluble AgNO3. Mice were necropsied on GD10 (10-nm AgNP and AgNO3 groups) or GD16 (10- and 50-nm AgNP groups). Blood, brain, kidneys, liver, spleen, uterus, placenta, visceral yolk sacs (VYS), and embryos were analyzed for Ag content by ICPMS. Ag was significantly increased (p<0.001) in tissues from all AgNP-treated mice compared to controls. For 10-nm AgNP-treated mice necropsied on GD10 or 16, Ag content was significantly increased (p<0.05) in liver, spleen, VYS, and endometrium (GD10 only), and placenta compared to other tissues from these treated mice; for 50-nm AgNP-treated mice necropsied on GD16, Ag content was significantly increased (p<0.05) in liver and spleen. Ag tissue distribution patterns after AgNP exposure were similar regardless of particle size or time after injection, with highest levels in liver, spleen, and VYS. Very low Ag levels were detected in embryos after 10- and 50-nm AgNP treatments. Ag tissue distribution after AgNP treatment followed a similar pattern. In conclusion, Ag after AgNP exposure distributed mainly to liver, spleen, and VYS; AgNPs do not appear to cross the placenta in significant amounts; and VYS appears to sequester Ag, minimizing transfer to embryos.

ZnO has potential to be used as a coating in food packaging and medical devices, should be further evaluated for potential adverse health responses.
Airborne engineered nanoparticles (NPs) that enter the respiratory tract are likely to be deposited in the alveolar region. To better understand mechanisms of inhaled NP toxicity in vivo, we exposed alveolar epithelial cells to aerosolized NPs at the air-liquid interface. Using this approach we recently found that aerosolized ZnO NPs induced toxicity in the ALI at nearly the same dose that was required to induce toxicity in submersed cultures. However, a fundamental difference was found in the pattern of oxidative stress elicited by aerosolized NPs at the ALI and by NP suspension in submersed cultures. A small and transient response that peaked 6 hours post exposure was observed at the ALI. However, a robust response was observed in submersed cultures as early as 2 hours post exposure, which was nearly 10-fold higher than the response at the ALI. To test the hypothesis that toxicity in submersed cultures is due, at least in part, to the readily available Zn ions that are shed into the growth medium, while toxicity at the ALI is largely due to the intact NP, we quantified and compared intracellular Zn concentrations in the two exposure systems. Using a fluorescent indicator for zinc and quantifying intensity by flowcytometry, we found that the average fluorescence intensity of cells exposed in submersed cultures was 15-20 fold higher than the average intensity of cells exposed at the ALI to the same toxic dose of ZnO NPs. These observations support the hypothesis that toxicity at the ALI is mainly induced by the intact NP. To further understand mechanisms of airborne ZnO NP toxicity we currently investigate intercalization pathways and cellular fate of NPs presented at the ALI, and measure the expression levels of pro-inflammatory proteins to identify response patterns that are relevant to inhaled ZnO NPs. This work is funded by NIEHS grant R01ES018786-01 (to GO).

Chlorpyrifos (CPF) is an organophosphate insecticide largely used in the last 4 decades for the protection of crops in agriculture and for indoor use. This pesticide is not classified as toxic to development although there are some doubts concerning the potential effects of chlorpyrifos on the developmental process. In this study, the effects of CPF and its toxic metabolite, chlorpyrifos-oxon (CPO) on the development have been assessed by evaluating the modifications of the expression of several genes in embryos treated with 1.0 μg/animal/day estradiol cyclopentylpropionate was reversed to background control at doses of 15, 50 and 90 mg/kg body weight on gestation day (GD) 6 through GD 63. A control group of 30 time-mated female guinea pigs was dosed with the vehicle (1% CMC) in parallel. At terminal sacrifice on GD 63 nineteen to twenty three females per group had implantation sites. In addition to the standard investigations as required by OECD test guideline 414, blood was taken from all pregnant females on GD 63 for hematological, clinicalchemistry and steroid hormone investigations, immediately prior to sacrifice of the animals. Following gross pathology, weights of selected organs and of the unopened uteruses were determined. For each pregnant female, corpora lutea were counted and number and distribution of implantation sites (differentiated by resorptions, live and dead fetuses) were determined. The fetuses were removed from the uterus, sexed, weighed and investigated for external, soft tissue and skeletal (incl. cartilage) findings. The high dose of 90 mg/kg bw/d (half the lethal dose in guinea pigs) caused a mild anemia and signs for a higher androgen and corticoid hormone production of the adrenals, possibly related to the aromatase inhibition and stress in pregnant guinea pigs. A low dose of 10 mg/kg bw/d caused no increased effects. In pregnant females were limited to increased serum levels of androstenedione, testosterone and 11-desoxy cortisol. Thus, the no observed adverse effect level (NOAEL) for maternal toxicity is 50 mg/kg bw/d. No test-substance related effects in guinea pig fetuses were noted in this study. Particularly there were no indications for the well-known triazole-related craniofacial malformations described for murid rodents (Mengola et al, 2006).

Epoxiconazole has been shown to cause an increase in late fetal resorptions when administered to pregnant rats by oral gavage from gestation days (GD) 7-19 or 7-21 at the maternally toxic dose level of 50 mg/kg bw/day. As its mode of action (aromatase inhibition) will result in a reduction of estradiol (E2) levels, we investigated the effect of co-administration of E2. The increase of fetal mortality could be prevented by co-supplementation of estradiol cyclopropylinopropionate. In earlier studies with epoxiconazole, the occurrence of late fetal resorptions was accompanied by placental weight increases. In order to investigate these changes histopathologically, placentas from control and treated rats were sampled on GD 18 and 21 and routinely processed for standard light microscopy. Dose-dependent degenerative changes in the labyrinth and trophospongium were found. Generally, changes in the labyrinth were characterized by cystic dilation of maternal sinusoids and concomitant degeneration, congestion and necrosis in the trophospongium. The pattern of placental degeneration was the same for placentas with live fetuses as for placentas with late fetal resorptions, however with different grades of severity, which was massive in placentas with late resorptions (>70% of the labyrinth affected) leading to rupture of interhemal membranes. The enlargement of the placentas was attributed to these changes. Late fetal resorptions were attributed to the massive rupture of interhemal membranes and loss of feto-maternal compartmentalization. The number of late resorptions and the severity of placental changes increased from GD 18 to GD 21. By supplementation of estradiol cyclopropylinopropionate, the severity of degenerative placental changes decreased dose-dependently with increasing estradiol dose and the number of late fetal resorptions in dams receiving 1.0 μg/animal/day estradiol cyclopropylinopropionate was reversed to background control levels.

Chlorpyrifos (CPF) is an organophosphate insecticide largely used in the last 4 decades for the protection of crops in agriculture and for indoor use. This pesticide is not classified as toxic to development although there are some doubts concerning the potential effects of chlorpyrifos on the developmental process. In this study, the effects of CPF and its toxic metabolite, chlorpyrifos-oxon (CPO) on the development have been assessed by evaluating the modifications of the expression of several genes in embryos treated with 1.0 μg/animal/day estradiol cyclopentylpropionate was reversed to background control levels.
marker genes in the differentiation process of D3 mouse embryonic stem cells. D3 cells seeded in monolayer and in differentiation were incubated during 12-hours in the presence of CPF and CPO. Tested concentrations always caused loss of viability lower than 50%. The treated cells were collected after 12-hours. The expression of different genes was quantified using qPCR. The expression of Kdr (kinase insert domain protein receptor), gene marker of the differentiation to endoderm was significantly decreased (p<0.01) by 85% and 82% times after exposure to 100 μM CPF and 400 μM CPO, respectively. These exposures were the maximum tolerable concentration under citotoxicity criteria, causing only slight cytotoxicity with reduction of cell viability in both cases lower than 20%. The same exposure to CPO caused significant (p<0.05) increases by 4 and 190 times, in the expression of Nestin (marker of neuroectoderm) and α-fetoprotein (marker of differentiation to mesoderm), respectively. In addition, some other markers have been assessed in order to characterize the embryotoxic potential of these organophosphates.

**1398 EFFECT OF ORGANOPHOSPHATES PESTICIDES ON PON1 ACTIVITY AND GENE EXPRESSION IN HEPG2 CELLS.**

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Human paraoxonase (PON1) is a member of proteins family widely expressed in mammals, including man. PON1 is involved in the metabolism of some endobiotics and xenobiotics such as organophosphates pesticides. PON1 could be an inducible enzyme since its expression is highly regulated by environmental factors such as diet, smoke, physical activity, certain drugs, as well as genetic factors including certain genetic polymorphisms in the PON1 gene. The aim of this study was to determine whether chlorpyrifos (CP) and methyl parathion (MeP) are capable of modulating the expression of PON1 in HepG2 cells. HepG2 cells were treated with CP or MeP at different concentrations (2–8 uM) for 24, 48, and 72 h, and the cellular metabolic activity was evaluated by MTT assays. To determine the expression of PON1, quantitative real-time PCR (rtPCR) assay of the transcripts was performed using gene-specific fluorescent labeled probes. Paraoxonase-arylesterase (PON1-aryl) activity was measured spectrophotometrically. The CP and MeP treatments did not affect cell viability in culture cells. CP treatment (8 uM) increased PON1 activity at 72 h compared to control (untreated cell cultures). MeP, however, reduced PON1 activity in all concentrations at 48 h. The gene expression preliminary results showed that treatment with organophosphates pesticides modulate PON1 mRNA. Studies are in progress to validate and define the molecular mechanisms underlying the modification of the expression of PON1 by these pesticides.

**1399 COMPARATIVE SENSITIVITY OF NEONATE AND ADULT SPRAGUE DAWLEY RATS TO CHOLESTERASE INHIBITION DUE TO DIAZOXON.**

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The susceptibility of infants and children to chemicals compared to that of adults is considered in human health risk assessments. While the parent diazoxon has been tested in young and adult rats, there is concern that the active metabolite, diazoxon, may show differential sensitivity based on developmental age. The effect on acetylcholinesterase activity in neonates and young adult rat (approximately two months old) to diazoxon was determined. In preliminary studies, time to peak effect was determined to be 6 hours in neonates and 1 hour in young adults; the highest repeat dose tolerated in neonates and young adults based on general toxicity was 6 and 45 mg/kg/day, respectively. The definitive 7-day repeat dose study was performed using gene-specific fluorescent labeled probes. Paraoxonase-arylesterase (PON1-aryl) activity was measured spectrophotometrically. The CP and MeP treatments did not affect cell viability in culture cells. CP treatment (8 mg kg⁻¹ day⁻¹) increased PON1 activity at 72 h compared to control (untreated cell cultures). MeP, however, reduced PON1 activity in all concentrations at 48 h. The gene expression preliminary results showed that treatment with organophosphates pesticides modulate PON1 mRNA. Studies are in progress to validate and define the molecular mechanisms underlying the modification of the expression of PON1 by these pesticides.

**1400 EFFECT OF ENDOPHYTIC BACTERIA AND METALAXYL ON METABOLIC PROFILE OF POTATO SEEDLINGS.**

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One of the most serious diseases in potato cultivars is caused by the pathogen Phytophthora infestans. Metalaxyl is one of the most effective agents against Phytophthora infestans. In Mexico, farmers apply metalaxyl 35 times during the cycle of potato production. This represents a risk to consumers. Metalaxyl has been detected in potato in New Zealand and United States. In Mexico, there are no records related to the detection of metalaxyl in vegetables and fruits. The LD50 of metalaxyl in mice is 669 mg kg⁻¹ (oral) and >3100 mg kg⁻¹ (subcutaneous). Metalaxyl provokes cell alterations in mouse liver at 2.5 mg kg⁻¹ day⁻¹. In dogs at 0.8 mg kg⁻¹ day⁻¹, it alters alkaline phosphatase levels in blood and provokes an increase in liver and brain weight. Due to extensive applications there is a high possibility that Mexicans are consuming potatoes with metalaxyl residues. In the present study, we evaluated the effect of Acinetobacter sp. on metalaxyl degradation in potato seedlings. The effect of bacteria and metalaxyl on the growth of potato seedlings was also evaluated. A metabolic profile analysis was conducted to determine potential molecular biomarkers produced by potato seedlings in the presence of Acinetobacter sp and metalaxyl. Acinetobacter sp strongly affected the growth of inoculated seedlings. LC-MS/MS analyses of metalaxyl residues in potato seedlings suggest that Acinetobacter sp did not degrade metalaxyl. PCA analysis demonstrated that the metabolic profiles of treated and control groups are distinctly clustered and suggest the alteration of metabolic pathways by both Acinetobacter sp infection and metalaxyl treatment. Levels of twenty-one known and other unknown metabolites were found to be altered by treatments.

**1401 INDUCTION OF MOUSE CARBOXYLESTERASE EXPRESSION AND FUNCTION FOLLOWING DEVELOPMENTAL DELTAMETHRIN EXPOSURE: ROLE OF THE CONSTITUTIVE ANDROSTANE RECEPTOR.**

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Previously, we reported that developmental exposure to the pyrethroid pesticide deltamethrin increased Phase I enzyme carboxylesterase (Ces) activity (139%) along with Ces enzyme activity (215%) in male offspring at 10-12 months of age. Developmental deltamethrin exposure significantly increased mRNA expression levels of multiple Ces isoforms by 85-100%. Induction of Ces was associated with activation of the nuclear receptor-mediated pathway involving the constitutive androstane receptor (CAR). Here, we sought to determine whether developmental deltamethrin exposure results in persistent CAR activation. Pregnant C57BL/6j mice were administered 0 or 1 mg/kg deltamethrin every three days throughout gestation and lactation. Livers were collected from four month old male offspring for mRNA and protein analysis. Ces activity was increased 58% at the 1mg/kg dose. Developmental deltamethrin exposure increased mRNA levels of Ces1d, 1g and 2a by 28%, 31% and 43%, respectively. The mRNA expression for the CAR target gene Cyp2b10 was also increased, by 90%. CAR mRNA expression was not increased in male offspring at this age. However, total CAR protein was increased by 32% at the 1 mg/kg dose. Taken together these data suggest, the Ces induction may be mediated by stabilization of CAR protein resulting in sustained target gene signaling. Supported by a BMS Predoctoral Research Fellowship, NIH ES015991 and ES050522.
The objective of this study was to determine the absorptive and transport properties of pyrethroid insecticides using Caco-2 cells as a model for intestinal enterocyte absorption. Cellular accumulation of deltamethrin (DLM), cis-permethrin (CIS), trans-permethrin (TRANS) was evaluated using the human colon adenocarcinoma cell line, Caco-2. For cellular uptake linearity studies, 25 nM solutions of 14C-radiolabeled DLM, CIS and TRANS were independently prepared in transport buffer. Caco-2 cells were preincubated with transport buffer for 15 minutes prior to addition of each compound for up to 5 minutes at 37°C. Cells were then rapidly washed with ice-cold phosphate buffer saline and lysed with 1% Triton X-100. An aliquot of the cellular lysate was quantified by liquid scintillation counting, and total protein measured using the bicinchoninic acid protein assay for normalization of cellular accumulation. For assessing concentration-dependent uptake, a series of concentrations of DLM, CIS and TRANS solutions in transport buffer were prepared by mixing radiolabeled and non-radiolabeled compound together. Uptake of all 3 compounds was linear from 0.5 and 5 minutes, therefore, all subsequent transport studies were performed at 3 minutes. Temperature-dependent transport assays were conducted by preincubating cells in each solutions of each compound at 37°C and cells at 4°C on ice. Concentration-dependent transport of DLM, CIS, and TRANS was assessed up to 2.5 μM at 37°C. For all 3 compounds, transport appears to saturate with increasing concentrations. In addition, uptake of DLM, CIS and TRANS was reduced at 4°C, suggestive of an active transport process. Cellular accumulation of DLM, CIS and TRANS demonstrated saturable- and temperature-dependence, suggesting the operability of a specific membrane transport process. Further work is required to identify specific transporters mediating the influx and/or efflux of these common pyrethroids. Supported by the Council for the Advancement of Pyrethroid Human Assessment.

**1404 ABSORPTION AND TRANSPORT OF THE PYRETHROID INSECTICIDES DELTAMETHRIN, TRANS-PERMETHRIN, AND CIS-PERMETHRIN BY CACO-2 CELLS.**

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The objective of this study was to determine the absorptive and transport properties of pyrethroid insecticides using Caco-2 cells as a model for intestinal enterocyte absorption. Cellular accumulation of deltamethrin (DLM), cis-permethrin (CIS), and trans-permethrin (TRANS) was evaluated using the human colon adenocarcinoma cell line, Caco-2. For cellular uptake linearity studies, 25 nM solutions of 14C-radiolabeled DLM, CIS and TRANS were independently prepared in transport buffer. Caco-2 cells were preincubated with transport buffer for 15 minutes prior to addition of each compound for up to 5 minutes at 37°C. Cells were then rapidly washed with ice-cold phosphate buffer saline and lysed with 1% Triton X-100. An aliquot of the cellular lysate was quantified by liquid scintillation counting, and total protein measured using the bicinchoninic acid protein assay for normalization of cellular accumulation. For assessing concentration-dependent uptake, a series of concentrations of DLM, CIS and TRANS solutions in transport buffer were prepared by mixing radiolabeled and non-radiolabeled compound together. Uptake of all 3 compounds was linear from 0.5 and 5 minutes, therefore, all subsequent transport studies were performed at 3 minutes. Temperature-dependent transport assays were conducted by preincubating cells in each solutions of each compound at 37°C and cells at 4°C on ice. Concentration-dependent transport of DLM, CIS, and TRANS was assessed up to 2.5 μM at 37°C. For all 3 compounds, transport appears to saturate with increasing concentrations. In addition, uptake of DLM, CIS and TRANS was reduced at 4°C, suggestive of an active transport process. Cellular accumulation of DLM, CIS and TRANS demonstrated saturable- and temperature-dependence, suggesting the operability of a specific membrane transport process. Further work is required to identify specific transporters mediating the influx and/or efflux of these common pyrethroids. Supported by the Council for the Advancement of Pyrethroid Human Assessment.
Imidacloprid photodegradation was significantly increased in the presence of benzo-phenone, 2-acetonaphthone and xanthone in a concentration-dependent manner. Overall, understanding mechanisms of neonicotinoid metabolism and photodegradation to potentially toxic metabolites plays an important role in defining the safest and most effective use of these major insecticides.

**1407 ENDOSULFAN UP-REGULATES CYCLOOXYGENASE-2 EXPRESSION MEDIATED THROUGH ACTIVATION OF NF-κB TRANSCRIPTION FACTOR.**

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Endosulfan is an organochlorine pesticide and it is correlated with endocrine disruption, reproductive and immune dysfunctions, and neurological symptoms. Recently, t6-endosulfan was known to inflammatory effect, but the influence on cyclooxygenase-2 (COX-2) expression is unclear. This study investigated the effects of COX-2 and molecular mechanisms by t6-endosulfan in murine macrophage RAW 264.7 cells. t6-Endosulfan induced COX-2 protein, mRNA and transcriptional activity in RAW 264.7 cells. Additionally, t6-endosulfan increased the production of prostaglandin E2 (PGE2) and transcription factor nuclear factor κB (NF-κB) luciferase activity. Moreover, t6-endosulfan enhanced NF-κB p65 protein expression, but IkB-α protein was reduced by t6-endosulfan. t6-endosulfan also increased phosphorylation of phosphoinositide 3-kinase (PI3K)/Akt and extracellular signal regulatory kinase (ERK) 1/2 1/2 within activated protein (MAP) kinase. Taken together, t6-endosulfan induced COX-2 expression and it was mediated through activation of ERK MAP kinase and NF-κB transcription factor.

**1408 MOLINATE TESTICULAR TOXICITY: EFFECT OF MOLINATE ON TESTOSTERONE AND CHOLESTEROLE LEVELS IN RAT TESTES.**

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The thiocarbamate herbicide, molinate (MOL), provokes testicular toxicity in rats. The exact mechanism of MOL toxicity is not clear, previous studies have shown that there are two main routes by which MOL is metabolized: hydroxylation of the ring and oxidation of the thiol moiety. MOL is converted first to MOL sulfoxide and then to MOL sulfone. In rat testes and liver, sulfoxide metabolite has been shown to inhibit the carboxylesterase, hydrolase A that hydrolyze endogenous esters such as cholesterol. Hydrolyase A is expressed in high levels in Leydig cells of testis, where cholesterol is sequestered in the form of cholesterol esters. Hence it is proposed that MOL could limit testosterone biosynthesis by inhibiting the esterase-dependent formation of free cholesterol. To evaluate this hypothesis, we examined the effects of MOL and molinate sulfoxide (MSO) on testosterone production in cultured Leydig cells by ELISA and on non-specific esterase (NSE) activity. The IC50 for NSE inhibition by MOL and MSO (3.42 μM and 1.9 μM, respectively) were more than two orders of magnitude below that required to inhibit testosterone production (750 and 60 μM, respectively). Further, in vivo studies were performed with Sprague-Dawley rats to ascertain the effect of MOL (200 mg/kg) on testicular testosterone production and cholesterol esters in testis. Levels of testosterone in testes decreased by 87% at 6 and 12 h time points, whereas only a 16% decrease in cholesterol esters was observed. In addition, a metabolic profiling study using ultra performance liquid chromatography-tandem mass spectrometry is underway to determine changes in hormonal levels in testis of controls and treatment group. Results from these studies will be presented in detail.

**1409 ETHYLENE BISDITHIOCARBAMATE PESTICIDES MANEB AND MANCOZEB CAUSE TOXICITY IN NORMAL AND TRANSFORMED COLON CELLS VIA AN APOPTOTIC MECHANISM.**

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Ethylenebisdithiocarbamate pesticides Maneb (MB) and Mancozeb (MZ) are broad range contact fungicides widely used for eradication of fungal infection on a variety of crops. While these agents are reported to possess low human toxicity, scientific studies have suggested that human toxicity to these agents does occur. Previous testing in our laboratory has established the toxicity of these compounds to transformed colon cells, HT29 and Caco2, and to normal colon cells, CCD-18Co. Significant decreases in cell viability were observed at the 200-400 μM concentrations and in Caco2 and CCD-18Co cells in concentrations ranging from 100-400 μM for both MB and MZ. The purpose of the present study was to elucidate the mechanism through which this toxicity occurs. To this end, the ability of MB and MZ to induce apoptosis was investigated by assessing caspase 3/7 induction. Caco2 cells were exposed to concentrations of both agents ranging from 100-200μM for 12hrs, and CCD-18Co cells were exposed to 80-200μM of MB and MZ for 12hrs. Significant increases in caspase 3/7 activity were observed upon exposure to 60μM for Caco2 cells and 200μM in HT29 for both agents. CCD-18Co cells showed significant increases in caspase activity at the 120 and 200μM doses for MB and the 200μM dose for MZ. To determine if the intrinsic or extrinsic pathway of apoptosis was activated upon exposure to these agents, caspase 9 activity was also measured. Significant increases in caspase 9 activity were observed following exposure to 60μM in Caco2 cells, from 160-200μM in HT29, and in CCD-18Co cells in concentrations of 100 and 200μM for both compounds. We conclude that the death observed in transformed colon cells HT29 and Caco2, and CCD-18Co normal colon cells, occurs through an intrinsic apoptotic mechanism.

**1410 IN VITRO SCREEN FOR PESTICIDE INTERACTIONS WITH HUMAN MDR1 AND BCRP EFFLUX TRANSPORTERS.**

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Interference with the function of the multidrug resistance protein 1 (MDR1) and breast cancer resistance protein (BCRP) transporters can render organs, such as the brain and placenta, susceptible to chemical exposure and toxicity. The ability of pesticides to interact with human MDR1 and BCRP as substrates or inhibitors is not well understood. The purpose of this study was to screen and classify organochlorine pesticides (dieldrin, endosulfan, DDT and its metabolites DDE and DDD), pyrethroid pesticides (cypermethrin, deltamethrin, permethrin, resmethrin), and a neonicotinoid pesticide (imidacloprid) as potential substrates and/or inhibitors of human MDR1 and BCRP using an in vitro method. The col- orimetric ATPase assay uses plasma membranes of S9 insect cells transfected with a human efflux transporter and indirectly measures the interaction between compound and transporter by quantitation of phosphate released during transport. Four of the organochlorine pesticides inhibited MDR1 and BCRP transport of prototypical substrates between 3.8-33.6 μM and 3.0-6.9 μM, respectively (MDR1: DDT=DDE>DDE=DDE=DDE; BCRP: DDO=DDE>DDE=endosulfan). The pyrethroid pesticides, permethrin and resmethrin, moderately inhibited BCRP transport near 10 μM. Interestingly, cypermethrin activated MDR1 ATPase hydrolysis, suggesting that it may be a substrate of MDR1. Imidacloprid did not in- teract with either transporter in the ATPase assay. Future studies will characterize the kinetics of MDR1 and BCRP inhibition by pesticides in a cell-dependent transport system. Knowledge of the in vitro interactions of pesticides with MDR1 and BCRP provides initial insight into how tissues that express the transporters may become vulnerable to chemical accumulation and toxicity. Supported by NIH ES-0205022, ES-005022, ES-015991, DK-080774.

**1411 KINETIC INTERACTION OF PMSF (A NEUROPATHY PROMOTER) WITH MEMBRANE-BOUND ESTERASES THAT ARE SENSITIVE OR RESISTANT TO ORGANOPHOSPHORUS INDUCERS OF DELAYED NEUROPATHY.**

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PMF is a pro tease and esterase inhibitor which can protect or potentiate (“promotion”) of organophosphorus delayed neurotoxicity depending of if it is dosed previ- ously or after an inducer of delayed neurotoxicity and it is unstable in water solu- tion. Kinetic data of the esterase inhibition were obtained for phenylethylsulfonyl fluoride (PMFS) tested at different concentrations incubated for twenty minutes with particulate fraction (membranes) of chicken brain. A ki- netic model equation was deduced assuming a multi-enzymatic system with three different molecular phenomena occurring simultaneously: (1) Inhibition; (2) spon-
taneous chemical hydrolysis of the inhibitor; (3) ongoing (inhibition during the substrate reaction). A three-dimensional fit of the model was applied for analyzing the experimental data. The best fitting model is compatible with a resistant component (14%) and two sensitive enzymatic entities (44% and 41%). The corresponding second order rate constants of inhibition (ki=0.0076 nM-1 min-1 and 0.0014 nM-1 min-1, respectively) and the chemical hydrolysis constant of PMSF (kh=0.28 min-1) were simultaneously estimated. The consistency of results in fixed time and progressive inhibition experiments was considered an internal validation of the methodology. Mipafos resistant fraction was assayed with different concentrations of PMSF. The best fitting model is compatible with a one sensitive component (47% of total esterase activity) with ki=0.0014 nM-1 min-1 and a resistant one (12%) with chemical hydrolysis constant of kh=0.30 min-1. The results allowed to discriminate the esterase fractions sensitive or resistant to PMSF among those sensitive or resistant to mipafos or paraxon, in order to understand their role in the mechanism of induction, protection or potentiation of delayed neurotoxicity.

**1412 INDUCTION OF PLASMA ACETYLCHOLINESTERASE ACTIVITY AND APOPTOSIS IN MICE TREATED WITH ORGANOPHOSPHORUS TOXICANT, ETHANOL, OR COCAINE.**

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Organophosphorus compounds (OP) inhibit acetylcholinesterase (AChE) activity and cause cultured cells to undergo apoptosis. Live mice treated with OP have reduced AChE activity, but after a short recovery period, their AChE activity rebounds to levels that exceed baseline by more than 2-fold. To date no information is available on whether abnormally high AChE activity is characteristic of apoptosis in animals. Our goal was to determine whether induction of AChE activity is associated with apoptosis in live mice. For this purpose we treated mice with three chemically diverse agents that induce apoptosis: tri-o-cresyl phosphate, cocaine, and ethanol. Only tri-o-cresyl phosphate causes AChE inhibition.

On day one after treatment of mice with 1500 mg/kg tri-o-cresyl phosphate their plasma AChE activity was inhibited 50%. On day 4 after treatment, plasma AChE activity rebounded to a level 2.2-fold higher than pretreatment activity and remained elevated for about two months. On day 4, AChE activity in the lung was 1.5-fold higher than in controls. Cells in lung sections that were positive in the apoptosis TUNEL assay, stained heavily for AChE activity. Mice were treated with 25 mg/kg cocaine daily for 8 days. Plasma AChE activity increased 1.5-fold above baseline on days 7-9. TUNEL-positive cells in the livers of these cocaine treated mice stained heavily for AChE activity. Mice treated with 200 μl of 20% ethanol daily for 10 days had 2.5-fold elevated AChE activity in plasma on days 3-7. Apoptotic cells in the liver stained for AChE activity. In conclusion, AChE activity and apoptosis are induced in mice treated with OP, cocaine, and ethanol. Unusually high AChE activity may be a marker of exposure to apoptosis-inducing substances.

**1413 CHARACTERIZING STABILITY OF ORGANOPHOSPHATES IN HOME ENVIRONMENT DUST.**

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The purpose of this research was to identify and inform best practices for storage of dust from the home environment for agricultural contaminants, specifically organophosphate pesticides. As part of a 15-year children's cohort study at the University of Washington Center for Child Environmental Health Risks Research (CHC), home and vehicle dust samples were collected from farmworker households during late spring when organophosphate pesticides were being applied to orchards in the Lower Yakima Valley, WA. Briefly, dust samples were collected from homes and commuter vehicles using vacuum techniques. Samples were analyzed by LC/MS/MS using ESI. After analysis samples were stored at -20°C for 7 and 10 years. A reanalysis of 50 samples after storage shows reanalysis is highly correlated with the original analysis at both time points of collection (1999, 2002), with r2=0.93. Ratios of reanalysis to original analysis for azinphosmethyl were above 100% (p<0.0001), and showed a significant increase in concentration of OPs in dust for azinphosmethyl. The mean increase was 19% SE=4% for the samples stored for 7 years and 29% SE=4% for the samples stored for 10 years. This is potentially attributable to loss of mass due to drying during storage. Results for malathion, phosmet, and chlorpyrifos were consistent with the results for azinphosmethyl after storage for 7 and 10 years. Results are being used to inform the National Children's Study (NCS) in the development of methods for storage of dust before analysis for indoor environmental contaminants. Future studies include analysis for other indoor contaminants of interest to the NCS including metals and mold.

**1414 URINARY PYRETHROID METABOLITES AMONG PREGNANT WOMEN IN A RURAL AREA OF JIANGSU PROVINCE: RESULTS OF A PILOT STUDY.**

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Pyrethroid pesticides are widely used throughout the world and particular concern is exposure of pregnant women and their fetuses because little is known about the potential developmental hazards of such exposure. The present paper provides data on pyrethroid pesticide exposure based on questionnaire items and measurement of maternal urinary metabolite levels among 1149 pregnant women living in rural area of Jiangsu, China in 2009-2010. None of them reported occupational exposure to pyrethroid insecticide. Urine specimens were analyzed for three main metabolites for 3-phenoxbenzoic acid (3-PBA), cis- and trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (cis-DCCA and trans-DCCA) using a gas chromatographic-mass spectrometry method. The limit of detection for pyrethroid metabolites was 0.1 μg/L. All three pyrethroid metabolites were found in more than 95% of the urine samples. Median unadjusted (μg/L) and creatinine-adjusted values (μg/g cr) for urinary metabolites in these female exposures to pyrethroid pesticide were 1.01 and 1.55 for 3-PBA, 0.44 and 0.69 for cis-DCCA, 1.17 and 1.86 for trans-DCCA, respectively. Close to half (45.5%) of women self-reported that they or another household member had applied commercially available pesticides in the home during pregnancy. The questionnaire and laboratory data revealed that exposure to pyrethroid pesticides was considerably widespread in our subjects. The urinary metabolite concentrations among pregnant women in the present study were about 5 times higher than those in the general population from the developed countries. Interestingly, we found there was a seasonal variation trend between summer and autumn or winter, especially the levels of pyrethroid metabolites in summer were significant higher than those in the winter. These data indicated the need to assess the potentially adverse effects of pyrethroid pesticides exposure on fetuses and infants and the adequate measure to protect pregnant women to reduce pesticide exposures.

**1415 SUSCEPTIBILITY AND EXPOSURE BIOMARKERS OF PESTICIDES IN WOMEN FROM AN AGRICULTURAL COMMUNITY IN SOUTHERN MEXICO.**

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Epidemiological studies have shown acute and chronic adverse effects in agricultural workers and organophosphates (OP) pesticides still remain among the most used in developing countries. Agricultural families are potentially exposed to pesticides and poor attention has been given to the effects on children and women. Enzymes involved in OP metabolism, such as paraoxonase 1 (PON1) are polymorphic, which have shown to modify OP toxicity. We evaluated acetylcholinesterase (AChE) activity in women from an agricultural region in Southern Mexico, describing the exposure scenario to pesticides and the role of PON1Q192R genetic polymorphism on this biomarker. A transversal study was conducted in Muna, Yucatán (Southern Mexico). Participants donated a blood sample and responded to a structure questionnaire. AChE activity was determined by the Ellman method and PON1Q192R polymorphism by RFLP. Seventy-eight unrelated women (32±5.9 years old) who were born in Yucatán were included. AChE mean value was 36.7±6.1 U/gHb. PON1Q192R genotype frequencies were: 0.17/QQ, 0.55/QR and 0.28/RR.
0.28/RR. Almost all participants were housewives and only 10% worked in agriculture (none with 192QQ genotype). Seventy-two percent of women lived with a relative who worked with pesticides and 40% of them were married with a farmer; interestingly, AChE was lower in women not married with a farmer. AChE activity was marginally lower in 192QQ homozygote women (p=0.063). Our preliminary results show that pesticide exposure in women is complex and more studies are needed to avoid the adverse impact on health of agricultural families. (Supported by PROMEP-SEP-México).

1416 EVALUATION OF URINARY PESTICIDE BIOMARKERS AMONG CHILDREN AND ADOLESCENTS.


Pesticide use in the United States continues to attract negative public attention. In recent years, attention has focused on the chronic, low-level effects of pesticides that may have on children. Over the past decade, studies have attempted to correlate reductions in birth weight and length with detections of pesticide biomarkers in maternal biological media. The current research investigates the relationship between the detection of non-persistent pesticide urinary biomarkers of exposure and differences in height and weight of children age 6-11 from the 2001-2002 NHANES dataset. Three biomarkers were detected in more than 50% of the sample (n=3152): 3,5,6-trichloropyridinol (TCPY); para-aminophenol (PNP); and 3-phenoxybenzoic acid (3PBA). Mean values for weight and height were determined for each age group of children with detectable biomarker in the urine sample and compared to the equivalent mean of children that did not have a detectable level of biomarker in the same group. In most age groups, t-test comparisons did not indicate significant differences between children with a recorded biomarker detected compared with those with a non-detect. Significant differences of note: PNP recorded significant height differences for children age 8 with detect mean height=130.9 cm (n=49) and non-detect mean height=134.3 cm (n=38), p=0.0499; weight for age 7 in the 3PBA group recorded a detect mean weight=28.6 Kg (n=76) and a non-detect mean weight=25.6 (n=27), p=0.0088. These differences were not consistent among age groups. Additionally, mean biochemical concentrations for adolescents (age 12-18) with recorded biomarker detectors were examined and compared to the mean of those that did not have a detected biomarker to assess overall health status. Significant differences in biochemical concentrations were observed in each biomarker subgroup. Biochemical variations were not consistent across the subgroups and those groups with significant differences in mean concentrations did not have a mean value that exceeded the normal range.

1417 FOLIAGE RESIDUES OF MALATHION AND FENPROPRAZIN INSECTICIDES AS DETERMINANTS OF LOW-LEVEL STRAWBERRY HARVESTER EXPOSURES.

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During strawberry harvesting, leaf pesticide residues and their transfer to the hands of harvesters was studied intensively in summer 2011 at Santa Maria, CA. Pesticide Glove Residues (PRG) are the part of Dislodgeable Foliar Residues (DFR) and Transferrable Foliar Residues (TFR) accumulated during harvesting. Malathion (M) and fenpropazin (F) insecticides were sprayed at 1 lb/acre in two large field sites. DFR (μg/cm2; M: 0.238 to 0.027, F: 0.026 to 0.015) and TFR (μg/cm2; M: 0.029 to 0.0026, F: 0.021 to 0.0033) drop to 10% in the 3d between the first picking at the Pre-Harvest Interval and the second picking. By relating the same pesticide residues found on the hands of harvesters who picked either barehanded or used rubber latex gloves, we estimated exposures of barehanded and gloved harvesters of this cropland, as well as other ornamental and turfgrass areas. The applied pesticides can disperse into surrounding surface water. The purpose of our study was to determine if in California, on a per county basis, pesticide usage correlates with surface water concentrations. The California Environmental Data Exchange Network (CEDEN) has an online database of publicly accessible geocoded surface water chemical sampling data from 1993-2009. Using reverse geocoding, we were able to correlate the latitude and longitude points of each sample with California counties. By combining sampling data with median county usage data from the California Pesticide Information Portal (CPIP), surface water concentrations of pesticides in a given county were related to their historical use from 1993-2009. Graphs were produced for each pesticide and r2 values were used to correlate each variable against the median sampling concentration. Dichlor was found to have an r2 of 0.603 for usage in lbs., 0.546 for usage ranking of bordering counties, and 0.566 for the simplified usage ranking of bordering counties. Chlorpyrifos was found to have an r2 of 0.010 for usage in lbs., 0.302 for usage ranking of bordering counties, and 0.287 for the simplified usage ranking of bordering counties. Diazinon was found to have an r2 of 0.027, 0.038, and 0.055 respectively. Dieldrin's data was very similar to that developed for Diazinon and Chlordane. Our results show that for some pesticides, there is a trend between per county usage and surface water contamination. We believe the lack of a trend for certain pesticides is linked to environmental fate and transport, application method, and the ecology and topography of application sites. The chemicals that did show a strong correlation will allow identification of potential contaminated areas of concern and allow for improved utilization of limited sampling resources.

1418 PESTICIDE TOXICITY BIOMARKERS IN INDIGENOUS AGRICULTURAL WORKERS.

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Indigenous agricultural workers are a vulnerable population to pesticide toxicity. In Nayarit, Mexico, 40% of indigenous named “huicholes” leave their communities to work in agricultural fields, where organophosphorous pesticides (OP) are used. Several studies have demonstrated the role of human paraoxonase 1 (PON1) in modulating OP toxicity. The aim of this study was to evaluate pesticide toxicity biomarkers in agricultural workers. A pilot study was carried out in 66 indigenous agricultural workers. A structured questionnaire was applied. Three inclusion criteria were applied: All with the blood group type (ABO: O and Rh+: sharing the cultural tradition and speaking the indigenous dialect. Hematological parameters were analyzed using a Coulter; acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activities were determined spectrophotometrically. PON1 192 genetic polymorphism was determined by real-time PCR using TaqMan system. PON1 enzyme activity was evaluated using as substrates phenylacetate and 4-methyl phenylacetate (4-CMPA). The preliminary results show that lower values of erythrocyte count, hemoglobin, hematocrit and platelets than reference values in 5% of indigenous huicholes. Mean AChE and BuChE activities were 24.7±4.5 U/g Hb and 3833.8±74 U/L±80.5, respectively. Mean alyesterase and 4-CMPA activities were 120.1 U/mL±52.7 and 43.6 U/mL±12.09, respectively. PON1 192 polymorphism showed frequencies of 35.4 for RR, 18.5 for QQ and 46.1 for QR. PON1 192 polymorphism was associated with alyesterase and 4-CMPA activities. We are increasing the number of individuals in order to validate the association found in this preliminary study. Research in agricultural indigenous workers is needed to further understand pesticide toxicity and the range of exposure in this vulnerable population.

1419 CORRELATION OF PESTICIDE SURFACE WATER CONCENTRATIONS WITH PUBLICLY AVAILABLE USAGE DATA.


California has roughly 10,000,000 acres of cropland. Pesticides are applied to much of this cropland, as well as other ornamental and turfgrass areas. The applied pesticides can disperse into surrounding surface water. The purpose of our study was to determine if in California, on a per county basis, pesticide usage correlates with surface water concentrations. The California Environmental Data Exchange Network (CEDEN) has an online database of publicly accessible geocoded surface water chemical sampling data from 1993-2009. Using reverse geocoding, we were able to correlate the latitude and longitude points of each sample with California counties. By combining sampling data with median county usage data from the California Pesticide Information Portal (CPIP), surface water concentrations of pesticides in a given county were related to their historical use from 1993-2009. Graphs were produced for each pesticide and r2 values were used to correlate each variable against the median sampling concentration. Dichlor was found to have an r2 of 0.603 for usage in lbs., 0.546 for usage ranking of bordering counties, and 0.566 for the simplified usage ranking of bordering counties. Chlorpyrifos was found to have an r2 of 0.010 for usage in lbs., 0.302 for usage ranking of bordering counties, and 0.287 for the simplified usage ranking of bordering counties. Diazinon was found to have an r2 of 0.027, 0.038, and 0.055 respectively. Dieldrin's data was very similar to that developed for Diazinon and Chlordane. Our results show that for some pesticides, there is a trend between per county usage and surface water contamination. We believe the lack of a trend for certain pesticides is linked to environmental fate and transport, application method, and the ecology and topography of application sites. The chemicals that did show a strong correlation will allow identification of potential contaminated areas of concern and allow for improved utilization of limited sampling resources.
1420 PROFENOFOS METABOLISM AND ESTIMATES OF PROFENOFOS EXPOSURE IN EGYPTIAN COTTON FIELD WORKERS.

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Profenofos is a thioephosphate organophosphorus (OP) pesticide capable of inhibiting B-esters such as acetylcholinesterase, butryrylcholinesterase, and carboxylesterase. Detoxification of profenofos is known to be mediated by cytochrome P450s to form the biologically inactive metabolite 4-bromo-2-chlorophenol (BCP), which is excreted in the urine. The goal of the present study was to investigate the metabolism of profenofos by rat and human liver microsomes as well as determine the concentration of BCP, a profenofos specific metabolite, in urine from Egyptian cotton field workers involved in profenofos application. Rat and human liver microsomes were incubated with 5μM and 25μM profenofos and the rate of BCP formation and profenofos disappearance was assessed. Results indicate that profenofos is readily metabolized to BCP and other metabolites at a greater rate in rats than in humans. In vitro metabolism studies confirm that BCP is a sensitive and specific biomarker for assessing the rate of profenofos metabolism in humans. Daily urinary BCP concentrations were utilized as a biomarker of profenofos exposure and were determined for Egyptian cotton field workers during 8-10 consecutive days of profenofos application to cotton fields. Substantial interindividual variability was observed for Egyptian cotton field workers during 8-10 consecutive days of profenofos application to cotton fields. From these BCP levels, estimates of daily absorbed profenofos were also served in urinary BCP concentrations which ranged from 0.9 - 8,053 ng/mg creatinine. From these BCP levels, estimates of daily absorbed profenofos were also calculated and ranged from 0.037-372μg/kg/day. This is one of the first reports to estimate the internal dose of profenofos in humans. (NIH R01 ES016308 and ES016308-02S)

1421 CHLORPYRIFOS EXPOSURE IN ADOLESCENT EGYPTIAN AGRICULTURAL WORKERS.

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Chlorpyrifos (CPF), an organophosphorus (OP) insecticide, is a public health concern due to its widespread potential for human exposure. Blood acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) levels may be used as biomarkers of effect while urine 3,5,6-trichloro-2-pyridinol (TCPy) is a specific biomarker of CPF exposure. A longitudinal study was designed to assess ChE and TCPy levels in adolescent insecticide applicators in Egypt over 10 months from baseline (pre-spray) to recovery (post-spray). It is not known whether adolescent populations are at increased risk compared to adult workers. This study will quantify ChE and TCPy in occupationally exposed adolescents and age-matched controls who may have received environmental exposures. Participants included male insecticide applicators aged 12 to 19 years (N=58). Control participants were from the same villages but did not work in the cotton fields (N=40). Pre-scan data served as baseline. Blood for ChE analysis was drawn four times, while urine for TCPy analysis was collected frequently during the spray period and several times before and after. Urinary TCPy was analyzed by negative ion GC-MS and normalized to urinary creatinine levels. Pre-spray and mid-spray TCPy levels for the applicators ranged from 2.92 - 42.79 and 5.8 - 3915.16 ng/mg creatinine, respectively. Control group pre-spray and mid-spray TCPy levels ranged from 2.36 - 46.17 and 9.83 – 211.94 ng/mg creatinine, respectively, indicating that this group received environmental CPF exposure. AChE and BChE were measured using the EQM Test-mate kit, and both occupationally and environmentally exposed groups showed significant inhibition of ChE during CPF application. The quantitative data for OP biomarkers of exposure and effect presented here are the first to be reported for adolescent agricultural workers. (NEIHS R21 ES017223)

1422 PARAOXANASE 1 STATUS IN EGYPTIAN AGRICULTURAL WORKERS EXPOSED TO THE ORGANOPHOSPHORUS PESTICIDE CHLORPYRIFOS.

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Animal studies have shown that paraoxanase 1 (PON1) genotype can influence susceptibility to the toxicity of the organophosphorus pestcide chlorpyrifos (CPF). However, few human studies have assessed the influence of PON1 genotype and activity on CPF related toxicity. In the current study we sought to determine the influence of PON1 genotype on serum PON1 activity and blood cholinesterase inhibition in a population of agricultural workers exposed to CPF. Saliva, blood and urine were collected from agricultural workers (n = 120) from Egypt's Menoufia Governorate. Saliva was used to genotype participants for PON1 polymorphisms, blood was used to monitor cholinesterase activity and serum was used to determine PON1 activity towards chlorpyrifos-oxon (e.g., CPOase activity of PON1) and paraoxon (e.g., PCE activity of PON1). Urinary levels of the CPF metabolite 3,5,6-trichloro-2-pyridinol (TCPy) were determined as a marker of CPF exposure. The PON1 55 (p ≤ 0.05) but not the PON1 192 genotype had a significant effect on CPOase activity. However, both the PON1 55 (p ≤ 0.05) and PON1 192 (p ≤ 0.001) genotype had a significant effect on PCE activity. When adjusted for urinary TCPy excretion and stratified by PON1 genotype, baseline CPOase activity did not have a significant effect on cholinesterase inhibition. Together this suggests that workers retained the capacity to detoxify chlorpyrifos-oxon under the exposure conditions experienced by this study population regardless of PON1 genotype and activity. These findings will be helpful to future risk assessment efforts for CPF exposure. (NIH R01 ES016308 and ES016308-02S)

1423 EXPOSURE TO PESTICIDES, CYTOGENETIC DAMAGE IN BUCCAL MUCOSA CELLS AND ACTIVITY OF ACETYLCHOLINESTERASE IN NICARAGUAN PESTICIDE VENDORS.

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Pesticides are highly consumed in Central America and represent one of the major environmental health risks for people in Nicaragua, taking hundreds of lives every year. Consequently, pesticide vendors in Nicaragua are an interesting group for bio-monitoring studies due to the existence of low level pesticide exposure over long periods of time, lack of safety regulation compliance and common proximity of pesticides to homes and food markets. This study was performed to evaluate the genotoxic effects of pesticide exposure on pesticide vendors in Matagalpa, one of the most important farming departments in Nicaragua. Micronuclei in buccal cells (MNBC) were analyzed in 47 pesticide vendors and in 40 non-exposed individuals (control); all the subjects were men. Additionally, pesticide biomonitoring was performed in whole blood and acetylcholinesterase activity was monitored by a titrimetric method. The results revealed a significant difference in the MNBC frequency between vendors and controls (mean ± S.D., 6.0 ± 2.3 versus 3.6 ± 1.3; MNBC≤0.001; Mann-Whitney U-test). Furthermore, cholinesterase levels indicated a higher abnormal level in the vendor group compared to the control group; however, as indicated by pesticide biomonitoring, chlorinated pesticide concentrations were very low in both groups. Characterization of the vendor group and their working conditions by questionnaires revealed that most of these vendors did not use any protective equipment/measures during work and that pesticide vendors are routinely exposed to genetic damage in somatic cells. Additionally, our results indicate that exposure to a mixture of pesticides had a positive significant correlation with genetic damage within the tested population.

1424 ASSOCIATION BETWEEN URINARY CONCENTRATIONS OF DICHLOROPHENOL PESTICIDES AND OBESITY IN ADULTS.

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Exposure to environmental pesticides has been found to have a number of detrimental effects on humans. Many of these chemicals are considered endocrine-disrupting agents that may damage the body’s natural weight-control mechanisms; thus, exposure to pesticides might be associated with obesity. In this study, we assessed the potential associations between exposure to dichlorophenol pesticides and obesity in adults. A total of 10,914 human subjects aged 20-85 years were selected from the 2005-2006 and 2007-2008 National Health and Nutrition Examination Survey. Subjects were categorized as obese and non-obese based on body mass index. Urinary concentrations of dichlorophenol residues were used to determine level of exposure to environmental pesticides. Multivariate logistic regression was performed using the SAS 9.2 to assess the association between 2,4-dichlorophenol (2,4-DCP) and 2,5-dichlorophenol (2,5-DCP) levels in urine and obesity with adjustment for potential confounders, including age, gender, race, income, education, dietary fat intake, and amount of physical activity. A dose-dependent increase in the prevalence of obesity was observed for both dichlorophenol compounds. Logistic regression revealed a significant association between dichlorophenol (AOR: 1.25, 95% CI: 1.02, 1.52) and fourth (AOR: 1.44, 95% CI: 1.19, 1.75) inter-quartile range were significantly associated with obesity among the second (AOR: 1.23, 95% CI: 0.001) genotype had a significant effect on POase activity. When adjusted for urinary TCPy excretion and stratified by PON1 genotype, baseline CPOase activity did not have a significant effect on cholinesterase inhibition. Together this suggests that workers retained the capacity to detoxify chlorpyrifos-oxon under the exposure conditions experienced by this study population regardless of PON1 genotype and activity. These findings will be helpful to future risk assessment efforts for CPF exposure. (NIH R01 ES016308 and ES016308-02S)
Carbofuran is a carbamate insecticide that inhibits AChE. Although toxic by ingestion in mammals it has low dermal toxicity, with relatively few incidents of confirmed worker illnesses. This risk assessment describes its time of onset, time to peak effect and time to recovery in rats using brain AChE inhibition in acute and 21 day dermal toxicity studies; in vitro rat/human relative dermal absorption for granular (5G) and liquid (4F) formulations; occupational exposure estimates using the Pesticide Handlers’ Exposure Database (PHED) and Agricultural Handlers’ Exposure Database (AHED) as well as worker exposure data using Furadan 3G in rice paddy fields. The point of departure for acute risk calculation (BMDL10) was 6.6 mg/kg/day for brain AChE inhibition after 6 h exposure. In a 21-day study, the BMDL10 was 6.8 mg/kg/day, indicating reversibility. At 75 mg/kg/day, time of onset was ≤30 min and time to peak effect was 6–12 h. Rat skin had ca ten-fold greater dermal absorption of carbofuran (Furadan 5G or 4F) than human skin. Exposure estimates for 3G in rice and 4F in ten crops had adequate margins of exposure (≥100). In conclusion, rat dermal carbofuran toxicity was assessed in terms of dose and time-related inhibition of AChE. Comparative dermal absorption in rats was greater than in humans. Worker exposure estimates indicated acceptable risk for granular and liquid formulations of carbofuran although the liquid formulation required much more personal protective equipment.
where acute effects were observed (\#body wt & food consumption; \#tremors; NOEL, Sprague-Dawley rats). A 90 day dietary rat study was selected for the chronic duration (\#body wt & lifespan; \#mammary tumors; NOEL, 0.41 mg/kg/d). All definitive studies were IFRA Guideline acceptable. Neuroendocrine effects are a priority but systemic effects with lower NOELs would protect for other effects. Mammary carcinogenesis is unique to Sprague-Dawley rats and is not a risk for humans. Dermal exposure (likely route of concern) had no useful studies so oral NOELs were used (oral ab-
ge against the hypothesis that chlorpyrifos can cause neurodevelopmental effects at exposures below the threshold for inhibition of acetylcholinesterase (AChE) activity in the nervous system, which is an established mode of action (MoA) for chlorpy-
rifs neurotoxicity. We weighed all of the data from epidemiology, animal toxicity, and mechanistic studies in terms of quality and relevance to humans, allowing each data set to inform one another. We then evaluated all of the data together to deter-
mine whether there is a causal relationship between chlorpyrifos at low exposures and neu-
rodevelopmental effects in humans is plausible. Our analysis determined that: the epidemiology data do not consistently demonstrate associations between chlorpyrl-
of grasp on the concept. However, our data suggests a novel mechanism by which arsenic exposure may elevate the risk of cancer development.

\[ 1432 \text{ CHARACTERIZATION OF HBD1 DOWN-REGULATION FOLLOWING ARSENIC EXPOSURE.} \]

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Arsenic is a potent human carcinogen and a major drinking water contaminant for millions of people worldwide. We previously reported decreased urinary levels of β-
defensin-1 (HBD1) peptides in men exposed to arsenic in drinking water in two case-control populations based in Nevada and Chile, as well as suppressed DEFBI mRNA resulting from arsenic exposure in vitro. In the present study, real-time RT-
PCRs analysis was performed to investigate the effects of arsenic (AsIII) and its more toxic metabolite, monomethylarsonous acid (MMAIII), on DEFBI expression in immortalized human cells derived from skin and kidney. AsIII and MMAIII treatment

\[ 1433 \text{ LOSS OF PTEN IS PRECEDED BY CHRONIC INFLAMMATORY RESPONSES DURING MMA (III)-

INDUCED MALIGNANT TRANSFORMATION OF HUMAN UROTHELIAL CELLS.} \]

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Bladder cancer development is associated with chronic exposure to arsenicals. Deletions or mutations of tumor suppressor phosphatase and tensin homologue deleted on chromosome ten (PTEN) locus occur frequently in invasive bladder car-
cinoma, however the mechanisms involved are not fully understood. PTEN expression is suppressed after 18 wk of in vitro exposure to arsenite in a human prostate epithelial stem/progenitor cell line. To evaluate the role of PTEN in ar-
senic-induced bladder cancer we determined PTEN protein expression in an im-
mortalized human urothelial cell line (UROtsa) that was exposed in vitro to 50 nM of an arsenic metabolite, the monomethylarsonous acid (MMAIII) for 12, 24 or 52 wk. PTEN protein expression was completely suppressed only in those cells ex-
posed for 52 wk to MMA III (III). Interestingly our group had previously established a 12 wk exposure period as key time point for cells transformation by MMA (III) which is consistent with a sustained over-expression of inflammatory cytokines and transcription factors activation. These facts suggest that PTEN loss is preceded by a chronic inflammatory state and suggest that there is a link between these two events. PTEN loss was consistent in this study with a significant decrease in STAT3 phosphorylation at Tyr 705 and an increase of c-myc expression. miRNA-21 has been reported as a PTEN negative regulator, however expression assays showed that PTEN expression is not regulated by miR21 in our model. These and previous re-
sults suggest that PTEN loss is not the responsible for UROtsa cells transformation but maybe a consequence of a sustained inflammatory state, however it is likely that PTEN loss could contribute to keep the malignant phenotype in UROtsa cells. Further investigation will aim to determine the link between chronic inflammation and PTEN loss in the process of transformation induced by MMA (III) after 24 wk exposure.

\[ 1434 \text{ OVEREXPRESSION OF SPARC MODULATES CELL GROWTH AND MIGRATION IN MALIGNANTLY

TRANSFORMED HUMAN UROTHELIAL CELLS (UROTSAs).} \]

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SPARC (Secreted Protein Acidic and Rich in Cysteine) is a member of the matri-
cellular group of proteins that is known to modulate interactions between cells and the extracellular matrix. Influencing tumor growth and migration, the expression of
SPARC in human bladder cancer was found to be absent in the malignant urothelial cells comprising the growth front, but high amounts are recruited to the tumor. The purpose of this study is to examine the expression of SPARC and the role it plays in the formation and progression of bladder cancer in a model of heavy metal induced cell transformation. Our previous study showed SPARC expression was significantly down-regulated in Cd\textsuperscript{2+} and As\textsuperscript{3+} transformed UROtsa cells. To investigate the possible role of SPARC in bladder cancer, SPARC was stably transfected into As\textsuperscript{3+} and Cd\textsuperscript{2+} transformed UROtsa cell lines. The expression of SPARC in the transfected lines was analyzed using real time reverse transcriptase polymerase chain reaction, western blot analysis, and immunofluorescence to determine intracellular localization. The transfected cell lines were further characterized by determination of growth rates, the ability to form colonies in soft agar, secretion SPARC into growth media, and migration capabilities. It was shown that SPARC mRNA and protein expression was induced in the transfected cell lines, was localized to distinct vesicles within the cytoplasm, and was secreted in copious amounts into the medium. Several SPARC-transfected cell lines had significantly different growth and migration rates when compared with the parental line; and all transfected cells were capable of forming colonies in soft agar. This study suggests SPARC expression effects the growth and migration of UROtsa cells and SPARC may play a substantial role during the multi-step process of bladder cancer development.

1435 EXPRESSION OF EXTRACELLULAR MATRIX PROTEINS IN UROTS A BLADDER CELLS TRANSFORMED WITH ARSENIC OR CADMIUM.

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Matricellular proteins are extracellular matrix molecules that have non-structural roles including signaling and protein-protein binding. We have previously shown that the matricellular protein SPARC is expressed in normal bladder epithelium and in the UROtsa cell line. However, SPARC expression is shut down to an extent with cells transformed with arsenic. We expect that matricellular proteins, including SPARC, contribute to tumor cell invasion and metastasis in bladder cancer. In this study, we performed a screen for essential genes involved in arsenite toxicity using yeast deletion mutant library and found several arsenite resistant genes. However, involvement of essential genes in toxicity of arsenite remain to be clari
d. In this study, we performed a screen for essential genes involved in arsenite sensitivity using D\textsuperscript{AmP} yeast library (Open Biosystems), which has decreased expression of essential genes through mRNA perturbation. We studied 17 genes (\textit{RRT1, YPT1, TAD3, POB3, EGR8, CEG1} and \textit{YBR089W}) which confer yeast cells hypersensitive to arsenite, when their expression were repressed. \textit{RRT1} is ribose-5-phosphate-ketoisomerase in the pentose phosphate pathway (PPP). Disruption of each gene in the non-oxidative branch of PPP, which is important for generating ribose-5-phosphate, enhanced arsenite toxicity. Moreover, arsenite repressed expression of these genes. Our results suggest that metabolite derived from the non-oxidative branch of PPP might be involved in protection against arsenite toxicity.

1438 DISFUNCTIONAL REGULATION OF METABOLIC AND MITOCHONDRIAL GENE EXPRESSION FOLLOWING ARSENIC EXPOSURES.

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Consumption of low to moderate levels of arsenic promotes a number of diseases (cardiovascular disease, neurodegeneration, insulin resistance, and diabetes) that stem from altered metabolism. However, it is unclear how arsenic enhances metabolic disease, especially in different target tissues. We explored the hypothesis that the pathogenesis of low to moderate arsenic exposure is promoted by transcriptional and post-transcriptional shifts in protein and microRNA programs that regulate mitochondrial function and insulin sensitivity. In support of this hypothesis, we observed that arsenic exposures (10 – 250 ppb in drinking water for 2 wk) increased adipose tissue

1437 INVESTIGATING OF ESSENTIAL GENES INVOLVED IN ARSENITE TOXICITY IN SACCHAROMYCES CEREVISIAE.

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In an effort to identify novel mechanisms responsible for toxicity of arsenite, we have previously searched for factors that determine sensitivity of cells to arsenite using yeast deletion mutant library and found several arsenite resistant genes. However, involvement of essential genes in toxicity of arsenite remain to be clari
d. In this study, we performed a screen for essential genes involved in arsenite sensitivity using D\textsuperscript{AmP} yeast library (Open Biosystems), which has decreased expression of essential genes through mRNA perturbation. We studied 17 genes (\textit{RRT1, YPT1, TAD3, POB3, EGR8, CEG1} and \textit{YBR089W}) which confer yeast cells hypersensitive to arsenite, when their expression were repressed. \textit{RRT1} is ribose-5-phosphate-ketoisomerase in the pentose phosphate pathway (PPP). Disruption of each gene in the non-oxidative branch of PPP, which is important for generating ribose-5-phosphate, enhanced arsenite toxicity. Moreover, arsenite repressed expression of these genes. Our results suggest that metabolite derived from the non-oxidative branch of PPP might be involved in protection against arsenite toxicity.

1439 EXPOSURE TO ARSENIC AND PREVALENCE OF DIABETES IN CHIHUAHUA, MEXICO.

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We have recently shown that exposure to inorganic arsenic (iAs) in drinking water is associated with increased prevalence of diabetes in Zamapan and Lagunera (Mexico). Here, we report preliminary results of an ongoing study in Chihuahua (Mexico). To date, we have recruited 554 subjects (360 women, 194 men) who drink water containing 0.1 to 298 μg As/L. Diabetic individuals were identified using fasting plasma glucose (FPG≥126 mg/dL), oral glucose tolerance test (2-hour plasma glucose, 2HPP≥200 mg/dL), and reported diagnosis or medication for dia
etes. The metabolites of iAs were analyzed in spot urine samples by hydride genera
tion-atomic absorption spectroscopy. Sum of iAs metabolites in urine ranged from...
5 to 425 ng/ml. Associations between diabetes and iAs exposure and urinary metabolites of iAs have been estimated by logistic regression with statistical adjustment for age, sex, hypertension and obesity. We found that both FPG and 2HPP are significantly associated with iAs concentration in drinking water and urine, with sum of iAs metabolites in urine, and with the concentrations of methylated metabolites of iAs, methylarsenic (MAs) and dimethylarsenic (DMAs) in urine. Odds ratios of 3.58 (95% CI 1.38-9.30) and 2.86 (95% CI 1.02-8.01) were found for the exposure to iAs in drinking water greater than 95th percentile (>186 μg As/l) and for urinary DMA concentrations greater than 95th percentile (>136 ng/As/ml), respectively. These preliminary results are consistent with previous reports from this and other laboratories linking the risk of diabetes to moderate or high exposures to iAs in drinking water. Future studies need to examine possible associations of diabetes with low iAs exposures.

**1440 PROLONGED IAS EXPOSURE LEADS TO ABBERRANT INSULIN SIGNALING IN L6 MYOCYTES.**

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Diabetes mellitus is a metabolic syndrome characterized by inappropriate production of insulin or the inability of cells to respond to insulin. It is estimated that by the year 2050 1 in 3 U.S. adults will have diabetes mellitus. Insulin is the principal hormone involved in lowering blood glucose and functions by suppressing liver gluconeogenesis and glycogenolysis in the liver and by stimulating the uptake of glucose into skeletal muscle and adipocytes. Recent epidemiological studies both in the USA and abroad have linked chronic ingestion of low levels of inorganic arsenic (iAs), an environmental toxicant, to the onset of diabetes mellitus. Although these observations have been met with some skepticism, there are few mechanistic studies that have tried to elucidate the mechanisms by which iAs perturbs insulin signaling. Here we show that L6 myocytes, an insulin responsive cell line, exposed to low to moderate levels of iAs (0.25 to 2 μM) for 4 days show decreased insulin stimulated glucose uptake as well as increased phosphorylation of IRS-1 at serine 307, thereby impairing insulin stimulated uptake. These data support the epidemiological evidence that chronic exposure to low physiologically relevant levels of arsenic can contribute to insulin resistance and type 2 diabetes states. And while the etiology of type 2 diabetes has yet to be elucidated these data show that in addition to pharmaceuticals such as Metformin and insulin, arsenic-exposed macrophages showed decreased pro-atherogenic cholesterol efflux. To better characterize the arsenic-enhanced plaque, we exposed ApoE−/− mice, a well-described model of atherosclerosis, to arsenic (200 ppb) for 13 weeks and characterized the macrophage composition within the plaque. Our observations may lead to a better understanding of the role of macrophages in arsenic-induced atherosclerosis.

**1441 ACUTE ARSENIC CARDIOTOXICITY ALTERS CARDIAC CYCTOME P450 EXPRESSION AND ARACHIDONIC ACID METABOLISM IN C57BL/6 MICE.**


Arsenic (AsIII) cardiotoxicity has received increasing attention as human exposure to As(III) was associated with myocardial damage, heart failure, and cardiac arrest. We have previously shown that the formation of the cardioprotective cytochrome P450 (CYP) epoxycygenase products (epoxycytoxicanic acids, EETs) is reduced in several cardiac pathologies. Nevertheless, the effect of acute As(III) toxicity on the expression of cardiac CYP enzymes has never been reported. Therefore, in the current study, we investigated the effect of acute arsenic toxicity on the expression of CYP enzymes as well as CYP-mediated arachidonic acid metabolism in mice hearts. In addition, we investigated the effect of acute As(III) toxicity on soluble epoxide hydrolase enzyme (sEH) which metabolizes the cardioprotective EETs to the less biologically active dihydroxyxycosainic acids (DHETs). Acute As(III) toxicity was induced by a single intraperitoneal injection of 12.5 mg/kg of As(III). Our results showed that As(III) treatment caused a significant induction of the cardiac hypertrophic markers, atrial natriuretic peptide, brain natriuretic peptide, and cardiotoxin-1. In addition, As(III) treatment caused a significant reduction of Cyp1b1, 2b9, 2b10, 2b19, 2c38, 2c40, 4f15, 4f18, and sEH gene expression in the heart of AsIII-treated mice. In the heart microsomes, the formation of the cardioprotective 11,12-, and 14,15-EETs was significantly reduced, whereas the formation of 8,9-, 11,12-, 14,15-DHETs was significantly increased. The decrease in the cardioprotective EETs can be attributed to the increase of sEH activity parallel to the induction of the sEH gene expression. In conclusion, acute As(III) toxicity alters the expression of several CYP and sEH enzymes with a consequent decrease in the cardioprotective EETs which may represent a novel mechanism by which As(III) causes progressive cardiotoxicity. Supported by NSERC Discovery Grant RGPIN 250139-07.

**1442 PRO-ATHEROGENIC ARSENIC EXPOSURE ALTERS MACROPHAGE POLARIZATION.**

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Environmental arsenic exposure is linked epidemiologically to increased atherosclerosis. Moreover, we showed that arsenic exposure altered plaque composition. However, the mechanisms by which arsenic enhances atherosclerosis are still unknown. Monocytes and macrophages are key players in atherosclerosis. Different macrophage phenotypes (M1, M2 or Mox) with different biological functions are present within atherosclerotic plaques. M1 are classical macrophages with inflammatory characteristics, M2 are reparative macrophages and Mox respond to oxidative stress in an mrf2-dependant manner (nuclear erythroid related factor-2). As arsenic activates mrf2, we hypothesize that it increases atherosclerosis by skewing macrophage polarization toward a Mox phenotype through mrf2 activation. Therefore, we investigated the effects of arsenic on murine bone marrow-derived macrophages by first culturing these cells in M-CSF into resting macrophages and then polarizing these into M1 with IFNγ or into M2 with IL-4. Macrophages or polarized macrophages were then exposed to arsenic (1.33 μM) and gene expression, phagocytosis and cholesterol transport were evaluated. Arsenic-exposed naïve macrophages showed a significant increase in phenotypic marker of Mox, including being higher expression of mrf2. Furthermore, arsenic increased Mox markers in M1 and M2 macrophages while decreasing markers of M1 and M2 (GNOS and arginase1), respectively, regardless of their primary polarization. Although these data suggest that arsenic skewed macrophage differentiation toward Mox, characterized by a decrease in phagocytosis, which was not seen at chronic arsenic exposure. Moreover, arsenic-exposed macrophages showed decreased pro-atherogenic cholesterol efflux. To better characterize the arsenic-enhanced plaque, we exposed ApoE−/− mice, a well-described model of atherosclerosis, to arsenic (200 ppb) for 13 weeks and characterized the macrophage composition within the plaque. Our observations may lead to a better understanding of the role of macrophages in arsenic-induced atherosclerosis.

**1443 SUPPRESSION OF ADIPOGENIC DIFFERENTIATION BY ARSENIC INVOLVES INDUCTION OF CHOP10 VIA ENDOPLASMIC RETICULUM STRESS RESPONSE.**


Adipogenesis is regulated by a complicated network of transcription factors that coordinate expression of hundreds of proteins required for establishing the mature fat-cell phenotype. Early in adipogenesis, the CCAAT/enhancer-binding protein β (C/EBPβ) and C/EBPα are rapidly induced to express and later activate expression of peroxisome proliferator-activated receptor γ (PPARγ) and C/EBPα. While the expression of C/EBPα rises quickly in preadipocytes in response to adipogenic hormones, its DNA binding activity is initially suppressed through binding with C/EBPβ, a constitutively expressed protein (C/EBPΔIII, also known as C/EBPβ or GADD153). The expression of CHOP10 is downregulated along with adipogenesis and results in activation of C/EBPα. Exposure of 3T3-L1 preadipocytes to noncytotoxic levels of arsenic, including inorganic arsenite (iAs3+, up to 5 μM), inorganic arsenate (iAs5+, up to 20 μM), monomethylarsonic acid (MMA3+, up to 1 μM) and dimethylarsonic acid (DMA3+, up to 2 μM) dose-dependently decreased adipogenic hormone-induced adipogenesis. In addition, iAs3+ exhibited a strong inhibitory effect on adipogenesis in primary cultured preadipocytes isolated from mouse white adipose tissues (WAT) and mesenchymal stem cells derived from human WAT. Time-course studies in 3T3-L1 cells revealed that inhibition of adipogenesis by arsenic occurred in the early stage of adipogenic differentiation and was substantially correlated with CHOP10 induction. Induction of CHOP10 by arsenic, followed by a reduction in DNA binding activity of C/EBPα, was associated with arsenic-triggered endoplasmic reticulum stress response. Taken together, our studies indicate that low-level iAs and its methylated trivalent metabolites trigger endoplasmic reticulum stress response and upregulate CHOP10, which inhibits C/EBPα transcriptional activity, and thus impairs adipogenesis.

**1444 LIPIDOMICS OF SUBCHRONIC LOW-LEVEL INORGANIC ARSENIC EXPOSURE IN THE RAT.**

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Recent epidemiological evidences indicate close association between inorganic arsenic exposure via drinking water and cardiovascular diseases. However, the exact mechanism of this arsenic-mediated increase in cardiovascular risk factors remains...
in enigmatic. In order to investigate the effects of inorganic arsenic exposure on lipid metabolism, rats were divided into 3 groups (50 ppm, 150 ppm, and 200 ppm) sodium arsenite (100, 150 and 200 ppm) in their drinking water for 12 weeks. Dyslipidemia induced by the two arsenicals exhibited different patterns. Hypocholesterolemia characterized the effect of arsenite at all the doses while arsenate induced hypercholesterolemia at the 150 ppm dose. Hypertriglyceridemia was the hallmark of arsenate effect whereas plasma free fatty acids was increased by the two arsenicals. Reverse cholesterol transport was inhibited by the two arsenicals as evidenced by decreased HDL cholesterol concentrations whereas hepatic cholesterol was increased by arsenite (100 ppm), but decreased by arsenate (150 ppm) and arsenate (100 ppm) respectively. Brain cholesterol and triglyceride were decreased by the two arsenicals; arsenate decreased the renal contents of the two lipids whereas arsenite increased the renal contents of the two lipids. Arsenite (150ppm) and arsenate (100ppm) inhibited hepatic HMG CoA reductase. At other doses of the two arsenicals, hepatic activity of the enzyme was up-regulated. The two arsenicals however up-regulated the activity of the brain enzyme. We observed positive associations between tissue arsenic levels and plasma FFA, and negative associations between tissue arsenic levels and HDL cholesterol. Our findings indicate that in contrast to strengthening a dose-dependent effect phenomenon as observed with many other compounds, the two forms of inorganic arsenic up- or down-regulate different pathways in the lipid metabolism spectrum at ‘low’ or ‘high’ doses and this may be responsible for their cardiovascular effects.

1445 ALTERED ARSENICAL DISPOSITION IN EXPERIMENTAL NONALCOHOLIC FATTY LIVER DISEASE.

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Nonalcoholic fatty liver disease (NAFLD) is the most common form of chronic liver disease in Western society. NAFLD represents a spectrum of liver damage ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), which may alter the ability of the liver to properly metabolize and eliminate xenobiotics. Arsenic is a well known environmental toxicant that is dependent primarily on liver metabolism for proper elimination from the body. The purpose of the current study was to determine whether NASH increases the elimination and metabolism of the toxic metalloid arsenic. Male C57Bl/6 mice received either a high fat or methionine and choline deficient diet to model simple steatosis and NASH, respectively. At the conclusion of the dietary regimen, all mice were given a single, oral dose of either sodium arsenate or arsenic trioxide. Mice with NASH exerted significantly higher levels of arsenic in urine (24h) compared to controls. Total arsenic retained in liver and kidney of NASH mice was not different from control; however both monomethyl (MMA) and dimethyl (DMA) arsenic metabolites were differentially retained in both kidney and liver of NAFLD mice. Specifically, NASH livers retained significantly higher levels of MMA, whereas DMA is retained significantly less in the kidneys of NASH mice. Interestingly, urinary elimination of the more toxic, trivalent inorganic arsenic species (iAsIII) was higher in mice with NASH compared to control; whereas pentavalent inorganic arsenic (iAsV) was preferentially retained in livers of NASH mice. Although no change in the protein expression of hepatic arsenic (+3 oxidation state) methyltransferase was detected in NASH, protein expression of Mrp1, a membrane transporter known to transport trivalent inorganic arsenic species, was increased in the livers of NASH mice. These results suggest that NASH alters the normal disposition and elimination of arsenical species, and implicates cellular transport rather than biotransformation as a possible mechanism.

1446 OCNOCGENIC TRANSFORMATION OF NORMAL PROSTATE STEM CELLS BY NEARBY, NONCONTIGUOUS MALIGNANT PROSTATE EPITHELIA POTENTIALLY INVOLVES INTERLEUKIN-6.

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Stem cells (SCs) likely play a key role in carcinogenesis. Malignant cells often release extracellular factors which influence SC microenvironment and modify tumor behavior. Thus, in this study we determined if normal SCs (NSCs) might be influenced by nearby arsenic-transformed malignant epithelia (ME) without actual physical contact. Tranwell, non-contact co-culture allowed study of effects of nearby but non-contiguous arsenic-transformed malignant human prostate epithelia (CAE-PE cells) on the isogenic normal prostate NSC line, WPE-STEM, NSCs exposed to the isogenic normal human prostate epithelial cells (RWPE-1) served as the control. After a few weeks of co-culture with ME, NSCs showed hyper-secretion of metalloproteinasises (MMPs), suppression of the tumor suppressor gene PTEN and pathway activation resulting in abnormal growth, increased colony formation and formation of highly branched duct-like structures in Matrigel, all indicative of a malignant SC phenotype. A time-related dysregulation of SC self-renewal genes occurred along with morphological and genetic evidence of epithelial-to-mesenchymal transition (EMT) in SCs during co-culture with ME. Interleukin-6 (IL-6), a cytokine involved in control of tumor microenvironment, was hyper-secreted by CAE-PE cells and NSCs previously transformed by inorganic arsenic into cancer SCs (CSCs). After 1 week of exposure to IL-6, NSCs hyper-secreted MMP, showed decreased PTEN expression and underwent EMT, similar to the response of NSCs co-cultured with ME. Taken together, our data indicate that ME can drive nearby NSCs towards a malignant phenotype, in effect creating putative CSCs without any actual physical contact. This transformation is likely due to factors secreted by the arsenic-transformed ME, potentially including IL-6. This tumor “recruitment” of NSCs into CSCs potentially constitutes a new phenomenon in cancer growth, extension and metastases and deserves further study.

1447 P53 AND BCL2 RESPONSES TO INORGANIC ARSENIC AND METHYL METHANESULFONATE IN HUMAN HT-1080 FIBROSARCOMA CELLS.

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Exposures to drinking water contaminated with inorganic arsenic have been associated with increased incidence of cancer in humans. In vitro and in vivo data have demonstrated that arsenic can act as a co-mutagen and co-carcinogen by inhibiting DNA repair. Methyl methanesulfonate (MMS) is a well known mutagen and mutagenic. The present study examined the responses of p53 and Bcl2 to 24 hr treatments with different concentrations of inorganic arsenic and MMS to determine whether arsenic affects the cellular response to DNA damage by MMS. p53 is a key protein in the cellular response to DNA damage, including apoptosis, and Bcl2 is a protein associated with an anti-apoptotic, survival response. Concentrations of 3, 10, 30, 100, and 300 nanomolar arsenic, 1, 3, 10, 30, and 100 micromolar MMS, and combinations of the lowest and highest arsenic concentrations with all of the MMS concentrations were assessed in a human fibrosarcoma cell line with functional p53 (HT-1080). MMS induced a statistically significant increase in p53 at 100 micromolar, but not at lower concentrations, while arsenic alone had no effect on p53 expression at concentrations up to 300 nanomolar. However, co-exposure of cells to 300 nanomolar arsenic resulted in a significantly increased p53 response to MMS at concentrations as low as 1 micromolar. Bcl2 was increased significantly in response 300 nanomolar arsenic, but not to MMS. Surprisingly, the combination of 300 nanomolar arsenic with MMS did not significantly increase the Bcl2 response except at the highest MMS concentration. These results are generally consistent with a co-mutagenic/co-carcinogenic mode of action for arsenite. The effect of arsenic co-exposure on other proteins in the cellular response to DNA damage will be investigated in future studies.

1448 ARSENIC INDUCES P53-DEPENDENT APOPTOSIS THROUGH THE DOWN-REGULATION OF UBE2D FAMILY GENES IN RENAL TUBULAR CELLS.

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We recently found that cadmium (Cd)-induced apoptosis result from overaccumulation of p53 through the suppression of Ube2d family (Ube2d1, Ube2d2, Ube2d3 and Ube2d4) genes expression in normal rat proximal tubule cells (NRK-52E cells). Ube2d family is one of the ubiquitin-conjugating enzyme and leads to degrade p53 by ubiquitin-proteasome system. In this study, we examined the effect of arsenite and inorganic mercury, inducing metals, on the expression of Ube2d family genes and cellular accumulation of p53 protein using NRK-52E cells. NRK-52E cells were treated with 20 μM NaAsO2, (As[III]) and 50 μM HgCl2, (Hg[II]) for 24 h, and cell viability was measured at 24 h. As[III] suppressed the gene expression of Ube2d family except Ube2d3 at 6 h treatment. P53 protein was markedly accumulated and apoptosis was detected in cells treated with As[III] for 24 h. On the other hand, Hg[II] treatment for 6 h decreased Ube2d1 mRNA level but increased Ube2d3 mRNA level. Although p53 protein level was not elevate, apoptosis was drastically observed in cells treated with Hg[II] for 24 h. These results suggest that As[III] as well as Cd induces p53-dependent apoptosis through suppression of the expression of Ube2d family genes in renal tubular cells, whereas Hg[II] induces p53-independent apoptosis.
1449 ARSENIC AND THE EPIGENOME: LINKED BY METHYLATION.

K. Bailey1, L. Smeester1, W. Ward2, J. Rager1, X. Guan4, N. Smith1, G. García-Vargas3, L. Del Razo4, Z. Drobná5, K. Hemant1, M. Styblo1 and R. Fry1, 2Environmental Sciences and Engineering, University of North Carolina at Chapel Hill, Chapel Hill, NC; 3Department of MT-3 gene. For this purpose, UROtsa cells were cultured either for 48 hours or for an extended period of time in the presence of 1 μM cadmium or 1 μM arsenite. Chromatin immunoprecipitation analysis showed that both arsenite and cadmium can increase acetyl H4 and H3K4me3, and can decrease H3K9me3 and H3K27me3 in the MT-3 promoter. These changes are more pronounced after extended exposure of the cells to the heavy metals. However, both arsenite and cadmium could not induce the expression of the MT-3 gene unless the cells were exposed to the histone deacetylase inhibitor MS-275. These results suggest that the histone modifications induced by heavy metals may not be sufficient to induce the expression of MT-3 in the bladder cells and some in vivo alterations may be necessary to induce the expression.

1450 EPIGENETIC REGULATION OF MT-1X OVER-EXPRESSION IN METAL-INDUCED BLADDER CANCER.

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Many bladder cancers have been shown to over-express metallothioneins (MT) which are small molecular weight metal-binding proteins that can confer resistance to chemotherapeutic agents. Cadmium and arsenite are bladder carcinogens that were recently used to transform the normal human urothelial cell line, UROtsa. In the metal induced transformed cells as well as in bladder cancer, MT-1X was shown to be over-expressed. In this study, chromatin immunoprecipitation analysis was used to assess the binding of transcription factors and to assess the modification of histone tails in chromatim preparations from normal and metal-transformed UROtsa cells. For this purpose, RNA was isolated from the parent as well as transformed cell lines and real time RT-PCR was performed to determine the expression level of MT-1X. The results obtained suggest that MT-1X is highly over expressed in the arsenite-transformed cells compared to the normal parental as well as the cadmium-transformed cells. There was also an increase in acetyl H4 and H3K4me3 modifications and a decrease in H3K27me3 modifications in the metal responsive region of the MT-1X promoter of the arsenite-transformed cells. Furthermore, transcriptional factors such as p300, NF-1, Sp1 and LBP1 also showed an increase binding to this region. Our data suggests that the transformation of the UROtsa cells with arsenite may have resulted in epigenetic modifications within the promoter of the MT-1X gene which resulted in its increased expression.

1451 ARSENITE AND CADMIUM-INDUCED EPIGENIC MODIFICATIONS IN THE PROMOTER OF THE METALLOTHIONEIN 3 GENE.

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Cadmium and arsenite are environmental carcinogens that have been implicated in the development of bladder cancer. A previous study from this laboratory has shown that both cadmium and arsenite can cause the malignant transformation of an immortalized, but non-tumorigenic, human urothelial (UROtsa) cell line. These transformed cell lines in culture do not express metallothionein-3 (MT-3); despite the fact that most human bladder tumors over-express this gene. However, when they are transplanted as tumors they show strong expression of MT-3 similar to what is observed in human urothelial cancers. This difference in MT-3 expression suggests that the mechanism of MT-3 gene silencing between the parental and transformed urothelial cells are different. The goal of this study was to determine if direct exposure to cadmium or arsenite could cause epigenetic modifications in the promoter of the MT-3 gene. For this purpose, UROtsa cells were cultured either for 48 hours or for an extended period of time in the presence of 1 μM cadmium or 1 μM arsenite. Chromatin immunoprecipitation analysis showed that both arsenite and cadmium can increase acetyl H4 and H3K4me3, and can decrease H3K9me3 and H3K27me3 in the MT-3 promoter. These changes are more pronounced after extended exposure of the cells to the heavy metals. However, both arsenite and cadmium could not induce the expression of the MT-3 gene unless the cells were exposed to the histone deacetylase inhibitor MS-275. These results suggest that the histone modifications induced by heavy metals may not be sufficient to induce the expression of MT-3 in the bladder cells and some in vivo alterations may be necessary to induce the expression.

1452 CHRONIC EXPOSURE TO MONOMETHYLSARONOUS ACID LEADS TO SUBSTANTIAL CHANGES IN GENE EXPRESSION AFTER 12 WEEKS.

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Bladder cancer has been associated with chronic arsenic exposure. Monomethylarsonous acid [MMA(III)] is a metabolite of inorganic arsenic and has been shown to transform an immortalized urothelial cell line (UROtsa) at concentrations 20-fold less than arsenite. MMA(III) was used as a model arsenical to examine the mechanisms of arsenical-induced transformation of urothelium. A microarray analysis was performed to assess the transcriptional changes in UROtsa during the critical window of chronic 50 nM MMA(III) exposure that leads to transformation at three months of exposure. The analysis revealed only minor changes in gene expression at one and two months of exposure, contrasting with substantial changes occurring at three months of exposure. These results indicate that there is a strong association between the acquired phenotypic changes that occur with chronic MMA(III) exposure and cellular features that are indicative of a malignant transformation. (NIHES 04940, NIH ES07091)

1453 CHRONIC EXPOSURE TO ARSENIC RESULTS IN RETENTION OF METHYLARSONONITE (MASHI) AND DIMETHYLSARNITE (DMASHI) IN UROTHELIAL CELLS.

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Current evidence suggest that the methylated trivalent metabolites of inorganic arsenic (iAs), MAsIII and DMAIII, are more toxic than either their pentavalent counterparts or iAs. We have shown that both MAsIII and DMAIII are relatively stable in cellular environment, suggesting that analysis of cells or tissues could be used to characterize the internal exposures to these toxic metabolites. Here, we examined the retention of tri- and pentavalent arsenicals in urinary bladder exfoliated cells (BECs) isolated from urine of 105 residents of Chihuahua (Mexico) who are exposed to iAs in drinking water. Arsenic species in BECs were analyzed by the oximate state-specific hydride generation (HG)-inductively coupled plasma-mass
Arsenic, a human carcinogen, affects millions of people worldwide. Inorganic arsenic (iAs) and its methylated metabolite, monomethylarsonic acid (MMAIII), a highly toxic form of the metalloid, may both have carcinogenic potential. However, the mechanisms of these arsenicals and whether or not they are shared or different, is not fully known. Thus, arsenic methylation-capable (TRL1215) and methylation-deficient (RWPE-1) cell lines were chronically exposed to low-level iAs (1.0–5.0 μM) or MMAIII (0.25–1.0 μM) and observed for oncogenic changes. Oxidative DNA damage (ODD), various metrics of transformation, and gene expression of factors involved in transformation and/or DNA damage/repair were periodically assessed. iAs induced a cancer phenotype in both cell lines. However, only methylation-capable cells showed an increase in ODD, and based on matrix metalloproteinase activity, cellular invasive ability, and colony formation, methylation-capable cells acquired this altered phenotype much more rapidly (~18 wks) than methylation-deficient cells (~30 wks). In contrast, MMAIII caused consistent increases in ODD levels (~500%) in both cell lines but this took several weeks (~20 wks) to reach a significant level. Increased invasive ability and colony formation also occurred at a similar time (~18–20 wks) in both MMAIII-exposed cell lines. Methylation-deficient cells acquire a cancer phenotype much more rapidly with MMAIII exposure compared with inorganic arsenic, while both iAs and MMAIII induced transformation at similar time-points in methylation-capable cells. For both arsenicals, several DNA damage/repair factors appear to be enhanced as cellular ODD increased. Thus, MMAIII causes more rapid transformation associated with ODD but arsenic methylation is not required for this transformation. Moreover, the observations that both MMAIII and iAs induce a malignant phenotype, and iAs does not induce ODD, indicates arsenic may have both genotoxic and epigenetic mechanisms dictated by target cell ability to methylate arsenic.

**1456 CYTOTOXICITY AND GENOTOXICITY OF ARSENIC IN PRIMARY HUMAN LUNG CELLS.**

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Arsenic originates from both geochemical and numerous anthropogenic activities including mining, combustion of fossil fuels, wood preservation, agriculture and metallurgy. Exposure of the general public to significant levels of arsenic is widespread. Arsenic is a well-documented human carcinogen. Long-term exposure to low levels of arsenic in drinking water has been linked to bladder, lung, kidney, liver, prostate, and skin cancer. Among them, lung cancer is of great public concern. However, little is known about how arsenic causes lung cancer. The purpose of this study was to determine the cytotoxicity and genotoxicity in human primary bronchial fibroblasts cells (NHBF). Our data show that arsenic induces a concentration-dependent increase in cell death after acute (24 h) or chronic (120 h) exposure, 0.1, 1 and 10 μM arsenic for 24 h induced 85, 64 and 35 percent relative survival, respectively, and induced 73, 57 and 11 percent of relative survival, respectively after 120 h treatment. 24 h exposure of 5 and 10 μM arsenic induced 15 and 32 chromosome damage compared to the control. Comet assay was used to measure DNA damage concentration of DNA damage repair enzymes. Chronic (120 h) exposure induced a concentration-dependent increase in double strand breaks. Future study will focus on arsenic cytotoxicity and genotoxicity in primary bronchial epithelial cells.
LUNG DISTRIBUTION OF ARSENIC ANDHEME OXYGENASE EXPRESSION IN RATS INTRATRACHEALLY EXPOSED TO ARSENITE.
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The anthropogenic exposure to inorganic arsenic (iAs) is mainly via inhalation. However, there is limited information about the tissue distribution of arsenic by this route. The induction of heme oxygenase-1 (HO-1) in tissues has been used as a sensitive biomarker of effect for iAs exposure. HO-1 induction depends on the kinetic and dynamic capacity of arsenical specie to reach and accumulate on the tissues. Kinetic studies in rodents have shown that a single oral dose of iAs results in the following distribution of iAs: liver-kidney-lung during the first 6 h after exposure, maintaining iAs as the main arsenical specie. The objective of this study was to evaluate the HO-1 induction in the lung of rats exposed to a single intratracheal dose to arsenite. Sprague Dawley male rats were exposed intratracheally (0.5 mg/kg) to sodium arsenite. Lungs were obtained after 3 and 24 h post-exposure for Western blot analysis to determine HO-1 levels and lung distribution of iAs and their metabolites (by hydride generation atomic absorption spectrometry). No changes in HO-1 in rat lung were observed after exposure to arsenite. Rat arsenic concentration in the lung at 3 h was 726 ng/g of tissue being iAs the prevalent specie (62%). While at 24 h arsenic concentration in lung was 200 ng/g of tissue were only methylated species were present, suggesting that iAs was complete biotransformed at this time. In conclusion rats exposed intratracheally to 0.5 mg/kg arsenite did not induce HO-1, this could be explained by the low distribution of arsencals in the lung and the quick biotransformation of iAs.

ARSENITE EXPOSURE DECREASES ENOS PROTEIN LEVELS AND DISRUPTS ITS PHOSPHORYLATION PROFILE IN A MODEL OF HUMAN PLACENTAL ENDOTHELIAL CELLS EA.HY 926.
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Arsenic exposure has been associated with a high incidence of hypertension and multiple cardiovascular diseases. Recent studies have shown that mice chronically exposed to low level arsenic through drinking water develop hypertension and consequent concentric left ventricular hypertrophy. In order to understand the underlying mechanism or arsenic related hypertensive pathology, a human placental endothelial cell line, EA.hy 926 was used to explore the effects of arsenic exposure on endothelial nitric oxide synthase (eNOS) function and stability. Phosphorylation of key residues necessary for adequate nitric oxide production and reduced total eNOS protein levels after a 6 hr exposure. This suggests that arsenic exposure affects eNOS function by increasing its degradation rate and altering phosphorylation of key residues necessary for adequate nitric oxide production and vasomotor regulation. (NIH ES 04940; ES06694; SWEHSC P30ES006694)

APOFERRITIN INHIBITS THE FORMATION OF ARSENIC-INDUCED REACTIVE OXYGEN SPECIES IN J774 MURINE MACROPHAGES.
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Arsenic (As) causes oxidative damage to a myriad of cellular targets via production of reactive oxygen species (ROS), although the precise mechanism is not known. Previously we described a model where As(III)-induced ROS are produced by the release of redox active iron from ferritin by the following redox reaction: Fe(III) + As(III) → As(V) + Fe(II). The resulting Fe(II) from this redox reaction is now free to produce ROS via the Fenton reaction. In order to study the relationship between Fe(II) and As(III)-induced ROS we employed the H2DCF-DA assay to measure ROS in J774 murine macrophages treated with 100 μM As(III), 400 μM H2O2, and 4 μM apoferritin. The data indicated that (i) As(III) by itself does not significantly increase ROS in J774 cells, (ii) H2O2 elevated ROS generation ~3-fold relative to control J774 cells, (iii) As(III) potentiates H2O2 mediated ROS production, and (iv) apoferritin reduces arsenic induced ROS in the presence of H2O2 by ~11%. Our finding that As(III) does not increase ROS levels by itself but increases ROS levels in the presence of H2O2 suggests that Fenton chemistry is involved. Since our previous study has shown that the iron chelator apoferritin does not bind As(III), the observed reduction in As(III)-induced ROS in the presence of apoferritin suggests that the apoferritin is chelating redox active iron and is supportive of our hypothesis. Because ferritin is found throughout the body and ROS have been implicated in As-induced cancer and cardiovascular disease, As induced ROS produced by As-Fe redox reactions in ferritin may represent a major and novel mechanism involved in arsenic toxicity against a broad range of cellular targets and diseases. (P30ES006694)
Heavy metal exposure can cause a wide variety of health problems such as cancers of the skin, bladder, lung, and liver, cardiovascular and peripheral vascular disease, neurological disorders, and diabetes. The health issues caused by heavy metal exposure are frequently linked to the activation of transcription factors. Expression of the human UGT1A1 gene, part of the UGT1 locus, is regulated by a host of xenobiotic receptors (XR), such as PXR, CAR, PPARα, the Ah receptor and the Nrf2-Keap1 pathway. We predicted that in vivo control of the human UGT1A1 gene might serve as an effective broad-spectrum bioassay system for the detection of the environmental toxicants. This was tested in recently developed humanized UGT1A1 (hUGT1A1) mice, created by expression of the human UGT1A1 locus in a Ugt1-null background. Neonatal hUGT1A1 mice develop severe hyperbilirubinemia resulting from inadequate expression of hepatic and intestinal UGT1A1. Induction of liver or intestinal UGT1A1 by agents that activate the XR leads to a rapid decrease in total serum bilirubin (TSB), which can be accurately and easily measured. When we examined metal exposure by oral administration towards As³⁺, Cd²⁺, Pb²⁺, Fe²⁺ and Cu²⁺, only As³⁺ and Cd²⁺ led to reductions in TSB levels. These metals did not induce hepatic UGT1A1 gene expression, but dramatically induced UGT1A1 in the small intestine. Along with UGT1A1 gene expression, As³⁺ and Cd²⁺ also induced intestinal Cyp2b10 gene expression. Interestingly, neither As³⁺ nor Cd²⁺ induction of UGT1A1 or Cyp2b10 gene expression was associated with activation of CAR, as demonstrated in UGT1A1 mice that were also Car-null. However, Cd²⁺ induction of Cyp2b10 is controlled by the intestinal NF-κB/IKK pathway, as confirmed in mutant mice with conditional deletion of IKKe/B in enterocytes. We suggest that UGT1A1 mice can be used as a biosensor for environmental toxicant exposure. (Supported by USPHS grant GM086713 and Superfund P42ES010337)

The Atlantic killifish (Fundulus heteroclitus) is a model environmental organism that is extremely tolerant to arsenic. As a first step in elucidating the mechanism behind this phenomenon, we used PCR to identify aquaglyceroporins, which are arsenic transporters, in the gill and intestine, the major organs exposed to environmental arsenic. Aquaporin3 (kAQP3) was the most highly expressed AQP in that organ. Our study indicated that AQP3 is the most highly expressed AQP in the intestine. Expression of kAQP3 and human AQP3 (hAQP3) in Xenopus oocytes significantly enhanced water, glycerol and urea uptake. However, hAQP3 expressed in HEK293T cells did not enhance arsenic uptake whereas hAQP3 significantly enhanced arsenic uptake. Thus, kAQP3 is a novel aquaglyceroporin because it does not transport arsenic. This observation may partially explain arsenic tolerance in the Atlantic killifish. By contrast, we demonstrated that a variant of AQP3 found in the Atlantic killifish does enhance arsenic transport into HEK293T cells.

The Atlantic killifish is a model environmental organism for assessing aquaporin variants as a potential sensor for environmental arsenic exposure. First, it has been well documented that some aquaporins have been functionally characterized in vivo and in vitro assays than are the non-transformed inorganic precursors. Second, it would appear that individuals with higher ratios of MMA to DMA in their urine are more at risk for adverse outcomes of As ingestion. Moreover, certain polymorphisms in the human arsenic (+3 oxidation state) methyltransferase enzyme (hA3MT) that performs such methylation have been associated with higher levels of MMA, suggesting this is a genetic risk factor. We have pioneered the engineering of hA3MT alleles into Drosophila as a means of comparing the differential metabolic and phenotypic effects that such allelic mixtures might bestow on flies. Moreover, because of the sophisticated genetic analyses available in this higher organism we can screen for genes and/or pathways that interact differentially with iAs (using wild type, non-methylating flies) as compared to the methylated As species produced in vivo (using the hA3MT transgenics). While we have found using in vivo RNA interference that NF-κB-mediated pathways are an important defense response to As in this system (as in humans), disruption of NF-κB function leads to extreme toxicity occurring in the hA3MT transgenic flies as compared to the wild type flies when both are similarly exposed to iAs. Our hypothesis is that other pathways will likewise show strong genetic interaction with methylated arsenicals, since we find these species exhibit significantly and varied binding to cellular proteins as compared to iAs alone.

There is a potential association between traffic-related pollution and the exacerbation of childhood asthma. As part of the Detroit near-road exposure to urban air pollutants Study (NEXUS), we are interested in evaluating the toxicological outcomes associated with downwind and upwind size-fractioned freeway impacted samples. A Chem-Vol sampler collected on a 2-in-3 day schedule for coarse, fine and ultrafine particles at 900 lpm at a site located 100m from the 1-96 freeway in Detroit, Michigan (32°44'11-5/11). The sampler was customized to switch between two separate sampling heads based on wind direction to isolate upwind (background) and downwind (roadway) contributions. The PUF and polypropylene filters will be extracted in methanol for both particulate characterization and toxicological studies. Colocated high time resolution measurement data at the 1-96 site were used to evaluate the potential upwind and downwind impacts. Upwind and downwind PM2.5 concentrations were 7.5 and 8.5 μg/m3, respectively (p<0.0001), as measured by a Thermo SHARP sampler. A Magee AE22 Aethalometer measured black carbon concentrations, a strong indicator for diesel emissions; upwind and downwind concentrations were 238 and 394 ng/m3, respectively (p<0.0001). Significant differences in vehicle emission contributions were also detected in the NOx data (Thermo 42C); upwind and downwind concentrations were 50.1 and 73.6 ppb, respectively (p<0.0001). Analysis of trace elements, ions, organic compounds and toxicological outcomes are still underway. These preliminary findings indicate that there is not only a difference in the PM mass influencing the sampling site, but that the composition and therefore potential toxicological effects could also differ based on the traffic-induced particles. We anticipate that our potential source contributions by using novel wind-activated sampling will enhance our understanding of the toxicological effects in a real-world exposure study.

This study evaluates how equilibrium vapor concentrations above petroleum solvent mixtures are affected by liquid aromatic content and the implications for estimating benzene vapor exposures. Mixtures with liquid benzene content ranging from 0.01 to 1.0% (by weight) and varied percentages (0, 1, 5, 10, 20 and 100%) of 1,2,4-trimethylbenzene in n-nonane were studied. Headspace vapor concentrations 150 min. after mixing in glass-sight vials were assessed using a direct-injection gas chromatography/flame ionization detection method that showed good precision. The measured values were compared to predictions based on Raoult’s Law, with and without non-ideality corrections using activity coefficients. Ratios of vapor to liquid benzene concentrations decreased with increasing total aromatic content; i.e., mixtures with 10% to 20% trimethylbenzene simulating non-hydrocarbon solvent mixtures had much lower ratios compared to the >99% aliphatic mixtures that simulate hydrotreated mineral spirits. Positive deviations from Raoult’s Law were greatest at liquid benzene concentrations below 0.1%, particu-
Flow cytometry-based hematotoxicity matrix readily integrates with studies of the effects of ionizing radiation or chemical exposure in multiple species.

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The ability to rapidly and efficiently generate data on the condition of peripheral blood cell populations has obvious value in studies designed to investigate the impact of agents like ionizing radiation or chemicals. A hematotoxicity response matrix, or HARM assay, has been devised that involves the addition of a minimal volume of whole blood to a solution containing a fluorescent nucleic acid dye, mitochondrial membrane potential dye and counting beads. Following a short incubation, the sample is analyzed by flow cytometry and cell populations are identified based on light scatter characteristics and nucleic acid content. Initial assessment of this method involved exposure of rats to a wide range of radiation doses and demonstrated time- and dose-dependent alterations in several important peripheral blood cell populations including lymphocytes, neutrophils, reticulocytes and platelets. As examples of the utility of the HARM assay, data from two study designs will be discussed. Mice were injected with 100 μCi 137Cs as a model of internal, systemic exposure to radiation. Absolute lymphocyte counts were reduced as early as 6 hrs and up to 4 weeks post exposure, however no effects were observed in the erythroid population. Integration of the HARM assay into the standard 28-day repeat dose rat study used in pharmaceutical and industrial product safety assessment was investigated. Exposure of rats to the antineoplastic agents chlorambucil, cyclophosphamide, melphalan, or thiotepa for 28 consecutive days elicited changes in lymphoid and erythroid populations that were dose and compound specific. The ability of the HARM assay to readily assess a multitude of hematological endpoints that display varying responses to different exposures highlights the utility of this assay for situations involving industrial hygiene and safety assessments and environmental biomonitoring.

Comparison of the sensitivity of GC/EI-MS to GC/ECNI-MS for the quantification of BDE-47, BDE-49, BDE-52, BDE-95, BDE-99, BDE-100, BDE-136, and BDE-153 in maternal plasma and cord blood.


Autism spectrum disorders affect about 1% of children in the US, with high economic costs to families and society. Little is known about the non-genetic causes of autism, and even less about the role of environmental chemical exposures. The use of polybrominated diphenylethers (PBDEs) rose rapidly in the US over the last two decades and, with it, the concern about their developmental neurotoxicity. Of the 209 PBDE congeners, tetra-, penta- and hexa-BDEs are most commonly present in human tissues. Previous analytical methods only evaluated a limited number of these PBDE congeners without particular focus on their neurotoxicity. Therefore, a highly sensitive and selective analytical method to simultaneously detect the most biologically active PBDE congeners at pg/g to ng/g plasma concentrations in small volume human plasma samples was developed. In this study, the sensitivity and specificity to determine PBDE congeners in human plasma using gas chromatography–mass spectrometry with electron ionization (GC/EI-MS) and electron capture negative ionization (GC/ECNI-MS) were compared. Electron energy, emission current, source temperature, focus lens, and choice of ECNI reagent gases were optimized. By monitoring bromide ion ([Br]−) using ECNI source, the limit of quantification of targeted PBDE congeners was 1 pg/g plasma, which was two orders of magnitude lower than the LOQs when using EI. The method was validated and used to quantify the targeted PBDEs in human plasma and cord blood samples. The concentrations of tetra-BDEs were much greater than those of penta- and hexa-BDEs, which is in contrast to their levels in the environment. Supported by R01ES020392, P42ES04699 and P01 ES011269 JB Foundation.

Comparison of two different ELISA-based methods for the detection of microcystins in human blood.

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Microcystins (MCs) are known contaminants of water bodies due to cyanobacterial blooms all over the world and the public is at risk of being intoxicated by exposure to contaminated water, food (e.g., fish) or algal food supplements. Subsequent to the 1996 Cariarua event in Brazil, where patients were intravenously exposed to high MC doses during dialysis in two dialysis clinics using cyanobacterial bloom contaminated water, several techniques (LC-MS, GC-MS and ELISA) were established to detect MCs in serum. Persons chronically exposed to low doses of MCs show very low toxin serum levels. Thus far, low MC levels were analyzed using LC-MS, albeit MC detection with the latter method is primarily dependent on the levels of free (non-covalently bound) MC and the availability of MC standards. Conversely, little is known about the suitability of highly sensitive ELISA for qualitatively and quantitatively determining MC in human blood. The aim of the current study was to determine whether commercially available ELISAs and the respective sample preparation methods would allow MC quantification in human blood serum. For this, two different MC-ELISAs, the Adda-ELISA kit or the MC serum-ELISA kit (Abraxis, Warminster, PA, USA) were used and the concentration-dependent recovery of MCs determined. Human blood serum samples were spiked with varying concentrations of microcystins (MC-LR, MC-YR, MC-RR, MC-LA, MC-LW, MC-LF and defined MC mixtures) and extracted with two different methods (methanol extraction followed by SPE and kit-based sample clean-up). In addition, MC spiked bovine serum and standard cell culture medium containing 10% FBS were used to investigate matrix effects. The results indicate that the serum ELISA is more suitable for MC analysis compared to the standard method (extraction, SPE and Adda-ELISA). Due to the detection range of the ELISA, sample concentrations of MCs and/or spiking methods required to allow detection of low levels of MCs in human blood or in cell culture samples.

Analytical method validation of tris(2-chloroisopropyl)phosphate (TCP) in Harlan Sprague Dawley maternal rat blood.

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The commercial flame retardant (FR), tris(2-Chloroisopropyl)phosphate (TCP) is added in rigid polyurethane foam for use in furniture products. TCP replaced the previous FR, pentabromodiphenyl ether, which had been found to accumulate in the environment and in human tissue. However, due to the prevalent use of TCP in the construction industry and the way in which organophosphate esters release into the surrounding environment, persistent levels of TCP may result in broad human exposure, leading to the selection of TCP for study by the National Toxicology Program. In this work, an analytical method was developed and validated for all four TCP structural isomers extracted from Harlan Sprague Dawley maternal rat blood; ranging from ~4 to ~100 ng/mL. After extracting samples using ethyl acetate, evaporating to dryness and reconstituting in hexane with an isoelectric internal standard, analysis was performed using gas chromatography with mass spectrometric detection. The results confirmed the method was linear (r ≥ 0.99), accurate (% RE: –15.7 to 18.7%), and precise (% RSD ≤ 10.3%) for all four isomers. Storage study results established that TCP spiked blood samples were stable for a minimum of 14 days without significant TCP loss when stored under freezer (–20°C) or ultra-freezer (70°C) conditions; while extracts were stable if stored under ambient, refrigerated or freezer conditions; thus allowing this validated method to be used to analyze samples from a future toxicological study.

An LC/MS/MS method for determination of various drugs of abuse and metabolites in municipal wastewater effluent samples.


A method was developed using a liquid chromatography-tandem mass spectrometry (LC/MS/MS) for the quantification and confirmation of 8 drugs of abuse (cocaine, codeine, MDMA, methadone, methamphetamine, morphine, nicotine, and...
Paraoxonase-1 (PON1) is capable of hydrolyzing the oxons of organophosphate pesticides (OP). PON1 genotype and phenotype have been proposed as markers of susceptibility to OP and health effects of OP exposure. In 2005-2006, we conducted a longitudinal study involving farmworkers and non-farmworkers in the Yakima Valley, WA, and evaluated the potential for OP exposure in farmworkers. Farmworkers had significantly higher levels of azinphos methyl (AZM) in blood than non-farmworkers, and ChE inhibition was significantly related to levels of AZM and urinary azinphos methyl metabolites in a dose-dependent manner. We tested whether PON1 192 genotype or PON1 phenotype, as represented by arylesterase (AREase) activity, modified the relationship between ChE inhibition and exposure biomarkers. Linear regression analyses of 109 individuals showed no differences in acetylcholinesterase (AChE) inhibition by PON1 genotype or phenotype. PON1 phenotype did not affect butyrylcholinesterase (BuChE) inhibition, but differences in the slope of BuChE inhibition based on levels of AZM, total dimethylphosphates, or dimethyl thiophosphate were observed with AREase activity. Post-hoc analysis showed that the slope of inhibition/unit exposure metric for the low AREase activity group (<91 Units/ml) was significantly lower than for the middle activity group (91-112 U/ml), while the high activity group (>112 U/ml) was not different from the other two. The lack of an effect of PON1 genotype and dose-response relationship for PON1 phenotype suggests that PON1 status does not modify the level of ChE inhibition after exposure to AZM, and confirms the in vivo predictions of the relationship between PON1 and AZM.

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**1473 ASSOCIATION BETWEEN PON1 GENOTYPE AND PHENOTYPE AND BLOOD CHOLINESTERASE ACTIVITIES IN FARMWORKERS.**

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**1474 PROBABILITY EXPOSURE ASSESSMENT OF DIOXINS VIA BEEF, PORK, CHICKEN MEAT, AND MILK IN KOREAN POPULATION.**


Introduction: More than 90% of human exposure to dioxin derives from food of animal origin. Many studies have been focused on the exposure assessment of dioxin via food. But, to our knowledge, few studies on probabilistic dioxin exposure assessment have been conducted until now, while there were several studies on distribution of dioxin in food. Quantitative exposure assessment is a useful technique to investigate the risk from contaminants via food intake. The present study was carried out to develop a probabilistic exposure model to dioxins (PCDD/Fs) of meat and milk and to assess the exposure of the general population.

Methods: We used probabilistic model to assess exposure to dioxins. As model inputs, dioxin concentration, meat and milk consumption and Koreans’ body weight data were used. The monitoring data by NVRQS on the dioxin contamination in meat and milk were used as input data. The uncertainty of the exposure assessment. Results: The simulated model estimated maximum exposure for dioxin in the range of 0.02 - 0.43 WHO-TEQ kg bw-1 per day based on the age groups of Koreans. The sum of sensitivity value of milk contamination and consumption were over half (72.4-97.6%) in all the age groups. Conclusions: This study showed that the mean exposure to dioxins from consumption of meat and milk is below the Korean provisional maximum tolerable daily intake of 0.66 WHO-TEQ kg bw-1 per day recommended by WHO. There are uncertainties in model outputs due to variations in contamination and consumption of milk rather than meat. This result will provide the useful information for further risk assessment and dioxin surveys in the future.

**1475 PBPK MODELS IN ACUTE CHEMICAL INCIDENTS.**


Rationale: Determining the risks of exposure to hazardous chemicals for humans in emergency situations is not straightforward. Use of physiologically based pharmacokinetic models (PBPK) could be of interest to better characterize risks. However, numerous chemicals can be involved in such incidents. In this study, we therefore evaluated available data on incidents with hazardous chemicals in the Netherlands in order to determine which compounds and which types of incidents are the most often observed. For these particular chemicals, we will consider the implementation of PBPK models.

Methods: We reviewed reports of acute intoxications with chemicals from 2008-2010 from the Environmental Incident Service of the National Institute for Public Health and the Environment (MOD) and the Environmental Calamity Service Rijnmond (DCMR) in the Netherlands. Cases of Dutch incidents were compiled in a database that contains sections including incident location, its purative cause, and the measured compounds. Results: In 2008-2010, 521 reports were available but only 2008 reports have been evaluated so far. In 2008, 212 incidents occurred. Region Rotterdam-Rijnmond ranked in first place (> 80% of the incidents). The two most common types of incidents were spills (N=87, 41%) & fires (N=84, 40%). Concerning the involved compounds, the results are as follow: ‘missing’ in 26% (often the compounds are not systematically measured in case of fires); benzene derivatives or dioxins in 11% (N=24); gases in 8.7% (N=20 e.g. CO, H2, H2S); acids in 6.1% (N=14) & metals in 1.8% (N=9) of the incidents.

Conclusion: This study will be of use for clinical toxicologists to chemical disaster planning and preparedness in the future. In particular, it will help selecting chemicals for which a PBPK model might be useful in acute situation of chemical incidents. The results are also of interest for other highly industrialised regions in the world.
ng/mL for TBBPA and 2,4,6-TBP in 100uL serum. The calibration curves are linear, while the accuracy, precision, stability and reproducibility of both approaches confirm that this method can be used as required for biomonitoring purposes and other studies.

1477 EVALUATING THE PLAUSIBILITY ESTROGEN RECEPTORS AS MEDIATORS OF BPA TOXICITY IN THE CONTEXT OF HUMAN SERUM CONCENTRATIONS OF UNCONJUGATED BPA.

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Bisphenol A (BPA) is a monomer used in the manufacture of polycarbonate plastics and epoxy can liners found in trace quantities in some consumer products and foods. The binding of unconjugated BPA with steroid hormones is hypothesized to mediate subsequent developmental and reproductive toxicity. Receptor binding is a plausible mechanistic step in humans only if serum concentrations of the bioactive (unconjugated) form of BPA are near the dissociation constants for estrogen receptors. Human exposure to BPA is widespread, but efficient first pass glucuronidation limits the bioactive fraction of BPA to ~0.1% of total BPA. Here we derive estimates of BPA serum concentrations from more than 30 human urine biomonitoring studies and external exposure estimates, representing greater than 12000 individuals across 9 countries, including adults, children, and pregnant and non-pregnant women, by applying the ratio of blood to urine concentrations of total BPA and the fraction of total serum BPA in the unconjugated form. Serum concentrations are also calculated directly from estimates of human oral exposure and the relationship between dose and peak serum concentration, and separately from PBPK modeling. The three methods converge in a range of plausible human serum concentrations of bioactive, unconjugated BPA. These concentrations are multiple orders of magnitude below existing disassociation constants for all estrogen receptors considered candidates for mediating the toxicity of BPA.

1478 INFANT RISK AND EXPOSURE ASSESSMENT OF BISPENOL A IN POLYCARBONATE AND BPA-FREE PLASTIC BOTTLES.

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Bisphenol A (BPA) is a nearly ubiquitous chemical in the environment, present at very low levels in many materials, including polycarbonate and possibly some BPA-free plastics (i.e., not manufactured with BPA). Previous risk assessments have evaluated infant exposures to BPA from polycarbonate baby bottles, but we are not aware of any that have evaluated potential BPA exposures from infants to non-poly carbonate plastic baby bottles. In order to evaluate the risks to infants posed by BPA in materials made with and without BPA, we conducted an exposure and risk assessment. Infant exposures to BPA in plastics were estimated using formula concentration and bottle migration data in the literature and three different exposure scenarios (breast milk, BPA-free, and polycarbonate). The calculated exposures were compared to toxicity criteria values developed by public health agencies in the United States, other countries, and Europe. For each of the three scenarios, the exposures calculated did not exceed the toxicity criteria values, and in some cases they were orders of magnitude lower. Although exposures calculated in the breast milk category were the lowest overall, all scenarios resulted in exposures that were below the toxicity criteria guideline values. Overall, the evidence does not support a reduction of risks from using BPA-free products.
were significantly correlated to the frequency of deep-fried french fries consumption during the month preceding blood collection ($r = 0.28 - 0.31$, $p < 0.05$). Biomarkers of AA exposure were not correlated to DNA strand breaks in peripheral lymphocytes.

1482 OCCURRENCE OF INSECTICIDE BIOMARKERS OF MALATHION IN PRODUCE AND THEIR ABSORPTION AND EXCRETION IN THE RAT.

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Malathion is an organophosphorus (OP) insecticide extensively used for agricultural pest control. Malathion diacit (MDA) and monooacit (MMA), dimethylphosphate (DMP), dimethylthiophosphate (DMTP) and dimethyldithiophosphate (DDMTP) are important urine biomarkers of malathion and organophosphorous insecticide exposure. However, these metabolites also are formed in produce. In 131 produce samples from the channels of trade, 127 contained more biomarkers than malathion. Under field conditions in Santa Maria, CA, malathion, MDA and MMA dissipated, while DMP, DMTP and DDMTP increased during a 20d study period. Mole ratios of Biomarker/(Malathion + Malaoxon) were always > 1 and increased from Day 4 to Day 23. The absorption and excretion of preformed biomarkers by consumers would falsely indicate pesticide exposure. Therefore, their disposition was studied in rats. Female Holtzman rats (240-300g) were gavaged with malathion and MDA, MAA, DMP, DMTP and DDMTP at equal molar doses (73μmol/kg). Urine and feces were collected every 24h for 8d. Methods were developed and validated for biomarker analysis in urine. Recoveries of 94% ± 10% at 0.01, 0.01 and 1 μg/mL. LOQs of MDA, MAA, DMP, DMTP and DDMTP were 0.005, 0.01, 0.005, 0.002 and 0.002 μg/mL, respectively. MDA (20 mg/kg) was recovered as 36±5% MDA, 0.05±0.02% DMP, 54.0±3.1% DMTP and 53±10% of administered dose in urine (>94% in first 24h). DMTP (10.4 mg/kg) yielded 88±8% DMP, 47±17% DMTP, and 55±10% of administered dose in urine (>92% in 24h). Results were similar with other biomarkers. Preformed biomarkers of organophosphorous insecticides in produce are readily absorbed and excreted. Low-level human dietary and non-occupational biomonitoring studies may be confounded by preformed biomarkers used to infer human pesticide exposure. (Support of California Strawberry Commission is acknowledged.)

1483 APPLICATION OF METHODS TO FACILITATE ENVIRONMENTAL CONTAMINANT MONITORING BY LOCAL RESIDENTS.


Identifying and solving environmental contaminations at the local level involves a series of complex issues; local resident support and participation, technology transfers, training, and documentation of progress. The goal of this research is to develop a framework that can be applied to numerous contamination problems in several countries. As a pilot program, two communities with similar contamination issues from local steel industries but different local environments and social contexts were utilized; West Berkeley, California (WBC) and Monterrey, Mexico (MM). Experience in WBC identified specific technology and air sample collection procedures to be applied in MM, although the size and demographics of the local population in the critical zone of high exposure were quite different. Both wind and other weather conditions had to be predicted in a differential manner as these parameters directly affect the number and timing of collection filters to be used for sample capture. At MM, the operation of the monitor and collection of air samples were done by local community members under daily guidance and training, a critical component of eventual technology transfer. In addition, detailed questionnaires and video interviews with local residents provided documentation of the research team – local resident relationship and potential associated health issues. Standard PM10 and XRF methods were used for contamination level quantification. This study demonstrates how each community has different sets of issues that require detailed knowledge and participation of community members. Guidance of a few specialists can overcome these constraints using the same or similar air monitoring procedures documented from other sites. It was found that cinematography was essential to document weather conditions, credibility of sampling and training procedures, and chain-of-custody of collected samples. Cinematography also provides a valuable tool that can be used in preliminary discussions with other communities.

1484 OBSERVATIONAL EXPOSURE MONITORING OF DICHLORETHYL ETHER (DICHLORETHYL) IN ADULTS AND CHILDREN FOLLOWING SPOT-ON FLEA MEDICATION APPLICATION: DESIGN CRITERIA AND SUMMARY RESULTS.


The US EPA is required to characterize potential human health risks for pesticides, including a variety of residential exposure scenarios. The risk assessments are based on GLO Toxipology data for purposes of hazard identification, but exposure estimation often relies on "professional judgment.” We report here the first GLP-conducted, IRB-approved observational exposure monitoring study for a spot-on flea insecticide product for dogs. Children age 3-6 and adult applicators living in the same household provided 10-day morning void urine samples (3 pre and 7 post) which were analyzed using HPLC MS/MS for 3-phenoxynbenzoic acid (3-PBA) as an indicator of exposure to cyphenothrin. This study was designed to satisfy accuracy criteria of 3-fold (at 95% titiles) and required participation of a minimum of 32 households. Ethical, logistical, study design and analytical methods and results are summarized. This study provided valuable experience and data including how to satisfy EPA’s current requirements for children’s observational exposure monitoring studies and the application of these data to quantify conservative bias incorporated into predictive exposure estimation methods. Pre and post-application 3-PBA samples indicated that exposures typically peaked within 1-3 days post dosing of the family dog and that exposure distributions are associated with reasonable certainty of no harm in comparison to appropriate toxicological benchmarks. 3-PBA levels from participants in this study fell within the range reported by the Centers for Disease Control in its most recent national report. Data interpretation, including pharmacokinetics and metabolism deconvolution are also discussed.

1485 DEHP EXPOSURE ASSESSMENT.

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The study describes a mechanistic approach for assessing dynamically in time-dependent source-to-dose exposure to DEHP. The overall modelling framework was built on the AclXtreme simulation environment, comprising: Multimedia indoor air quality models for estimating DEHP concentrations in gas, particles and dust phase starting from gaseous emissions; exposure assessment models incorporating all possible exposure pathways and routes (inhalation of DEHP, dermal exposure through rubbing of dust, non-dietary oral exposure through dust ingestion and object to mouth behavior of plastics containing DEHP), internal dosimetry models, for the assessment of DEHP and its 3 major metabolites (MEHP, 5-OH MEHP and 5xO-MEHP) in human tissues and urine through a multi-compartmental PBPK model. Uncertainty and variability quantification across all stages of the assessment. Under a typical scenario of a common resident dwelling (size of 90 m2 and air exchange rate 0.5) characterized by DEHP gaseous concentration of 500 μg/m3 (from vinyl flooring and other plastic equipment), the concentrations of DEHP in the gaseous, particles and dust phase are equal to 1.5 μg/m3, 21 μg/m3 and 4400 μg/dust. For these media concentrations, overall daily intake varies between 0.2 to 10 μg/kg-bw depending on the exposure scenarios considered. These values are age dependent: adults are exposed mostly through inhalation and infants mostly through non-dietary oral intake. For a common repeated aggregate exposure scenario of 2 μg/kg-bw, the DEHP internal dose in venous blood and adipose tissue (where bioaccumulation is clearly observed) reaches a quasi-state equilibrium at 0.07 and 0.4 μg/g. The expected urinary concentrations of MEHP 5-OH MEHP and 5xO-MEHP are 3.1, 6 and 4 μg/g respectively, allowing also the use of Biomonitoring Equivalents for Risk Characterization.

1486 EXPLORING PHthalate Biomonitoring DATA WITHIN THE 2007–2008 NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY (NHANES) DATASET FOR COEXPOSURE PATTERNS.

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As regulatory initiatives increasingly call for understanding cumulative risks from chemical mixtures, it is imperative that exposure data from large biomonitoring programs which may inform these assessments be evaluated to better understand occurrence and patterns of coexposures. We analyzed the urinary metabolite data for 6 phthalates (butyl-benzyl phthalate, BBP; di-buty phthalate, DBP; di-isobutyl
phthalate, DiBP; bis(2-ethylhexyl) phthalate, DEHP; di-isooctyl phthalate, DNOP; and di-n-octyl phthalate, DIDP) in the 2007/2008 NHANES dataset. To explore the distribution of coexposures, urinary metabolite concentrations of the 6 phthalates were converted to their daily intake values and exposure distributions were developed for each individual phthalate (daily intake was converted to its percent value). For the total dataset (N=2507), the co-occurrence of multiple phthalates at the upper percentile of exposure distribution is rare. There are no cases where all 6 phthalates examined had exposure values > 95th percentiles of individual exposure distributions. In 4 cases (0.16%), 5 phthalates fell above the 95th percentiles. In 5 cases (0.2%), 4 phthalates fell above the 95th percentile. For 78% of individuals, none of the six phthalates were above the 95th percentile of their respective exposure distributions. A cumulative distribution of the averaged exposure percentiles across 6 phthalates was developed to explore what percentile, on average, of the individual phthalate exposure distribution would provide a summed coexposure estimate related to various percentiles of the summed exposure distribution for the NHANES data set. Results indicate that 95% of individuals have total exposures that fall below an averaged percentile of 80. These coexposure patterns observed above, however, were based on an un-weighted survey data. Caution must be taken when applying these findings generally to the US population.

Typically, exposure to contaminated sediment in a human health risk assessment is represented without regard to bioavailability or particle size. We used an in-vitro assay to estimate lead bioavailability of sediment size fractions, which were selected based on particle sizes that are likely to adhere to skin. For arsenic, a factor of 0.6 was applied as a protective estimate of relative bioavailability. Studies have demonstrated that, under dry conditions, fine particles (<125 mm) are more likely to adhere to skin than larger particles. Conversely, the size of adhered particles increases with moisture content. At each of 33 locations, we collected 5 surface sediment samples, each consisting of 12 homogenized subsamples. All samples were analyzed for metals in the < 2 mm size fraction. The dry EPC for each of the four grain size intervals was also determined. One sample per beach also included an in-vitro bioaccessibility measure for lead and metals analyses in each of the 4 size ranges. The exposure point concentration (EPC) was estimated as a mass-weighted average by assuming the differences between enrichment ratios for each particle range relative to the < 2 mm size fraction do not change for a given beach and metal. A dry EPC was based on particles <.125 mm. A wet EPC was based on particles <0.25 mm. Risks were driven by lead and arsenic, with concentrations in the dry EPC consistently elevated as much as twice as high as the wet EPC. Uncertainties are associated with using adherence as a proxy for ingestion although more direct measures of ingested particle sizes are lacking. Although concentrations of lead and arsenic are lower in the wet EPC, the concentration reduction may be offset by increases in mass of dermal loading with potential increases in ingestion rate.

Acute fumonisin B1 (FB1) toxicity in swine is well established and managed in production facilities. However, the limited long-term exposure assessments of FB1 have resulted in recommended guidelines for animal and human dietary exposures versus action levels. We investigated long-term exposure (27 weeks) in pigs by using quantitative dietary exposure assessment modeling in an attempt to understand the lifetime exposure. The swine production stages from wean to nursery and in grower-finisher are considered. Our analytical approach involved both deterministic and semi-stochastic modeling for dietary comparative analyses of FB1 exposures from Bt-corn, conventional non-Bt corn and distiller's dried grains with solubles (DDGS) derived from Bt and/or non-Bt corn. A chronic toxicological incipient level of concern (LOC) of 1.0 mg FB1/kg diet was determined from the literature, which represented a decrease in average daily gain in nursury and grower-finisher pigs. All nursery and grower-finisher diets exceeded the LOC to varying degrees depending on the feeding scenario. Nursery semi-stochastic results where the corn fraction was entirely from Bt-corn showed the least FB1 exposure with a mean of 0.95 mg FB1/kg diet, whereas diets using non-Bt grain and DDGS sources demonstrated the highest exposures with a mean of 2.63 mg FB1/kg diet. Grower-finisher semi-stochastic results predicted the lowest FB1 exposure for Bt grain with a mean of 1.5 mg FB1/kg diet and the highest FB1 exposure for a diet consisting of non-Bt grain and non-Bt DDGS with a mean of 5.08 mg FB1/kg diet. Deterministic results closely mirrored but slightly under predicted the mean for the semi-stochastic analysis. Our novel comparative model reveals that diet scenarios where the source of grain is derived from Bt corn presents less potential for FB1 toxicity than diets containing non-Bt corn and DDGS. Assessment of the uroto stage needs to be further investigated to understand lifetime exposure.
1491 METAL BIOACCESSIBILITY IN THE TRADITIONAL FOODS OF FIRST NATIONS PEOPLE IN CANADA.

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Background: Traditional foods are important to the physical and cultural wellbeing of First Nations in Canada and abroad. Long-range and point-source contaminants can be detected in traditional foods hunted and harvested in the wild; however, it is not yet known to what extent this exposure poses health risk.

Objective: The goal of this work is to measure the bioaccessibility of metal contaminants in traditional foods for the calculation of dietary exposure. Methodology: A cross-sectional study titled the First Nations Foods, Nutrition, and Environment Study (FNFNES) recruited participants from 125 randomly selected households in 21 reserves in British Columbia, Canada. From these households, 1103 self-identifying First Nation adults returned a completed survey that included a Food Frequency Questionnaire (FFQ). In addition, up to 30 composite traditional food samples, with each sample coming from 5 individual plants or animals, was provided by each community for metal analysis by ICP-MS. Of these 431 samples, 45 (including sockeye and Chinook salmon, salmon eggs, clams, seaweed, rabbit meat, moose meat, liver and kidney, deer meat and liver) were selected for bioaccessibility tests. An in vitro model that simulates the physicochemical conditions of the human gastrointestinal tract was used to extract the bioaccessible fraction of each food. Extracts were then measured by ICP-MS. Results: In brief, bioaccessible Cd concentrations were highest for moose kidney (8.5 μg/g), moose liver (2.0 μg/g), rabbit meat (0.9 μg/g), and seaweed (0.8 μg/g). Bioaccessible As concentrations were very high in moose kidney (0.3 μg/g). Conclusions: In vitro models offer risk assessors a rapid and inexpensive tool to refine dietary exposure estimates since only a fraction of an ingested contaminant is likely to be absorbed into systemic circulation. Future work will incorporate these results with speciation and food intake data for calculation of dietary exposure.

1492 A VALIDATED METHOD FOR THE QUANTITATION OF CHLORFENPYR IN LIVER BY GC/ECD.

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Chlorfenapyr is the first commercial pesticide derived from microbial-produced halogenated pyroles. It is a pro-insecticide, requiring bioactivation by CYP450 enzymes to a toxic metabolite that uncouples oxidative phosphorylation. Due to concerns regarding potential toxicity in non-target species and the fact that this insecticide is being used more prevalently, a GC/ECD method has been developed and validated for the detection of chlorfenapyr in biological samples. This method briefly involves the following: 1) samples are extracted in acetonitrile and NaCl (10:1, v/v), 2) the acetonitrile extract is concentrated to 1 mL and subjected to ENV1-Carb™-HPLC solid-phase extraction, and 3) SPE eluates are evaporated to dryness under nitrogen and reconstituted with 0.5 mL toluene for GC/ECD analysis. The GC/ECD conditions used included an injector port temperature of 220°C, column flow set to 1.0 mL/min, an initial column temperature of 100°C with a 20°C/min ramp to 300°C, and the ECD temperature was set at 300°C. Using this procedure, chlorfenapyr has a retention time of 15.75 ± 0.02 minutes and calibrators range from 0 ppm to 0.75 ppm. This method has shown specificity for chlorfenapyr and results in a limit of detection of 10 ppb and a limit of quantitation of 25 ppb. Recoveries of fortified samples are within 80% - 120% acceptance criteria at fortifications levels ranging from 25 ppb to 600 ppb. The sensitivity and simplicity of this method provides a fast and accurate means by which chlorfenapyr can be quantitated in biological samples, particularly in cases of non-target species exposure or toxicity.

1493 A VALIDATED ASSAY FOR SEVEN COMMON CARBAMATE PESTICIDES IN FEEDS, BAIT, AND STOMACH CONTENTS USING QUENCHERS AND GAS CHROMATOGRAPHY/MASS SPECTROMETRY.

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Carbamate pesticides are systemic insecticides with agricultural, commercial, and residential indoor (bait) and outdoor uses. Carbamates vary in their spectrum of activity, persistence in the environment, and toxicity. Their mode of action is similar to organophosphates in that they inhibit acetylcholinesterase (AchE), an enzyme responsible for breaking down acetylcholine, a neurotransmitter, at cholinergic nerve endings in the central nervous system. Carbamate poisoning of mammals can occur unintentionally through promiscuous eating behaviors of pets and livestock, or intentionally by illegal baiting of nuisance wildlife. Accordingly, the Michigan State University Diagnostic Center for Population & Animal Health has developed and validated a qualitative fast extraction QuEChERS method for seven common carbamate pesticides, including Aldicarb, Bendiocarb, Carbofuran, Methiocarb, Methomyl, and Propoxur, in bait, feed/food, and stomach contents. Identification and confirmation of the seven carbamate compounds is by gas chromatography/mass spectrometry (GC/MS). The limits of detection for these compounds are 1 ppm for Aldicarb, 0.5 ppm for Bendiocarb, Carbofuran, Methiocarb, and Propoxur, and 10 ppm for Methomyl. Compared to the traditional liquid/liquid extraction and gel permeation chromatography clean-up, the QuEChERS extraction procedure has many advantages including use of a smaller sample size, enhanced lab safety, greater economy, and shorter turn-around time. We are currently looking to extend the QuEChERS approach to quantitation of these compounds in feeds and tissues, matrices of particular relevance to veterinary diagnostic laboratories.

1494 MEASUREMENT OF HYDROXY-PAHS IN URINE AND ATMOSPHERIC PARTICULATE MATTER.

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Humans are exposed to Polycyclic Aromatic Hydrocarbons (PAHs) through polluted air and diet. Broiled, smoked or grilled foods contain higher amounts of PAHs compared to uncooked or boiled foods. In air, PAHs are emitted from incomplete combustion of fuels and are adsorbed on fine particulate matter, which can be deposited in lungs, causing adverse health effects and cancer. When in the human body, PAHs are metabolized to hydroxy-PAHs and excreted via urine. By comparing the parent PAH composition of PM and the hydroxy-PAH composition of urine, the metabolic pathways of PAHs can be studied.

An analytical method was developed to measure 33 hydroxy-PAHs in urine and PM using gas chromatography/mass spectrometry (GC/MS). Urine samples were deglucuronidized with β-glucuronidase/aryl sulfotase and solid phase extracted (SPE) with Plexa stationary phase. PM was extracted from filters with the use of Accelerated Solvent Extraction (ASE). The urine extracts and aliquots of PM extracts were mixed with an internal standard and derivitized with N-methyl-N-tertbutyldimethylsilyl-trifluoroacetamide (MTBSTFA). The derivatized samples were analyzed in electron impact ionization mode with GC/MS. Stable isotope labeled surrogates were used to account for OH-PAH loss throughout the analytical method.

Recovery experiments were conducted using Plexa SPE columns and elution with a 1:1 mixture of Dichloromethane/Ethylacetate. Most of the OH-PAHs recoveries from urine ranged from 80% to 120%. Intra- and inter-day variability of the measurements was also investigated. The stability of the derivatized product was investigated as well. Results showed that the 33 OH-PAH formed stable products, over a two week storage period. The stability of OH-PAHs in frozen urine was also studied. Results showed that many OH-PAHs were stable in frozen urine for one week period. However, the dihydroxynaphthalenes were not stable in frozen urine for one week period.

1495 PULMONARY INHALATION OF ULTRAFINE TIO2 AND CARDIOVASCULAR EFFECTS: A NEURON-REGULATED PATHWAY.

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Inhalation of nano-sized air pollutant particles is a recognized risk factor for cardiovascular disease; however, the link between occupational nanoparticle exposure and adverse cardiovascular events remains unclear. We reported previously that pulmonary exposure of rats to ultrafine titanium dioxide (UFTiO2) enhances substance P synthesis in nodose ganglia, which was associated with an increased phosphorylation level of cardiac proteins. In the present study, we further investigated changes in cardiovascular function following pulmonary exposure to UFTiO2. Our study indicates that pulmonary exposure of rats to UFTiO2 (6 mg/m3) for 4 hours significantly increased heart rate and depressed diastolic function of the heart in response to isoproterenol. On the vascular side, pulmonary exposure to UFTiO2 elevated mean and diastolic blood pressure in response to norepinephrine. Pretreatment of the rats ip with the transient receptor potential (TRP) channel...
blocker, ruthenium red (2.5 mg/kg), not only inhibited substance P synthesis in nodose ganglia and the increased phosphorylation level in cardiac proteins, but also prevented the above cardiovascular changes induced by pulmonary exposure to UFTiO2. Our results suggest that the effects of pulmonary exposure to UFTiO2 on the cardiovascular system are most likely influenced by a lung-nodose ganglia-regulated pathway via the activation of TRP channels located on the endings of c fiber sensory neurons in the lung. Activation of this neuronal pathway may contribute to an increased incidence of cardiovascular diseases associated with pulmonary inhalation of small-sized particle components from ambient air.

**1496 CARDIOVASCULAR EFFECTS AFTER PULMONARY EXPOSURE TO WELDING FUME.**
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Epidemiological studies have found positive associations between air pollution and adverse cardiovascular outcome. Whether exposure to welding fumes cause similar cardiovascular dysfunction remains unclear. The present study investigated the effects of manual metal arc-hard-surfacing (MMA-HS) on the heart and the vascular system. Rats were intratracheally instilled with MMA-HS (2 mg/rat, once a week) or saline for seven weeks. On days 1 and 7 after the last treatment, rats were implanted with indwelling catheters and cardiovascular function in response to increases in adrenoreceptor agonists was assessed. Pulmonary exposure to MMA-HS decreased the basal level of left ventricular pressure and the positive dp/dt of the heart at 1 day post-exposure. The negative dp/dt of the heart in response to isoproterenol decreased 7 days after exposure to MMA-HS. Exposure to MMA-HS slightly reduced blood pressure in response to norepinephrine at 1 day post-exposure, but this change was not statistically significant. In addition, pulmonary exposure to MMA-HS reduced the phosphorylation level of cardiac troponin I in the heart. This was consistent with the reduced heart muscle contractility indicated by decreases in left ventricular pressure, positive and negative dp/dt. These findings suggest that the heart may be more prone to developing dysfunction than the vascular system after exposure to welding fumes.

**1497 NI IN AMBIENT PM CAUSES MICROVASCULAR DYSFUNCTION VIA NO AND NADPH PATHWAYS.**
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Exposure to PM2.5 (<2.5 μm) has been associated with endothelial dysfunction in humans and animal models. We have shown that systemic inflammation and athero-sclerosis in residents of two China cities, Jinchang (JC) and Zhangye (ZH), were linked to specific PM2.5 compositions. Despite similar PM2.5 concentrations, JC had levels of nickel, selenium, copper, and arsenic that were 76, 25, 17, and 7 fold higher than in ZH, respectively. The aim of this study was to use unique PM2.5 samples from these cities to identify the mechanism(s) that drive pulmonary and systemic effects. Male FVB/N mice received a single or repeated oropharyngeal aspiration of water or aqeous suspension of PM2.5 from JC, ZH or ZH spiked with Ni (ZHi+Ni) at the same concentrations found in the JC PM, followed by evaluation of pulmonary inflammation. Mesenteric arteries were isolated 24 hr post exposure for gene activity or functional response ex vivo. To investigate Ni-induced changes in NO and NADPH pathways, functional response was also assessed using LNAME or Apocinin, respectively. Plasma cytokine and cardiovascular markers were measured using a Mesoscale Discovery multiplex assay. Lung lavage revealed significant pulmonary inflammation from the JC and ZHi+Ni; p<0.001. No differences were seen in artery contractile function, however, there was significantly less artery relaxation in JC and ZHi+Ni; p<0.001. Percent relaxation was also altered among groups after LNAME and Apocinin incubation. There was significantly higher gene expression in JC and ZHi+Ni (TNF-α, IL-6, Nos2; p<0.01 for all; NOX4 p<0.005). Lastly, multiplex results showed significantly higher concentrations of VEGF and IL-10 in JC and ZHi+Ni (p<0.01, p< 0.001, respectively). These data suggest that changes in vascular responses can be driven by nickel found in PM2.5. Both short and long-term exposures can induce an acute systemic inflammatory response, trigger endothelial damage via eNOS uncoupling and NADPH oxidase pathways, and result in vascular dysfunction.

**1498 TREADMILL STRESS TEST AFTER DIESEL EXHAUST PARTICULATE EXPOSURE REVEALS A TIME-DEPENDENT SHIFT FROM PARASYMPATHETIC TO SYMPATHETIC DOMINANCE.**
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Epidemiological studies suggest that particulate matter (PM) air pollution is a major trigger of acute cardiac events—including arrhythmia—especially in those with preexisting cardiac disease. Diesel exhaust (DE) contributes the majority of urban fine and ultrafine PM, and is thus likely a key trigger of acute cardiac events. Research suggests that several interrelated mechanisms underlie the acute cardiototoxicity of PM, including autonomic nervous system imbalance. Abnormal heart rate (HR) and HR variability (HRV) responses to treadmill challenge are indicative of autonomic dysfunction and predictive of cardiovascular mortality. We hypothesized that a single intra-tracheal instillation (IT) of DE particles (DEP, 500 μg/kg) in hypertensive heart failure-prone rats would provoke abnormal HR and HRV responses to treadmill. Rats were monitored by radiotelemetry during treadmill challenge at 24-h pre-IT and 3- and 24-h post-IT. Relative to saline-instilled rats, DEP significantly decreased HR and low-to-high frequency ratio of HRV (LF/HF) while increasing time domain HRV parameters (SDNN & RMSSD) during treadmill deceleration and recovery at 3 h post-IT, suggesting parasympathetic dominance. Upon 24-h post-IT treadmill challenge, DEP significantly increased HR while decreasing SDNN and RMSSD during acceleration, indicating sympathetic dominance relative to saline. During treadmill challenge, DEP did not affect arrhythmia counts, peak HR, or HR recovery. The treadmill stress test is useful in unmasking the latent cardiovascular effects of air pollutant exposure, which in this study included autonomic imbalance characterized by early parasympathetic dominance followed one day later by an excess in sympathetic tone. Collectively, these effects indicate increased risk for adverse cardiovascular events. (Abstract does not reflect EPA policy; Supported by UNCEPA CR83323601.)

**1499 CHRONIC EXPOSURE TO DIESEL EXHAUST PARTICULATE INDUCES VENTRICULAR REMODELING AND DYSFUNCTION.**
J. Bradley and J. D. Gardner.
Louisiana State University Health Science Center, New Orleans, LA. Sponsor: T. Dugass.

In the United States, approximately 60,000 deaths annually are attributed to particulate matter (PM). Chronic PM exposure increases the risk of cardiovascular disease in urban residents, predisposing them to the development of diseases including heart failure. Diesel exhaust particulates (DEP) accounts for 90% of outdoor air pollution. DEP is a heterogeneous mixture composed of inorganic compounds and polyaromatic hydrocarbons (PAH). Chronic PAH exposure is associated with ventricular dilation and wall thinning. Although the mechanisms are unknown, we hypothesized that chronic exposure to DEP induces ventricular remodeling and dysfunction. Male Sprague-Dawley rats were exposed to nose-only nebulization of DEP (SRM 2975, 0.23 mg/mL) or vehicle for 20 min/day x 5 weeks. Echocardiographic measures of left ventricular (LV) end diastolic diameter and posterior wall diameter taken at baseline and weekly thereafter demonstrated DEP induced chamber dilation and posterior wall thinning compared to vehicle. After 5 weeks, LV function using pressure volume catheter indicated systolic dysfunction in these DEP animals. Morphological analysis using Picrosiris Red staining of LV collagen revealed that DEP reduced cardiac interstitial collagen. AHR activation has been linked to impaired extracellular collagen remodeling through suppression of the hypoxic inducible factor (HIF)-1α pathway. Our studies show that DEP exposure was associated with reduced expression of cardiac HIF-1α. Furthermore, these animals had reduced cardiac expression of VEGF and TGF-β; both mediated by HIF-1α activation and stimulate collagen production. Attenuation of either VEGF or TGF-β signaling is associated with chamber dilation, contractile dysfunction, and impaired cardiac growth. Moreover, DEP animals exhibited greater cardiac AHR/I HIF-1α expression suggesting that blockade of these signaling molecules may be due to the activation of the AHR pathway in the heart. Furthermore, activation of the AHR in the heart may play a significant role in ventricular remodeling and dysfunction through the impairment of collagen turnover.
DOSE-DEPENDENT INCREASES FOR THORACIC AORTA PLAQUE DEVELOPMENT IN APOE-/- MICE WITH CIGARETTE SMOKE INHALATION EXPOSURE.


ApoeE-/- mice fed a high-fat diet and exposed to cigarette smoke were previously shown to exhibit dose-related increases for thoracic aorta plaque incidence, high-grade plaque development and plaque volume. Subsequent validation studies were intended to refine the smoke exposure regimen, apply additional qualitative/quantitative methodologies for tissue analysis, and demonstrate portability for the testing protocol/experimental model. Female ApoE-/- mice were fed a high-fat diet and exposed to cigarette smoke (0, 0.12, 0.24, 0.36 mg WTPM/L; 3 h/d, 5 d/wk; 12-18 wks); select regions of the aorta (root sinus, arch, thoracic aorta, brachiocphalic trunk) were submitted for virtual histology and histopathology. Quantitative assessments (virtual histology, entire regions) indicated dose-dependent increases for thoracic aorta plaque volume (0.14, 0.16, 0.59 and 1.05 mm3), plaque surface area (4.23, 4.91, 12.67 and 18.87 mm2) and arterial occlusion (4.66, 5.81, 14.10 and 21.78 %) as smoke exposure concentration was increased; no meaningful differences between exposure groups were observed for the root sinus, arch or brachiocephalic regions. Findings were confirmed by histopathology (sampled areas), with dose-related increases observed for thoracic aorta plaque incidence (41, 48, 63 and 78 %), high-grade plaque development (grade 3/4 plaques: 0.05, 0.28, 0.54 and 0.79) and plaque volume (9.63, 19.63, 53.98 and 99.04 X 10-3 mm3). For normal chow-fed, cigarette smoke-exposed groups, no meaningful increases for thoracic plaque formation were confirmed. These data show that cigarette smoke inhalation exposure increases thoracic aorta plaque development in a manner suitable for comparative toxicology testing; that additional quantitative/qualitative methodologies can enhance tissue analysis and extend experimental findings; and, that findings based on the current testing protocol/experimental model can be replicated in a second laboratory (portability).

AMBIENT MOUNTAIN TOP MINING PARTICULATE MATTER ALTERS SYSTEMIC MICROVASCULAR FUNCTION.

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Air pollution particulate matter (PM) has been associated with cardiovascular health effects over a variety of geographical locales and concentrations. In the Appalachian region, PM from local mining operations represents a considerable health burden on the neighboring population. While the health effects are largely known, the mechanisms that govern these toxic responses remain to be clarified. Ambient PM was collected within 1 mile of an active mountain-top mining (MTM) site in southern WV on PTFE filters (5.0 μm pore size) over six weeks. The particles were extracted in water and then dehydrated. The extracted sample was primarily PM10 and largely comprised of sulfur (38%) and silica (24%) by weight. 24-hours after rats were intratracheally instilled with 300 μg MTM, arterioles were isolated from the mesenteric and coronary microcirculation. A subset of rats were also prepared for intravital microscopy. MTM exposure blunted endothelium-dependent (EDD; max % dilation 53%±4 control, 28%±7 MTM) and -independent (max % dilation 73%±7 control, 43%±11 MTM) arteriolar dilation in both mesenteric and coronary arterioles. In vivo, arteriolar function was assessed via EDD, perivascular nerve stimulation (PVNS) and active hyperemia (AH). MTM exposure significantly blunted EDD (max diameter 49±3 μm control, 33±3 μm MTM) as well as AH-mediated vasodilation (max % of control 157%±3 control, 122%±15 MTM), α-adrenergic receptor blockade inhibited PVNS-induced vasoconstriction in exposed animals, while only blunting constriction in control tissues (max % of control 22%±4 control, -2%±8 MTM). This indicates an activation of a compensatory vasodilatory pathway. Overall, these data suggest that MTM particle exposure alters normal microvascular function in disparate microvascular beds, likely through quenching NO and sympathetic nerve mechanisms. NIH RO1-ES015022, RC1-ES018274 (TRN), and NSF-1003907 (VCM)

SUBCHRONIC EFFECTS OF CONCENTRATED AMBIENT PARTICLES ON HEMODYNAMIC CHANGE AND ORGAN DAMAGE IN ISOPROTERENOL-INDUCED MYOCARDIAL INJURY RATS.

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Epidemiologic studies have shown that ambient particulate matter (PM) is associated with health effects of respiratory diseases, cardiovascular diseases (CVDs) and heart failure (HF). However, the mechanism is still unclear. The goal of this study is to investigate the sub-chronic effects of concentrated ambient particles (CAPs) on hemodynamic change and organ damage in rats with isoproterenol (ISO)-induced myocardial injury. Male Sprague-Dawley (SD) rats received 150 mg/kg ISO by subcutaneous injection to induce myocardial injury. Fine particle concentrator was used for sub-chronic CAPs exposure (Whole body inhalation exposure, 5 hours/day, 4 days/week for 13 weeks). Compared to filter air (FA) inhalation controls, CAPs exposure group had significantly higher fibrinogen level (190.7 ± 15.0 vs 160.5 ± 24.2 mg/dl, p <0.05). Heart rate variability and hemodynamic variables measured by LabChart were altered after CAPs exposure. PM also caused inflammatory and degenerative changes in the heart, and enhanced glomerulosclerosis in the kidneys, as detected by histopathology. Particulate air pollution may affect blood coagulation, hemodynamic change, and organ damage in susceptible populations. Further studies are needed.

PULMONARY EXPOSURE TO PARTICULATE MATTER LEADS TO OXIDANT STRESS IN EXTRAPULMONARY ARTERIOLES OF FEMALE RATS.

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Cardiovascular complications triggered by exposure to airborne particulate matter (PM) often involve microvascular dysfunction. In studies on male rats, we have established that acute pulmonary exposure to PM at occupational-relevant concentrations reduces the capacity for endothelium-dependent dilation in arterioles outside of the lung. In part due to scavenging of nitric oxide by reactive oxygen species (ROS). Because there can be profound gender differences in susceptibility to microvascular dysfunction in response to pathological stimuli, the aim of this study was to assess the effect of pulmonary PM exposure on the oxidant status of extrapulmonary arterioles in females. Sexually mature female rats were exposed to TiO2 nanoparticles (primary particle diameter ~21 nm) via intratracheal instillation at a deposition of 500 μg/rat. Twenty-four hours later, the spinotrapezius muscle was excised, and after exposure to dihydroethidium, fluorescence microscopy was used to detect ethidium bromide (EB) fluorescence, an indicator of ROS, in individual arterioles. EB fluorescence intensity was greater in arterioles of PM-exposed rats than in those of sham controls exposed to sterile saline (176±5 vs 160±3 greyscale units, p <0.01). However, arteriolar EB fluorescence was not elevated (15±4 units) if the muscles from PM-exposed rats were treated with the NAD(P)H oxidase inhibitor apocynin (10-5 M). In contrast, apocynin had no effect on arteriolar EB fluorescence in muscles from sham-exposed rats (159±4 units). There was no significant difference in arteriolar EB fluorescence between the PM-exposed and sham-exposed groups in the presence of apocynin. These results indicate that as in male rats, pulmonary PM exposure in female rats increases NAD(P)H oxidase-dependent ROS production in arterioles outside of the lung. Therefore, females are not protected against the adverse effects of PM exposure on the systemic microcirculation.

CARDIOMYOCYTE AUTOPHAGY IN SPRAGUE DAWLEY RATS TREATED WITH IMATINIB, A TYROSINE KINASE INHIBITOR, ASSESSED BY IMMUNOHISTOCHEMISTRY.

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Imatinib (Imb)-induced cardiotoxicity in rats is characterized morphologically by autophagy, apoptosis, and necrosis. It was suggested that cardiomyocyte autophagy is implicated in the pathogenesis of Imb-induced cardiotoxicity (Herman, E. H., et
and A. O. El-Kadi. To investigate the specific role of c-Abl in Imatinib-induced cellular calcium homeostasis and decreased contractility in the isolated guinea pig heart Langendorff assay. It was shown to accumulate in lysosomes. The cytotoxicity induced by Imatinib can be reduced by bafilomycin A1 pretreatment, demonstrating the involvement of lysosomal acidification in c-Abl toxicity. Imatinib was also shown to perturb intracellular calcium homeostasis and decreased contractility in the isolated guinea pig heart Langendorff assay. To investigate the specific role of c-Abl in Imatinib-induced cardiac toxicity, we performed targeted gene inhibition of c-Ab1 by RNA interference in NCMs, which did not lead to cytotoxicity or induction of ER stress markers. To further support our hypothesis, we designed Imatinib structural analogues that do not have appreciable c-Ab1 inhibition in NCMs. The c-Ab1 inactive analogues induced cytotoxicity, ER stress, autophagy perturbation, and contractility changes at similar potencies and magnitudes as Imatinib, demonstrating that the c-Abl inhibition is involved in Imatinib-induced cardiac toxicity. The results from our studies strongly suggest that imatinib induces cardiac dysfunction through disruption of intracellular calcium homeostasis, induction of ER stress and alteration of autophagy process, independent of c-Ab1 inhibition.

1505 CARDIOTOXICITY IN MALE SPRAGUE DAWLEY RATS TREATED DAILY FOR UP TO 4 WEEKS WITH IMATINIB.

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Imatinib (IMB) is an anti-cancer agent acting by inhibition of tyrosine kinase. Cardiotoxicity has occurred with clinical use of IMB as an unexpected adverse effect. The characteristics of IMB-induced cardiac toxicity are not well defined. The current study examined the effect of treatment period on the cardiotoxic effects of IMB in Sprague-Dawley rats (SD). Groups of male SD (5-6/group) were treated with 125 mg/kg IMB daily for 1, 2, 3 or 4 weeks. Tissues and blood samples were collected 24 hours after the last dosing. Serum levels of cardiac troponin I (cTnI) were monitored with the Erenna immunoassay system. Body weight declined only during week 1 of treatment. The red blood cell counts were significantly decreased from control at treatment week 3 and 4. Cardiac lesions were observed in all animals treated for 1-4 weeks with IMB. The lesions were characterized by cytoplasmic vacuolization, myofibrillar loss, and interstitial infiltration with inflammatory cells. Other cardiac myocyte alterations included necrosis, apoptosis and autophagy. Mean lesion scores (based on a scale of 0-3) were 1.5, 2.0, 1.7 and 1.4 after 1, 2, 3 or 4 weeks, respectively. Increased levels of cTnI were detected in all IMB-treated groups (mean pg/ml at 1 wk=7.0, 2 wk=14.3, 3 wk=17.0 and 4 wk=19.9 compared to 3.6-4.0 pg/ml for control groups). The results indicate that the SD rat is a sensitive animal model for detecting IMB-induced cardiac injury and that IMB can induce cardiac alterations following a treatment period as short as 7 days. In addition, monitoring serum levels of cTnI appears to provide a sensitive means of detecting IMB-induced cardiotoxicity.

1506 MECHANISTIC INVESTIGATION OF IMATINIB-INDUCED CARDIAC TOXICITY AND THE INVOLVEMENT OF c-ABL KINASE.

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The small molecule Bcr-Abl tyrosine kinase inhibitor Imatinib mesylate is the frontline therapy for chronic myeloid leukemia. Imatinib has been reported to cause left ventricular contractile dysfunction in the clinic and cardiac myopathy in preclinical studies, which has been proposed to be associated with its pharmacologic activity. In order to determine the role of Ab1 kinase in the reported cardiac toxicity, we measured the lowest toxin-associated with imatinib-induced toxicity in vitro. Imatinib treatment at or above the clinical Cmax (5 μM) induced apoptosis, ER stress, and dissipation of mitochondrial membrane potential in rat neonatal cardiomyocytes (NCMs), consistent with previous reports. Imatinib was shown to accumulate in lysosomes. The cytotoxicity induced by Imatinib can be rescued by bafilomycin A1 pretreatment, demonstrating the involvement of lysosomal acidification in c-Ab1 toxicity. Imatinib was also shown to perturb intracellular calcium homeostasis and decreased contractility in the isolated guinea pig heart Langendorff assay. To investigate the specific role of c-Ab1 in Imatinib-induced cardiotoxicity, we performed targeted gene inhibition of c-Ab1 by RNA interference in NCMs, which did not lead to cytotoxicity or induction of ER stress markers. To further support our hypothesis, we designed Imatinib structural analogues that do not have appreciable c-Ab1 inhibition in NCMs. The c-Ab1 inactive analogues induced cytotoxicity, ER stress, autophagy perturbation, and contractility changes at similar potencies and magnitudes as Imatinib, demonstrating that the c-Ab1 inhibition is involved in Imatinib-induced cardiac toxicity. The results from our studies strongly suggest that imatinib induces cardiac dysfunction through disruption of intracellular calcium homeostasis, induction of ER stress and alteration of autophagy process, independent of c-Ab1 inhibition.

1507 CHRONIC DOXORUBICIN CARDIOTOXICITY MODULATES EXPRESSION OF CYTOCHROME P450 AND SOLUBLE EPOXIDE HYDROXYLASE ACTIVITY IN RATS.


Doxorubicin (DOX, adriamycin) is an effective anticancer agent whose major limiting side effect is cardiotoxicity. Recently, we demonstrated that acute DOX toxicity altered expression of cytochrome P450 (CYP450) and arachidonic acid (AA) metabolism with a significant increase in soluble epoxide hydrolase (sEH) activity, which metabolizes the cardioprotective epoxyeicosatrienoic acids (EETs) to the less active metabolite dihydroxyeicosatrienoic acids (DHETs). However, the clinical situation involves chronic administration of the drug where the cumulative dose is the only currently used predictor of cardiotoxicity. Therefore, we investigated the effects of chronic DOX cardiotoxicity on the expression of CYP450 and sEH in DOX-induced cardiotoxicity in male Sprague–Dawley rats. The chronic toxicity was induced by multiple intraperitoneal injections for a cumulative dose of 15 mg/kg divided into six injections within two weeks. After 14 days of the last injection, the heart was harvested and the expression of sEH was determined by real-time PCR. sEH mRNA was amplified and different AA metabolites are analyzed by LC-ESI-MS. As a result, the chronic DOX toxicity significantly induced gene expression of hyporerophic markers (ANP β-MHC), apoptotic markers (Bax, p(53), CYP450 (CYP2E1, CYP4A3, CYP4F1, CYP4F5), and sEH. Also, the activity of sEH was increased by 50% with a minor but significant increase in the activity of epoxyeicosatrienoic acid (EET) formation. This caused a significant induction of 14,15-8,9 EETs with a massive induction in formation of 14,15-11,12-8,9 DHETs. In conclusion, the chronic DOX administration significantly modulates expression of CYP450 and sEH enzymes leading to imbalance between CYP-mediated cardiotoxic and cardioprotective pathways. Interestingly, sEH has a central role in DOX cardiotoxicity suggesting an innovative mechanism by which DOX causes progressive cardiac inflammation. Therefore, sEH might be considered as a novel target in treatment of DOX cardiotoxicity.

1508 COMPARISONS OF CARDIAC TROPONIN BIOMARKER ASSAYS AFTER A SINGLE ISOPROTERENOL DOSE IN MALE HAN WISTAR RATS.

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Myocardial necrosis is a frequent finding during preclinical toxicity studies with cardiac troponin (cTnI) being the clinically accepted biomarker for the emergency room setting and a prominent emerging biomarker in the preclinical setting. The goal of this study was to compare the qualified Centaur cTnI assay to a Luminex Millipore custom panel assay and the MesoScale Diagnost (MSD) assays Cardiac Injury Panel 3 and Muscle Injury Panel 1 (CIP3 & MIP1) using isoproterenol (ISO) as the positive control for inducing myocardial necrosis. Rats were dosed intraperitoneally with vehicle (deionized water), 100 or 500 μg/kg ISO (n=5/group) and blood and heart were collected at necropsy (2 hours post dose). Individual plasma samples were used to evaluate cTnI using the Centaur, MSD CIP3, MSD MIP1 and Luminex cardiac assays. All of the MSD cTnI assay values using both CIP3 and MIP1 were above the lower limit of quantification. No treatment-related cardiac hemotlogic changes were observed. The MSD cTnI assays had an r2 >0.82 when correlated with the Centaur assay. In addition to the cTnI comparisons, it was noted that the biomarkers Fatty Acid Binding Protein 3 (FABP3) and Myosin Light Chain 3 (Myl3) present in MSD CIP3 correlated at r2=0.95 and r2=0.73, respectively, with the MSD cTnI values. To verify between lab reproducibility, the MSD CIP3 was performed in two separate laboratories and produced consistent results. Based on consistency of each Centaur cTnI assay, the data supports the MSD assay qualification with the additional benefit that the MSD assays require less sample volume than the Centaur (25 vs 300 μL plasma). With this in
mind, the MSD assays not only had similar values between laboratories and assays, but also evaluated FABP3 and Myl3 with less sample volume than the Centaur re-
quired for just ctTn analysis.

1509 CHRONIC PROBUCOL TREATMENT DECREASES THE SLOW COMPONENT OF THE DELAYED-RECTIFIER POTASSIUM CURRENT IN CHO CELLS TRANSFECTED WITH KCNQ1 AND KCNE1: A NOVEL MECHANISM OF QT PROLONGATION.


Indirect effects of drugs on ion channel expression levels on plasma membrane are focused as the cause of QT prolongation, and we explored the chronic effects of QT-prolonging drugs on the slow component of the delayed-rectifier potassium current (IKs). CHO cells expressing IKs channels were constructed by transfecting KCNQ1/KCNE1 genes, and the IKs were measured using IonWorks Quattro in the population patch clamp mode. After 24 hours of treatment with IKs blockers (HMR1556, L-768673, or chromanol 293B) or hERG channel trafficking inhib-
itors (amiodarone, 17-AAG, brefaridin A, pentamidine, thiordizine, or prob-
col), only probucol, a cholesterol-lowering drug, produced a concentration-de-
pendent reduction in the IKs, with a half maximal inhibitory concentration of
149.1 nM. A reduction in the IKs by 1 μM of probucol was observed beginning 2 hours after treatment, and the current was reduced by about 80% at 24 hours. The current recovered in a time-dependent manner after the washout of probucol. Probucol did not directly inhibit IKs channels at concentrations of up to 10 μM, nor did it inhibit the hERG current at concentrations of up to 30 μM. In Western blotting analysis, however, probucol at 1 μM did not affect KCNQ1 level in the cell membrane. These results suggest that chronic probucol treatment may con-
tribute to QT prolongation in humans by decreasing the IKs without directly in-
hibiting the IKs channels.

1510 INCREASED EXPRESSION OF PRO-ANGIOGENIC GENES AND ANGIOTENSIN–CONVERTING ENZYME 1 IN THE CARDIAC TISSUES OF RATS FOLLOWING LONG-TERM EXPOSURE TO BISPHENOL A.

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Epidemiological studies report an association between exposure to the plastic com-
ponent bisphenol A (BPA) and cardiovascular disease. This study investigates the effects of BPA in vivo in rat cardiovascular tissues and in vitro in human cardiomy-
ocytes. Rats were exposed to 0.025, 0.25 or 2.5 μg/ml BPA in the drinking water from five to fifteen weeks of age and human cardiomyocytes were exposed to 10 nM-10 μM BPA for hours. The mRNA expression of markers for endothelial dysfunction, inflammation and angiogenesis in rat cardiac tissue and in human car-
diomyocytes were investigated using qRT-PCR. Plasma VEGF-A was investigated using an enzyme immunoassay and plasma nitrite/nitrate was investigated using the Griess reaction. Also, the expression of the BPA target receptors, ERα, ERβ, ERYr and GRF30, were examined in rat cardiac tissue and in human cardiomyocytes using immunohistochemistry and qRT-PCR.

Exposure of rats to ≥0.025 BPA increased the mRNA expression of vascular endo-
thelial growth factor A (VEGF-A), angiotsin converting enzyme 1 (ACE1), endothelial nitric oxide synthase (eNOS) and VEGF receptor 2 (VEGFR2) in the myocardium and exposure to 2.5 μg/ml BPA increased plasma VEGF-A. Exposure of human cardiac myocytes to 10 μM BPA increased the mRNA expression of ACE1 and eNOS.

These findings show that exposure of rats to environmentally relevant levels of BPA cause changes in cardiovascular tissues previously associated with coronary heart disease and that the effects of BPA in vivo in rat myocardium were in line with the effects in vitro in human cardiomyocytes.

1511 EXERCISE MODULATES POLYCHLORINATED BIPHENYL-INDUCED CARDIOVASCULAR TOXICITY IN MICE.

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Cardiovascular diseases such as atherosclerosis are the leading cause of mortality worldwide. Polychlorinated biphenyls (PCBs) are persistent environmental pollu-
tants that can contribute to the initiation of atherosclerosis. Previous work in our laboratory has examined the potential role of nutrition in modulating the toxicity of PCBs in vascular endothelial cells. It has been well-established that exercise can reduce the risk of cardiovascular diseases; however, it is not known whether exercise can modulate vascular inflammation and dysfunction induced by environmental pollutants, such as PCBs.

In the current study, LDLr/-/- mice were fed a Western high-fat diet for 12 weeks to promote an atherogenic phenotype, and then divided into sedentary and exercise groups at week 4. Mice in the exercise group were housed individually with a run-
ning wheel while their sedentary counterparts were housed individually with no wheel. The mice were further divided into two groups which were administered PCB77 at a dose of 170 μmol/kg mouse or safflower oil vehicle during weeks 6, 8, 10, and 12.

Relative to PCB exposure and sedentary behavior, exercise contributed to lower serum cholesterol levels, a reduction in body fat, and less systemic inflammation. Results from this study provide novel findings suggesting that regular physical ac-
tivity could be utilized as a therapeutic approach for the amelioration of adverse cardiovascular health effects induced by environmental pollutants such as PCBs.

1512 NEUROLOGICAL AND ARRHYTHMOGENIC EFFECTS OF 1, 1-DIFLUOROETHANE.

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Inhalant abuse is the intentional inhalation of chemical vapors in order to attain eup-
thetic effect. Many common household products are abused by inhalation and one of them is 1,1-Difluoroethane (DFE). DFE, also known as Freon 152A is a halo-
genated hydrocarbon used as propellant in dust-off spray and air brush painting. In this study, effects of DFE inhalation on muscle co-ordination and arrhytmogenic changes were monitored using Rotarod test and electrocardiographic (ECG) respec-
tively. Thus, an animal model study was used to simulate the clinical conditions of inhalation abuse that results in known DFE clinical sudden death.

To investigate arrhythmogenic and neurological effects Sprague dawley rats (n=8) were exposed to 30 s of 20 L/min of DFE. Arrhytmogenic effects were evaluated by continuous electrocardiographic monitoring (ECG test). These effects were ana-
yzed by evaluating the arrhythmia frequency and arrhythmogenic severity after multiple doses of DFE. ECG test with pretreatment of propranolol, epinephrine, and combination of propranolol and epinephrine before DFE administration was also performed. For the rotarod test, animals were divided in two groups (n=10) and given a dose of DFE 20 L/min. The first group was administered DFE dose for 30 s and the second group for 15 s. Electrocardiographic monitoring showed that DFE and epinephrine + DFE resulted in higher incidence of arrhythmias as com-
pared to the propranolol treated group. Moreover, severity of arrhythmias was higher in epinephrine + DFE group as compared to all the other groups. In Rotarod test, DFE dose for both the groups reduced muscle coordination and animals failed to maintain on the Rotarod. Analyses confirmed that after 15 min but exhibited signs of lethargy and sedation. These results suggest that DFE shows cardiotoxic effect in form of arrhythmias and CNS depressant effects in form of sedation and motor impair-
iment.

1513 EXPOSURE TO B(A)P IN UTERO PREDISPOSE LEH RAT OFFSPRING TO CARDIOVASCULAR DYSFUNCTION IN LATER LIFE.

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In utero exposure of fetuses to benzo(a)pyrene [B(a)P], a polycyclic aromatic hy-
drocarbon, is hypothesized to dysregulate cardiovascular development. To investi-
gate the effects of in utero B(a)P exposure on cardiovascular development, timed-
pregnant Long Evans Hooded (LEH) rats were exposed to B(a)P (0, 600 and 1200 μg/kg/BW) by oral gavage on embryonic (E) days E14-17. There were no signifi-
cant effects of in utero B(a)P exposure on the number of pups born per litter, the pre-weaning heart tissue metabolite profiles of B(a)P-exposed offspring. High performance liquid chromatography (HPLC) was employed to characterize the pre-weaning growth curves, or on the initial and final heart to body weight ratios. High performance liquid chromatography (HPLC) was employed to characterize the pre-weaning heart tissue metabolite profiles of B(a)P-exposed offspring. Systolic blood pressure in B(a)P exposed offspring was significantly elevated as compared to controls. Microarray and quantitative real-time PCR analysis demonstrated upreg-
ulation of mRNA expression for angiotensin (AGT) and endothelial nitric oxide synthase (NOS3/eNOS). Ingenuity Pathway Analysis (IPA) and Expression Analysis Systematic Explorer (EASE) software was used to identify potential signal-
1515 ANGIOTENSIN II/AT1 PLAYS A CRITICAL ROLE IN ALCOHOLIC CARDIOMYOPATHY THROUGH PKC/NADPH OXIDASE ACTIVATION PATHWAY.

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To investigate the mechanisms responsible for alcohol induced the cardiac cell death, we recently demonstrated that compared with control, hearts from mice with alcohol feeding for two months exhibited increased apoptosis along with significant nitrosative damage [measured by 3-nitrotyrosine (3-NT) abundance] and NADPH oxidase (NOX) 2 activity and NOX4 up-regulation. Direct exposure of H9c2 cells to alcohol induced apoptosis, in a dose-dependent manner via NADPH oxides (NOX) activation since pre-incubation of alcohol-treated cells with urate (peroxynitrite scavenger), 1-NAMe (nitric oxide synthase inhibitor), MnTMPyP (SOD mimic) and apocynin (NOX inhibitor) abrogated alcohol-induced oxidative stress and apoptosis. In the present study we further showed that PKC-β1 inhibitor and specific siRNA can prevent alcohol induced NOX activation, oxidative stress and cell death. Exposure to alcohol significantly increased the expression of angiotensin II (Ang II) and its type 1 receptor (AT1). AT1 blocker completely prevented alcohol-induced activation of PKC-β1 and NOX, indicating that alcohol-induced oxidative stress and cell death is mediated by PKC-β1-dependent NOX activation via AT1. To validate the in vitro findings in an in vivo condition, mice with knockout of AT1 gene (AT1-KO) and wide-type (WT) were simultaneously treated with alcohol for two months. Alcohol significantly increased systemic Ang II levels in both WT and AT1-KO mice. Knockout AT1 gene was found to completely prevent alcohol-induced cardiac PKC-β activation, NOX 2 up-regulation and NOX 4 activation, protein nitration, and cell death. In addition, alcohol also induced cardiac fibrosis (remodeling) and dysfunction significantly in the WT mice, but not in AT1-KO mice. These findings strongly suggest that alcohol-induced cardiac oxidative stress and apoptosis, which is mediated by Ang II interaction with AT1 and subsequent activation of PKC-β1-dependent NOX pathway, plays an important role in the development of alcoholic cardiomyopathy.

1516 METALLOTHIONEIN PROTEIN IN PERMITTED HYPOXIA INDUCED CARDIOMYOPATHY BY INHIBITION OF CARDIAC OXIDATIVE DAMAGE, APOPTOSIS, AND INFLAMMATION.

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Obstructive sleep apnea (OSA) is a highly prevalent condition associated with increased upper airway resistance during sleep, which is characterized by repeated oxygen desaturation events (intermittent hypoxia, IH) and by sleep disturbance (sleep fragmentation, SF). Cardiovascular disease is one of pathophysiologic sequelae in OSA patients. Oxidative damage may be the underlying mechanism for OSA. Metallothionein (MT) is a non-specific, efficient and endogenous antioxidant, and expresses in the heart. Therefore, we tested the hypothesis that MT can protect OSA-induced cardiomyopathy. To mimic the hypoxia/oxxygenation events occurring in human OSA, IH was applied to transgenic mice with cardiac-specific over-expression of MT gene (MT-TG) and the wild-type mice for 4 wk and 8 wk. The nadir FIO2 was maintained at 8% for 20 seconds and the IH paradigm consisted of 20.5% O2 / 8% O2 FIO2 alternation cycles (30 episodes per h) for 12 h a day during night day. IH was found to increase the ratio of heart weight to tibia length at 4 wk, and progressively decreased cardiac function from 4 wk to 8 wk. Cardiac fibrosis was evidenced by increased expression of CTGF, PAI-1 and collagen accumulation at 4 wk and 8 wk. Inflammation was significantly increased in IH-treated heart, shown by Western blot of ICAM, VCAM and neutrophile infiltration at 4 wk. Cardiac apoptosis was observed at 4 wk and 8 wk in IH-treated heart, reflected by increased TUNEL positive cells. CROPP expression and caspase-3 cleavage. IH also induced cardiac oxidative damage, shown by increased protein nitration and progressively decreased the antioxidant components (MT and SOD). However, all the above pathogenic changes were not observed in IH-exposed MT-TG mice. These results suggest that IH can induce cardiomyopathy (cardiac remodeling and dysfunction) associated with oxidative damage, apoptotic cell death and inflammation. MT can completely prevent IH-induced cardiomyopathy.

1517 REGULATION OF ENDOTHELIAL CELL-SPECIFIC NITRIC OXIDE SYNTHASE ACTIVITY BY THE PHOSPHODIESTERASE-4 INHIBITOR, CI-1044.

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The development of phosphodiesterase-4 inhibitors (PDE-4i) as anti-inflammatory and anti-asthma drugs has been hampered by the finding of drug-induced vascular injury (DIVI) in preclinical toxicology studies. The DIVI lesions are typically located in the mesenteries of rats and the coronary arteries of dogs, pigs or monkeys. It is not clear if similar lesions would be observed in humans. Hence, the identification of a clinically translatable biomarker for DIVI is essential for the further development of this class of drugs. Our previous data has shown that nitric oxide (NO) is elevated with PDE-4 administration in rats and that nitric oxide synthase (NOS) blockade prevents DIVI. The aim of this study was to further elucidate the mechanism by which PDE-4 inhibition leads to the elevation of NO and to DIVI, by examining the level of endothelial cell-specific NOS (eNOS) phosphorylation over time. Phosphorylation of eNOS at six different sites is known to regulate its activity. Rats were treated with the PDE-4i CI-1044, and serum and mesentery tissue collected over a 24-h time period after the last dose. Serum nitrate levels remained at baseline initially, but increased between 8-24 h. Mesentery tissue was examined for total eNOS and phosphorylation at residues Y83, S116, T495, S617, S632, and S1177. Phosphorylation of S617 was significantly increased at 2 h relative to other time points. In contrast, phosphorylation levels of Y83, S116, and S632 were increased similarly relative to controls at all time points. Interestingly, phosphorylation of S1177 was decreased at all time points relative to controls, while phosphorylation at T495 was increased. Our results suggest that treatment of rats with the PDE-4 inhibitor, CI-1044, leads to modulation of the phosphorylation of eNOS, with subsequent elevation in NO, as measured by serum nitrate levels. This work provides further evidence that NO production may be a useful biomarker for PDE-4 inhibitor-induced DIVI.

1518 MACROPHAGES AND TOXICANTS: EFFECTS ON CHOLESTEROL EFFLUX.

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Cholesterol cycles between two forms within cells, one is free (unesterified) and the other esterified to fatty acids. Only the free form is transported (effluxed) out of macrophages via ABC transporters to extracellular acceptors destined for the liver.
We have previously shown that a key enzyme in the cholesterol cycle (CES1) is inhibited by xenobiotics (organophosphates) and endogenous toxins (4-hydroxy-2-nonenal, HNE). We therefore hypothesized that these compounds would inhibit cholesterol efflux from cultured macrophages. To examine this possibility, THP-1 macrophages were preloaded with [3H]-cholesterol/acytethylated LDL, followed by an equilibration period in serum free medium to enable radiolabeled cholesterol to distribute into various cellular pools. The lipid-engorged cells were then treated with toxicants for 24 h in the absence of cholesterol acceptors, followed by a 24 h efflux period in the presence of either 10% fetal bovine serum (FBS), HDL, or ApoAI (toxicant present in culture medium for 48h). Efflux of [3H]-cholesterol from control (vehicle-treated) macrophages increased with time (25-30% efflux into the culture medium by 24h) when cultured in medium containing 10%FBS. Compared to control cells, no differences in efflux rates were found after treatment with paraoxon or HNE (up to 10μM). When HDL (ABCG1-mediated transport) was used as cholesterol acceptor, a modest 25% reduction (p<0.05) in efflux was found after treatment with paraoxon or chlorpyrifos oxon (10μM each), but not for HNE. Moreover, when apoAI (ABCA1-mediated transport) was used as cholesterol acceptor, a dose-dependent reduction in efflux (up to 50%, p<0.05) was found for paraoxon (0-10μM). Treatment of cells with HNE did not affect cholesterol efflux under any condition. Levels of ABCA1 and ABCG1 proteins are currently being evaluated by western blotting to determine whether they are downregulated. The results suggest that toxicants may interfere with key steps in cholesterol homeostasis and contribute to a pro-atherogenic macrophage phenotype (NIH R15ES015348).

### 1519 ARSENIC DOES NOT AFFECT CARDIAC GROWTH IN THE ANGIOTENSIN-II MODEL OF HYPERTENSION.

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Human exposure to arsenic occurs most commonly through drinking water, and can cause both acute and long-term cardiovascular health effects. Arsenic has been shown to interact with zinc-finger motifs of certain proteins. We hypothesized that if Arsenic inhibited muscle ring finger associated protein (MuRF-1), a zinc-finger-containing protein that induces cardiac atrophy, cardiac hypertrophic processes would become exaggerated. To test this, we examined the combined impact of arsenic and angiotensin-II on cardiac growth. Briefly, twenty-four mice were divided into 4 groups. Group 1 received phosphate buffered saline (PBS) via osmotic pump and was given tap water to drink. Group 2 received PBS and was given sodium arsenite (500 ppb) via drinking water. Group 3 was implanted with an osmotic pump containing protein that induces cardiac atrophy, cardiac hypertrophic processes were evaluated. The results do not support a role for arsenic in potentiating the cardiac hypertrophy induced by hypertension.

### 1520 TRICLOSAN INTERFERES WITH EXCITATION-CONTRACTION COUPLING OF SKELETAL AND HEART MUSCLE-IMPLICATIONS FOR HUMAN ENVIRONMENTAL HEALTH.

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Uses of triclosan (2,4,4′-trichloro-2′-hydroxy-diphenyl ether, TCS), a synthetic antimicrobial, in consumer products have burgeoned since 1972. Commonly present in consumer products, including soaps, deodorants, toothpastes, shaving creams, mouthwashes, cleaning supplies, kitchen utensils, toys, bedding, socks, and trash bags in concentration that can reach in excess of 20 μM, TCS is considered a high volume chemical with annual usage of >300 tons. TCS and its metabolites are found in human serum, urine, and breast milk, and aquatic and terrestrial ecosystems. Total TCS in the urine of Americans vary with age and household income with a 95th percentile concentration of 1.6 μM but reaching levels as high as 13 μM in some individuals. Little is known about the possible hazards associated with pervasive TCS exposures containing acute toxicity (29 mg/kg, iv; 84 mg/kg, ip) and the same range as picrotoxin. We report the discovery that TCS and its metabolite, methyl-TCS, potently impair the integrity of excitation-contraction coupling of both cardiac and skeletal muscle in a concentration-dependent manner, with nanomolar to low micromolar potency. Mice exposed to 3-25 mg/kg (ip) triclosan quickly develop impaired cardiovascular hemodynamics, and dosages >50 mg/kg ip cause frank heart failure. Triclosan as low as 0.25 μM in water significantly impairs fish swimming performance. Considering the ongoing scientific and public debate concerning the safety and efficacy of TCS as an antibacterial, the inherent intrinsic toxicity of TCS to interfere with proteins participating in excitation-contraction coupling provides a biologically essential to short and long-term health of skeletal and cardiac muscle, must be considered. Sponsored by NIH P42 ES04699, P01 11269, U.S. EPA R833292, R829388, and T32 training program in basic and translational cardiovascular science (HL 86350).

### 1521 AUTOMATED TYROSINE KINASE INHIBITOR CARDIOTOXICITY ASSAY IN ZEBRAFISH.

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The zebrafish embryos have recently gained relevance in biomedical research thanks to some of its characteristics including embryo transparency, small size, ease of manipulation and possibility to evaluate different internal organs avoiding invasive methodologies. Combined with the possibility to adapt the model with an automated device and the reduced cost associated to each assay, the model is an ideal killer experiment in early phases of drug discovery as well as a novel method to increase the selection arguments to reduce the candidates to enter into the Drug Development processes. Cardiotoxicity is one of the most important reasons for drug attrition during the process of Drug Development. Evaluation of cardiotoxicity and especially HERG channel inhibition is described in regulatory guidelines, but limitations demands the development of new complementary assays that can also evaluate the human heart from a holistic point of view. Biobide has set up a novel in vivo automated platform that allows testing compounds in zebrafish embryos. To evaluate and validate the quality of the analysis system, the model and the value of the information, we have used a panel of blind-coded Tyrosine kinase inhibitors that had been previously described in other in vitro and in vivo assays. The results indicate that our automated method provides with high informative and complementary data that can significatively improve the process of selection of new candidates with low or no cardiotoxicity.

### 1522 EMBRYONIC EXPOSURE TO 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN INHIBITS EPICARDIAL DEVELOPMENT IN THE ZEBRAFISH HEART.

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Early embryonic exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) disrupts cardiac development and/or function in a wide range of vertebrate species including fish, birds and mammals. In zebrafish, exposure to TCDD during embryogenesis results in decreased numbers of cardiomyocytes, heart looping defects, cardiac valve malformations, reduced cardiac output and ventricular standstill (heart failure). The window of TCDD sensitivity during zebrafish embryogenesis correlates with the formation of the outermost layer of the heart, the epicardium, which plays a critical role in cardiac development. The epicardium contains cardiac progenitor cells that contribute to the developing myocardium, valves and coronary vasculature. In addition, the epicardium is necessary for the maturation of the cardiac conduction system. We hypothesized that TCDD induced heart failure in zebrafish may result from disruptions in epicardial development. Using in situ hybridization, histology and fluorescence immunocytochemistry in conjunction with confocal microscopy, we have found that TCDD exposure immediately following fertilization causes a complete loss of the epicardium. In order to better assess how TCDD impacts epicardial development we characterized the progression of epicardium formation. Previous reports suggested that epicardial progenitors cells attach at the atrial-ventricular junction and spread over the atrium and ventricle simultaneously to form a continuous epicardial layer. We have found that epicardial cells first ensheathe the ventricle then cover the atrium. Exposure to TCDD during epicardial cell migration stops the normal progression of epicardial cells over the
ventricle and atrium. Exposure after the epicardium has formed does not result in death. When we are currently investigating in the molecular mechanism(s) underlying the observed epicardial toxicity. Our results show that TCDD exposure prevents the formation of an essential layer of the vertebrate heart. NIH Grant ES012716 and UW Sea Grant.

**1523** A. T. Cartus, I. Groh, L. W. Weishaupt, K. Merz, M. Esselen and D. Schenk, Food Chemistry and Toxicology, University of Kaiserslautern, Kaiserslautern, Germany.

ME and MIE are both natural constituents of herbal spices and flavoring substances in food products and cosmetics. The carcinogenicity of ME in rodents is supposed to result only from its metabolic activation to 1'-hydroxy-ME (1'OH). After sulfation, the unstable sulfate ester forms a highly reactive carbonation that can react covalently with DNA. In contrast, MIE (which bears a propenyl instead of an allylic side chain) was found to be noncarcinogenic in rats and claimed to be supposed to result only from its metabolic activation to 1'-hydroxy-ME (1'OH). TCDD is a potent environmental pollutant that disrupts normal embryonic development resulting from craniofacial malformation, unrolling of the heart, pericardial edema and culminating in heart failure in Danio rerio. TCDD toxicity is mediated by the aryl hydrocarbon receptor that is thought to mis-regulate developmental genes that require strict regulation for proper development. Our lab has shown that TCDD down-regulates sox9b, a transcription factor, in the jaw resulting in craniofacial malformations. We hypothesized that TCDD also induces a down-regulation of sox9b in the heart and that this down-regulation results in cardiotoxicity. To determine if TCDD down-regulates sox9b in heart cells, mRNA was extracted from TCDD and vehicle treated hearts. qRT-PCR analysis showed a 2 fold decrease in sox9b in TCDD treated hearts relative to vehicle. If sox9b is playing a role in mediating TCDD toxicity than down-regulation of sox9b should produce TCDD-like cardiotoxicity. This was assessed in sox9b null, and TCDD and vehicle treated AB embryos. Sox9b null larvae presented with pericardial edema and elongation of the heart relative to vehicle. The changes in heart morphology were statistically similar to TCDD treated fish. Since ablation of sox9b expression produces a TCDD-like cardiac phenotype then down-regulation of sox9b should also affect the ability of the heart to function. Heart function was determined by measuring the red blood cell (RBC) perfusion rate in the intersegmental vessels of the tail. Sox9b null fish exhibited a decrease in RBC perfusion rate while TCDD fish exhibited a total RBC standstill. Thus, down-regulation of sox9b could explain the TCDD induced morphological changes in the heart. However, the 2 fold decrease of sox9b expression cannot account for the severity of circulation or cardiac defects produced by TCDD exposure, suggesting that a reduction of sox9b in the heart can account for some but not all of the TCDD cardiotoxicity.

**1524** Y. Zhu, X. Ding and Q. Zhang, Wadsworth Center, Albany, NY.

Molecular and Environmental Toxicology, University of Wisconsin-Madison, Madison, WI and 2School of Pharmacy, University of Wisconsin-Madison, Madison, WI.

Cytochrome P450-mediated drug biotransformation is essential for drug clearance, but it may also convert inert compounds into toxic metabolites, leading to tissue toxicity. The intestine is a frequent target tissue for drug toxicity; however, the mechanisms underlying drug-induced intestinal toxicity are poorly defined for many drugs that are known to have gastrointestinal side effects. The aim of the present study was to determine the specific roles of small intestinal (SI) P450 enzymes in the metabolic activation of diclofenac (DCF), a widely used nonsteroidal antiinflammatory drug, and in DCF-induced intestinal toxicity. DCF induces intestinal ulcers in humans as well as mice. However, we found that the number of DCF-induced (at 50 mg/kg, p.o.) intestinal ulcers was significantly smaller in an intestinal epithelium (IE)-specific P450 reductase (CYP) knockout (IE-Cpr-null) mouse model, which has little P450 activity in the IE, than in wild-type (WT) mice, at 14 hours after DCF administration. The involvement of intestinal P450 enzymes in DCF metabolism was confirmed by our finding that the rates of in vitro formation in SI microsomal reactions of hydroxylated DCF metabolites and reactive intermediates, trapped as DCF-glutathione (GSH) conjugates, were reduced by >90% and >80%, respectively, in the IE-Cpr-null mice, compared with WT mice. Furthermore, the SI levels of DCF-GSH conjugates were also >80% lower in IE-Cpr-null mice than in WT mice, at 4 hours after DCF treatment, and the abundance of DCF-protein adducts, detected with an anti-DCF antibody on immunoblots, was significantly lower in IE-Cpr-null mice than in WT mice. In additional experiments, we found that pretreatment of mice with grapefruit juice (GFJ), which is known to inhibit SI P450 activity, significantly ameliorated the intestinal toxicity of DCF in WT mice. Taken together, our results not only strongly support the notion that SI P450 enzymes play an important role in DCF-induced intestinal toxicity, but they also illustrate the possibility of preventing DCF-induced intestinal toxicity through dietary intervention.

**1525** M. Foroozesh, J. Sridhar, J. Liu and C. Klein Stevens, Chemistry, Xavier University of Louisiana, New Orleans, LA.

Emodin, a natural anthraquinone isolated from Rheum emodi and known to be metabolized by cytochrome P450 enzymes, was one of the hits and was used as the lead compound. Emodin was found to inhibit CYP1A1, 1A2, and 1B1 with IC50 values of 12.25 μM, 3.73 μM, and 14.89 μM respectively. Further similarity searches of the PubChem and ZINC chemical databases resulted in the identification of 12 emodin analogs for testing against CYP1A1, CYP1A2, CYP2B1 and CYP2A6-dependent activities. 1-amino-4-chloro-2-methylanthracene-9,10-dione showed the best inhibition potency for CYP1A1 with an IC50 value of 0.40 μM, 1-amino-4-chloro-2-methylanthracene-9,10-dione and 1-amino-4-hydroxyanthracene-9,10-dione both inhibited CYP1A2 with the same IC50 value of 0.53 μM. In addition, 1-amino-4-chloro-2-methylanthracene-9,10-dione acted as a mechanism-based inhibitor of CYPs 1A1 and 1A2 with KI and KIvalues of 5.38 μM and 1.57 min-1 for CYP1A1, and 0.50 μM and 0.08 min-1 for CYP1A2. 2,6-Di-tert-buty1-5-hydroxynaphthalene-1,4-dione directly inhibited CYP2B1 with good selectivity and inhibition potency (IC50 = 5.66 μM). Docking studies using the 3D-structures of the enzymes were carried out on all of the compounds. The binding modes of these compounds revealed the structural characteristics responsible for their potency and selectivity as presented here.

**1526** SOT 2012 Annual Meeting 329
TCE is a well-known environmental and occupational toxicant contaminating air, water, and soil. One of the reasons why TCE is still a challenge is due to its variable toxicity across different species. TCE is a well-known environmental and occupational toxicant contaminating air, water, and soil. One of the reasons why TCE is still a challenge is due to its variable toxicity across different species. TCE is a well-known environmental and occupational toxicant contaminating air, water, and soil. One of the reasons why TCE is still a challenge is due to its variable toxicity across different species. TCE is a well-known environmental and occupational toxicant contaminating air, water, and soil. One of the reasons why TCE is still a challenge is due to its variable toxicity across different species. TCE is a well-known environmental and occupational toxicant contaminating air, water, and soil. 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1532 CHIRAL POLYCHLORINATED BIPHENYLS (PCBS) ARE ENANTIOSELECTIVELY OXIDIZED IN MOUSE LIVER TISSUE SLICES.

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Biotransformation by P450 enzymes may contribute to the developmental toxicity of PCBs through the formation of neurotoxic metabolites. Here we investigate the hypothesis that chiral PCB91 (2,2',3,3',4,4'-hexachlorobiphenyl), PCB95 (2,2',3,3',6,6'-hexachlorobiphenyl), PCB132 (2,2',3,3',4,4'-hexachlorobiphenyl), PCB136 (2,2',3,3',6,6'-hexachlorobiphenyl) and PCB149 (2,2',3,4',5',6-hexachlorobiphenyl) are enantioselectively metabolized to hydroxylated metabolites (OH-PCBs) in mouse liver slices. Liver tissue slices were obtained from female C57BL/6 mice and incubated for 4 h with Krebs-Henseleit buffer (pH 7.4) containing 50 μM PCBs. Lactate dehydrogenase release from the tissue slices was 25.1±5.4 %, indicating viability of the tissue slices. The parent PCBs accumulated in tissue slices (19-47% of total PCB) and only <1% were primarily metabolized to OH-PCBs containing the hydroxyl group in the 5-position of the 2,3,6-trichloro substituted phenyl ring in each of the PCBs investigated. The relative concentrations of these metabolites were 5-OH-PCB149 > 5-OH-PCB91 ≥ 5-OH-PCB132 > 5-OH-PCB136 > 5-OH-PCB95. Minor metabolites included 4-OH-PCB95, 4-OH-PCB132, 4-OH-PCB136, 4,5-diOH-PCB136, and the NIH shift products of PCB91 and PCB132. Enantioselective gas chromatography showed an enrichment of (+)-PCB136 and the first eluting enantiomer of 4-OH-PCB136, which is formed from (-)-PCB136. The enantiomeric fractions (EF) of PCB132 and 4-OH-PCB136 were 0.51 and 0.16, respectively. The first eluting peak of 5-OH-PCB149 was enriched in both medium and slices, with EF values of 0.68 and 0.70. Overall, the PCB metabolite patterns observed in the present study are comparable to in vivo disposition studies, which suggest that precision-cut tissue slices are a useful model for studying the enantioselective metabolism of neurotoxic PCBs [Supported by NIH grants ES05605, ES013661 and ES017425].

1533 BIOTRANSFORMATION OF TRANS-1-CHLORO-3,3,3-TRIFLUOROPROPENE (HCFO-1233zd).

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HCFO-1233zd is novel foam blowing and precision cleaning agent with a very low global warming and ozone depletion potential. Acute and subchronic (90-day inhalation NOEL 4000 ppm) toxicity studies in Sprague Dawley rats as well as negative Ames and micronucleus tests suggest a low potential for toxicity. For further toxicological characterization, the metabolism of HCFO-1233zd was determined in male Sprague Dawley rats, female New Zealand White rabbits and in vitro. Animals were exposed by inhalation to 2,000, 5,000 and 10,000 ppm of HCFO-1233zd for 6 hr and urine was collected for 48 hr after the end of the exposure. Microsomes were incubated with HCFO-1233zd at 37 °C for 20 hr. Urine samples and microsomal incubation supernatants were analyzed for metabolites using 19F-NMR, LC/MS-MS. A predominant in vitro biotransformation product, S-(3,3,3-trifluoro-trans-propenyl)glutathione was identified in rat, rabbit and human microsomal experiments (77% in rat, 81% in rabbit, 86% in human - total metabolites by 19F NMR signal intensities). On the other hand, the oxidative metabolites 3,3,3-trifluorolic acid (TFLA) and N-acetyl-(3,3,3-trifluoro-trans-propenyl)-L-cysteine (MA) were observed as the major in vivo metabolites, 32% for TFLA and 40% for MA (rat) and 46% for MA (rabbit) of the total metabolites by 19F NMR signal intensities in urine samples obtained within 6 hr after the exposure. TFLA was not found in rabbits. Quantification by LC/MS-MS showed rapid oxidations of both metabolites, with half times of less than 6 hr in both species. Based on metabolite recovery in urine and estimations of inhaled HCFO-1233zd, biotransformation extent of HCFO-1233zd was determined as 0.01% of received dose in rabbits and 0.002% in rats. The metabolite structure suggests HCFO-1233zd undergoes both oxidative biotransformation by CYP and direct glutathione conjugation. The data on toxicokinetics support prior studies that HCFO-1233zd has a low potential for toxicity in mammals.

1534 IMPACT OF AN INFLAMMATORY MICROENVIRONMENT ON THE ARYLAMINE-MEDIATED INDUCTION OF PHASE I ENZYMES IN HUMAN KERATINO CYTES.

J. Hennen, J. Clemens and B. Blomeke, Department of Environmental Toxicology, University of Trier, Trier, Germany.

Human skin may be exposed to monocyclic amines via skin painting, dark coloured textiles and hair dyes. Exposure to para-phenylenediamine (PPD) or paratoluenediamine (PTD) may lead to induction and elicitation of allergic contact dermatitis. Many small molecules need activation by air oxidation (prephatens) or enzymatic processes (prohaptens) in order to bind to proteins. In this context we previously found an induction of cyclooxygenases (COX) in HaCaT keratinocytes by PPD (Moeller et al., 2008). This study concentrated on the impact of these compounds on cytochrome P450 1 (CYP). Upon incubation of HaCaT1s with 100 μM PPD we detected a translocation of thearyl hydrocarbon receptor (AhR) from the cytoplasm to the nucleus. Both PPD and PTD induced cytochrome P450 1 (CYP) 1A1 and 1B1 mRNA and CYP activity in human keratinocytes. Knowing that PPD also induces COX expression in HaCaT keratinocytes we studied the influence of free arachidonic acid (AA) on the observed CYP1 induction in these cells. In contrast to what has been found in various tissues we found no decrease of CYP1A1 mRNA expression as well as CYP1 activity but rather an augmentation under this inflammatory condition. We therefore conclude that AA or inflammatory conditions do not per se inhibit the metabolic potential of keratinocytes. Reference: Moeller, R., Lichter, J. and Blomeke, B. (2008). Impact of para-phenylenediamine on cyclooxygenases expression and prostaglandin formation in human immortalized keratinocytes (HaCaT). Toxicology 249, 167-75.

1555 THE ROLES OF INTRONIC DNA ELEMENTS IN THE REGULATION OF HUMAN MICROSOMAL EPOXIDE HYDROXYLASE TRANSCRIPTION.

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Microsomal epoxide hydrolase (mEH, EPHX1) plays an important role in the detoxification of xenobiotic epoxide intermediates generated by CYP450 enzymes. However, it is also involved in the bioactivation of procarcinogens. Our laboratory...
Cytochrome P450 1b1 (Cyp1b1) is a member of the cytochrome P450 super family of mono-oxygenase proteins. Although mutations in Cyp1b1 gene have been reported in patients with congenital glaucoma, the role Cyp1b1 plays in the development and function of trabecular meshwork (TM) remains unknown. Here we have determined the impact of Cyp1b1 deficiency on TM cell function. Primary cultures of TM cells were prepared from Cyp1b1+/+ and Cyp1b1−/− mice. Expression of several proteins was used to confirm the identity of these cells as TM cells. The constitutive and inducible expression of Cyp1b1 protein was confirmed by Western blotting. The rates of apoptosis and survival, under basal and oxidative challenge, were determined using caspase 3/7 and MTS assays. Dihydroxyethidium (DHE) staining was used to determine the level of intracellular oxidative stress in Cyp1b1+/+ and Cyp1b1−/− TM cells. The adhesion to various matrix proteins and cell contractility were also evaluated. Cyp1b1−/− TM cells exhibited a significantly higher level of oxidative stress compared to Cyp1b1+/+ TM cells indicating increased oxidative stress in the absence of Cyp1b1. There was a significant decrease in the survival and increase in the apoptosis rate of Cyp1b1−/− TM cells incubated with hydrogen peroxide compared to Cyp1b1+/+ TM cells. This was alleviated by administration of N-acetylcysteine (NAC), a potent antioxidant. Furthermore, Cyp1b1−/− TM cells exhibited a significant increase in adhesion to various matrix proteins, which was reversed in presence of NAC. In addition, Cyp1b1−/− TM cells lost their ability to contract collagen compared to Cyp1b1+/+ TM cells. Thus, the lack of metabolic activity of Cyp1b1 may contribute to enhanced oxidative stress of TM cells with significant impact on their adhesive and contractile properties. In addition, our data suggest that the enhanced oxidative stress in the absence of Cyp1b1 may have a significant impact on the structural organization of TM tissue in vivo.

**1538 CYPIB1-DEFICIENCY PROMOTES THE DYSGENESIS OF TRABECULAR MESHWORK THROUGH ENHANCED OXIDATIVE STRESS.**

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Cytochrome P450 1b1 (Cyp1b1) is a member of the cytochrome P450 super family of mono-oxygenase proteins. Although mutations in Cyp1b1 gene have been reported in patients with congenital glaucoma, the role Cyp1b1 plays in the development and function of trabecular meshwork (TM) remains unknown. Here we have determined the impact of Cyp1b1 deficiency on TM cell function. Primary cultures of TM cells were prepared from Cyp1b1+/+ and Cyp1b1−/− mice. Expression of several proteins was used to confirm the identity of these cells as TM cells. The constitutive and inducible expression of Cyp1b1 protein was confirmed by Western blotting. The rates of apoptosis and survival, under basal and oxidative challenge, were determined using caspase 3/7 and MTS assays. Dihydroxyethidium (DHE) staining was used to determine the level of intracellular oxidative stress in Cyp1b1+/+ and Cyp1b1−/− TM cells. The adhesion to various matrix proteins and cell contractility were also evaluated. Cyp1b1−/− TM cells exhibited a significantly higher level of oxidative stress compared to Cyp1b1+/+ TM cells. This was alleviated by administration of N-acetylcysteine (NAC), a potent antioxidant. Furthermore, Cyp1b1−/− TM cells exhibited a significant increase in adhesion to various matrix proteins, which was reversed in presence of NAC. In addition, Cyp1b1−/− TM cells lost their ability to contract collagen compared to Cyp1b1+/+ TM cells. Thus, the lack of metabolic activity of Cyp1b1 may contribute to enhanced oxidative stress of TM cells with significant impact on their adhesive and contractile properties. In addition, our data suggest that the enhanced oxidative stress in the absence of Cyp1b1 may have a significant impact on the structural organization of TM tissue in vivo.
cially the CYP3A13/3A1. Furthermore, using the heterologous expressed human CYP proteins in the PBDEs metabolism in vitro, CYP1A2 and CYP4A4 showed the highest metabolic efficiency and the metabolic clearance, respectively, CYP1A1 and CYP2B6 displayed the low clearance. However, CYP2A13 and CYP2E1 seemed not to be associated with the PBDEs metabolism. Also, the docking calculations showed the similar results.

Conclusions: Our study demonstrated that the hydroxylated PBDEs might be their major metabolites and human CYP3A4 and CYP1A2 should be involved in the PBDEs metabolism.

**PS 1540 IN VITRO REGULATION OF HUMAN CYP3A4 mRNA AND ENZYME ACTIVITY BY DIETHYLSTILBESTROL: COMPARISON TO PERSISTENT ORGANIC POLLUTANTS (POPs).**

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Rational: The human pregnane X receptor (PXR) has been proposed as a xenosensor of endocrine disrupting chemicals. Alterations in CYP3A4 expression may affect endocrine function by altering steroid hormone metabolism. DPX2 cells (a human hepatic cell line) contain the human PXR FOXO3A and a portion of the human CYP3A4 gene promoter region containing a PXR DNA response element connected to firefly luciferase reporter gene. We compared the effect of diethylstilbestrol (DES), a known endocrine disrupting chemical (EDC), on the regulation of human CYP3A4 gene expression and enzyme activity in DPX2 cells with the effects of persistent organic pollutants (POPs).

Experimental Procedures: Cells were treated with DES and the induction of CYP3A4 mRNA was determined by quantitative real-time PCR. DPX2 cells were also treated with POPs including o,p-DDT, p,p-DDT, p,p-DDE, polychlorinated biphenyl mixture (aroclor 1254), lindane (γ-hexachlorocyclohexane). Human CYP3A4 enzyme activity and inhibition was determined using luciferin-IPA a CYP3A4 selective substrate (Promega Corp.).

Data: DES increased human CYP3A4 mRNA (three fold) and enzyme activity (two fold) at 10 μM in DPX2 cells. DES also inhibited CYP3A4 enzyme activity. Treatment of DPX2 cells with the POPs o,p-DDT, p,p-DDT, p,p-DDE, aroclor 1254, and lindane caused an increase in CYP3A4 enzyme activity (two to three fold) at concentrations similar to DES.

Conclusions: DES caused increased expression of human CYP3A4 mRNA and enzyme activity in DPX2 cells containing the human PXR. DES is also an inhibitor of CYP3A4 enzyme activity. POPs o,p-DDT, p,p-DDT, p,p-DDE acroclor 1254, and lindane also caused an increase in CYP3A4 enzyme activity in DPX2 cells, indicating that POPs can also alter human CYP3A4 expression, and support the role of the pregnane X receptor as a xenosensor for endocrine disrupting chemicals.

**PS 1541 SCREENING FOR MICRORNA REGULATORS OF THE ORPHAN P450, CYP4V2.**

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Cytochrome P450 4V2 (CYP4V2) is a gene linked to the ocular disease Bietti’s Crystalline Dystrophy (BCD). Sequence analysis of CYP4V2 in BCD patients identified potentially disruptive exonic and intronic mutations. Patients with BCD have characteristic crystalline deposits in the cornea and retina, degeneration of the retinal pigmented epithelium and sclerosis of the choroidal capillaries. Visual defects in BCD progress from nyctalopia to eventual blindness. In addition to vision loss, BCD patients exhibit crystalline deposits in fibrolasts and lymphocytes and alterations in plasma fatty acids. Enzymes of the CYP4 family are associated with metabolism of endogenous substrates, including ω-hydroxylation of fatty acids; CYP4V2 is also a ω-hydroxylase of fatty acids, including docosanoids. While progress has been made in determining the activity and function of CYP4V2, regulation of its expression has not been well-examined. Amongst the 57 CYP genes in humans, CYP4V2 stands out with regard to the length of the transcript 3’ UTR, extending over 2.8 kb in comparison to 1.6 kb of coding sequence. This led to the hypothesis that CYP4V2 may be subject to epigenetic regulation by microRNAs.

To test this hypothesis, we selected human liver samples from the UW Liver Bank that had highest and lowest CYP4V2 mRNA and subjected them to microRNA microarray analysis (n=6 per group). We identified miR-146b-5p to be over-expressed in liver samples with lower CYP4V2 mRNA, a miRNA that has a potential binding site in the CYP4V2 mRNA 3’ UTR. Interestingly, mir-146b expression has been found to be up-regulated by Resolvin D1, a docosanoid signaling molecule involved in acute inflammation. This raises the question of what role CYP4V2 may have in docosanoid signaling pathways in healthy individuals and the effects of CYP4V2 mutations in BCD patients.

**PS 1542 CATALYTIC FUNCTION OF YEAST-EXPOSED BAikal SEAL CYP3A1, 1A2 AND 1B1 PROTEINS.**

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Our previous studies have shown that hepatic cytochrome P450 (CYP) 1A1, 1A2 and 1B1 are induced by the accumulation of dioxins and related compounds in wild Baikal seals (Pusa sibirica). However, the catalytic functions of these Baikal seal (BS) CYP1s have not yet been characterized. The present study, therefore, attempted to analyze the catalytic activities of alkoxyresorufin O-dealkylase (AROD) including methoxyresorufin (MROD), ethoxyresorufin (EROD), pentoxysresorufin (PROD) and benzoxysresorufin O-dealkylase (BROD) by yeast-expressed BS CYP3A1, 1A2 and 1B1 proteins. Complementary DNAs of these BS CYP1s were inserted into a yeast expression vector, pYES-DEST52, and the vector was transfected to yeast cells. The expressions of BS CYP1A1, 1A2 and 1B1 proteins were confirmed by the reduced CO-difference spectra and by immunoblotting using the respective anti-human CYP1 monoclonal antibodies. The recombinant BS CYP1A1 protein showed the highest activity of EROD, followed by MROD, PROD and BROD. The AROD profile was similar to those of human CYP1A1. The kinetics analysis showed that the Vmax/Km ratios of all AROD activities catalyzed by BS CYP1A1 were lower than those by human CYP1A1, suggesting less efficient metabolic potential of BS CYP1A1. The enzymatic assay by BS CYP1A1 showed no or minimal AROD activities, while human CYP1A2 displayed higher activities for MROD and EROD. The result indicates lower contribution of BS CYP1A2 to AROD activities. BS CYP1B1 showed a similar AROD profile to human CYP1B1 (EROD > BROD > MROD >> PROD), but the Vmax/Km ratios of all the AROD activities by BS CYP1B1 were higher than those by human CYP1B1, indicating that BS CYP1B1 may have a greater metabolic capacity than human CYP1B1. The present study reveals that BS CYP1s have distinct metabolic capacities from human CYP1s.

**PS 1543 COMPARATIVE CYP AND UDP-GLUCURONOSYLTRANSFERASE mRNA EXPRESSION IN LIVER AND INTESTINE AND THEIR INDUCIBILITY BY PHENOBARBITAL AND A SELF MICRO EMULSION DRUG DELIVERY SYSTEM (SMEDDS).**


Many xenobiotics can affect Phase I and Phase II drug-metabolizing enzyme activity through directly or indirectly modulating transcription and subsequent translation to the protein. This effect on transcription may occur with a drug itself, but also with vehicles used to formulate drugs in non-clinical studies, particularly where vehicle includes components aimed to enhance drug solubility and bioavailability. The purpose of this study was to assess the effects of a Self Micro Emulsion Drug Delivery System (SMEDDS), comprising a mixture of Capryol 90/TranscutolTM/CremophorTM EL (13/28/59, w/w/w), on the mRNA expression of selected UDP-Glucuronosyltransferases (Ugt) and cytochrome P450 (CYP) in the rat duodenum, ileum and liver.戊ar rats were administered p.o. SMEDDS (4ml/kg/day), Phenobarbital (PB, 80 mg/kg/day) or purified water for 7 days. At the end of the study, the liver and the intestinal mucosa from duodenum and ileum were collected for RNA extraction and transcriptomic profiling. mRNA levels of 3 CYPs and 16 Ugt family genes were assessed by real time qPCR with Taqman Low Density Array (TLDA). Basal expressions of CYPs/Ugts were similar between duodenum and ileum, except for Cyp2b1 and Ugt2b17 which were more expressed in the ileum and duodenum, respectively. When comparing liver and duodenum or ileum basal expressions, Ugt2b showed higher, and Ugs 1a2, 1a7 and 1a8 showed lower expression in liver compared to intestines. The SMEDDS treated group mainly down-regulated mRNA expression at the intestinal level, mainly for Cyp1a1 in the duodenum, Cyp2b1 and 6 Ugt genes in the ileum (Ugs 1a2, 1a5, 2a3, 2b36, 2b37 and 2b5). In contrast, PB up-regulated the mRNA levels of.
Chlorine substituted PCBs than CYP2C. PCBs by Baikal seal CYP2s were estimated. Collectively, these in silico analyses sug-
netic or toxicological profiles of the carried drug.

IN UTERO BIPHENOL A EXPOSURE ALTERS PHASE II METABOLIZING ENZYME EXPRESSION IN HUMAN FETAL LIVER TISSUE.

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Proper establishment of molecular pathways, including xenobiotic metabolism and transport processes, during critical windows of development is necessary for the body’s adaptation to environmental change. Developmental exposures may alter the establishment of these essential pathways and thereby influence disease susceptibility later in life. In this study, we evaluated human fetal hepatic expression of phase I and phase II drug metabolizing enzymes using SBiosciences Drug Metabolism RT2 Profiler PCR Arrays in relation to bishenol A (BPA) exposure. Exposure to BPA, a ubiquitous endocrine active compound found in polycarbonate plastics and epoxy resin, is a growing public health concern especially among pregnant women and children. Herein, healthy 1st and 2nd trimester fetal liver samples obtained from the University of Washington Laboratory of Developmental Biology were analyzed for free and glucuronide-conjugated BPA tissue concentrations, and dichotomized into low and high BPA groups based on median BPA levels within the cohort. When we compared samples measuring high BPA levels to samples with low BPA levels (N>4 per group), only phase II metabolizing enzymes exhibited differential expression. The high BPA group displayed greater than two-fold reduction in expression of several metabolizing enzymes including carboxylesterase (CEST; p-value: 0.026; CES2; p-value: 0.034), catechol-o-methyltransferase (COMT; p-value: 0.037), and glutathione s-transferase (GSTA5; p-value: 0.025). Interestingly, phase II enzymes essential for BPA detoxification such as sulfotransferase (SULT1B1; p-value: 0.026; SULT2A1; p-value: 0.025) and glucurononitransferase (UGT1A9; p-value: 0.025; UGT1A10; p-value: 0.028) also demonstrated reduced expression within the high BPA group. Validation of these genes in a larger sample adjusting for gestational age, sex, and environmental exposure will provide insight into the developmental of the xenobiotic metabolism system and its contribution to toxicity in sensitive populations.

IN SILICO DOCKING OF POLYCHLORINATED BIPHENYLS WITH CYTOCHROME P450 2A, 2B, AND 2C FROM THE BAikal SEAL (PUSA SIBIRICA).

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Cytochrome P450 2 family (CYP2) enzymes play important roles in a variety of physiological and toxicological processes in animals. CYP2 genes are highly diverse and exhibit broad substrate specificities among species. It is also known that CYP2B and 2C genes are transactivated by phenobarbital-type chemicals and POPs in some mammalian models. Hence, knowledge on the molecular characterization of CYP2 genes in a variety of animals is necessary for our understanding of the evolution and functional divergence of these genes. However, the sequences and catalytic functions of CYP2 genes in aquatic mammals have not yet been characterized. This study aims at providing more information on CYP2 genes in Baikal seals which were found to accumulate high levels of POPs. The isolated cDNAs of Baikal seal CYP2A, 2B, and 2C encoded proteins containing 494 (molecular weight: 56.8kDa), 494 (56.3kDa), and 490 (55.5kDa) amino acids, respectively. Comparisons of the deduced amino acid sequences of these CYP2 genes with those of other mammalian CYP2 genes showed that the Baikal seal CYP2 amino acid sequences were most closely related to those of dog CYP2s. Using the Baikal seal CYP2 amino acid sequences, in silico homology models of these CYP2 proteins were constructed based on the protein crystal structures of human CYP2A6 (1Z10), rabbit CYP2B4 (1SUO) and human CYP2C9 (1R90) from the Protein Data Bank. Some of polychlorinated biphenyl congeners (PCBs) were docked into the binding pockets of the three CYP2 proteins using the Molecular Operating Environment (MOE) Program. The distance between the heme iron and the predicted hydroxylase site of each PCB was measured and the metabolic capacities of PCPs by Baikal seal CYP2s were estimated. Collectively, these in silico analyses suggest that Baikal seal CYP2A and 2B proteins may more efficiently metabolize ortho chlorinated substituted PCBs than CYP2C.

CONTRIBUTIONS OF ESTERASES AND CYP P450 3A ENZYMES TO THE METABOLISM OF BECLOMETHASONE DIPROPIONATE WHEN TREATING ASTHMA.

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Asthma causes chronic inflammation of the airways, bronchoconstriction, increased mucus production and occasional airway obstruction. As of 2009, 24.6 million people were reported with asthma; 7 million of those patients under the age of 18. The mainstay treatments for asthma are inhaled glucocorticoids which act on the glucocorticoid receptor in the lung to decrease gene expression of inflammatory agents and cytokines production. Although these treatments are effective for many, about 30% of patients do not respond to treatment. Beclomethasone dipropionate (BDP) is administered as an ester pro-drug. Esterases in the lung cleave an ester to form the biologically active beclomethasone 17-monopropionate. 3A enzymes also metabolize BDP, facilitating its clearance. Therefore, it is possible that differences in metabolism by either esterases or CYP3A enzymes regulate the efficacy of BDP. The goal of this study was to determine the kinetics of BDP metabolism by CYP3A4, 5 and 7 and to measure the effects of BDP treatment on cellular expression of these genes. Recombinant P450 incubations were performed and analyzed using LC/MS/MS to measure the disappearance of the parent compound and formation of the de-esterified, hydroxylated and dehydroxylated metabolites. The de-esterified product was of particular interest due to its electrophilic nature and possible toxicities. Preliminary results suggest 3A4 and 3A5 metabolized BDP at similar rates to produce de-hydroxylated and hydroxylated products, but BDP was not metabolized by 3A7. Treatment of A549, BEAS-2B and primary cells cultured from tracheal aspirates of ventilated pediatric patients showed treatment with BDP induced the transcription of CYP3A5, but not 3A4 or 3A7. Collectively, the results suggest that the metabolism of BDP by CYP3A enzymes represents an important clearance pathway for BDP. The individual differences in the expression of these enzymes may correlate with variable efficacy for BDP: Funding: NICHHD HD060559.

EXPRESSION AND FUNCTIONAL CHARACTERIZATION OF HEPATIC MU-CLASS GLUTATHIONE S-TRANSFERASES FROM TURKEYS (MELEAGRIS GALLOPavo) AND THEIR ROLE IN DETOXIFICATION OF AFLATOXIN B1.

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Turkeys are extremely sensitive to aflatoxin B1 (AFB1), putatively due to a deficiency of hepatic glutathione S-transferase (GST) mediated conjugation of exo-afatoxin B1-8,9-epoxide (AFBO), formed primarily by cytochromes P450 1A5 and 3A7. Due to the importance of GSTs in species susceptibility, we cloned and expressed hepatic mu-class tGSTs (tGSTM3, tGSTM4; GenBank JF340152, JF340153) in a heterologous system from turkeys. Predicted molecular weights of tGSTM3 and tGSTM4 were 25.6 and 25.8 kDa, respectively. Multiple sequence comparisons revealed four mu-class motifs and the mu-loop in both proteins. tGSTM4 has 89% amino acid sequence identity to chicken GSTM3, while tGSTM3 has 73% sequence identity to human GSTM3. Specific activities of E. coli expressed GSTM3 toward 1-chloro-2,4-dinitrobenzene (CDNB) and peroxidase activity toward cumene hydroperoxide were five- and two-fold greater, respectively, than GSTM4. The two enzymes displayed equal activity toward ethacrynic acid (ECA). Importantly, neither of the tGSTMs metabolized AFBO, suggesting that tGSTMs play no role in AFB1 detoxification in turkeys. Supported in part by NRI Competitive grant 2007-35205-17880.

PREDOMINANT ROLE OF CYP2B6 IN THE OXIDATIVE METABOLISM OF BDE-99 IN HUMAN LIVER MICROSONES.

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Polybrominated diphenyl ethers (PBDEs) are persistent, bioaccumulative, and toxic environmental pollutants frequently detected in human samples. The purpose of this study was to investigate the oxidative metabolism of 2,2',4,4',5,5-hexabromodiphenyl ether (BDE-99), the major component of a widely used commercial PBDE mixture, by human liver microsomes and to determine the cytochrome
PCB3 was a major metabolite and its sulfate conjugate was predominantly higher in serum and urine. (Supported by P42ES013661)

Phenolic metabolites were extracted by Supported-Liquid-Extraction chromatography and analyzed by LC-MS. We found that excretion in the form of free and conjugated phenols reached maximum within 24 hours of exposure by eliminating 70% of dose in feces and 1% in urine. Three quinone precursors, including 4'-hydroxy-4-chlorobiphenyl (4'-OH-PCB3) and 3'-hydroxy-4-chlorobiphenyl (3'-OH-PCB3), were identified. 4'-OH-PCB3 was a major metabolite and its sulfate conjugate was predominantly higher in serum and urine. (Supported by PA01ES03661)

There are substantial changes in expression of drug metabolizing enzymes that occur during maturation that can have a profound impact on drug pharmacokinetics and pharmacodynamics in the juvenile compared to adult. To further understand these changes, we collected liver and kidney from juvenile rats at different stages of postnatal development to evaluate changes in expression using Affymetrix whole genome microarrays (Rat230 2.0). Tissues were collected from untreated animals at postnatal days 7, 14, 22, 29 and 36, using 5 males and 5 females per group. Tissues from adult animals at 14 weeks of age were used for comparison. Analysis was primarily focused upon the Phase I and Phase II drug metabolizing enzymes as well as Phase III drug transporters. In the liver, there was a significant age-dependent increase in a number of metabolizing enzymes, including the rat homologs to human CYP2D6, CYP2C19 and CYP1A2 which were up-regulated in both males and females which was up-regulated over time in males only. Changes in expression were also noted in the liver for a number of common drug transporters such as MDR1, OCT1 and OAT2. In the kidney, there was a significant increase in most of the tubule-expressed metabolizing enzymes and transporters as nephrogenesis progressed over time. As with the liver, there were several genes that were regulated in a sex-dependent fashion, showing selective increase or decreased expression over time. This sexual dimorphism, which is rodent-specific, could be clearly visualized by principle component analysis, with divergence of the sexes occurring between postnatal days 22 and 29 in both liver and kidney. Results from this study support a better understanding of the effects of age on drug PK/PD which have implications in juvenile toxicity studies as well as potential impact for pediatric indications.
The placentas play a critical role in mammalian development. Syncytiotrophoblast cells, located within placentale tissue, represent the metabolically active cellular barrier between maternal and fetal blood supplies. These cells are largely responsible for nutrient and waste exchange between the developing embryo and mother. Syncytiotrophoblasts, acting much like a competent fetal liver, are also capable of biotransforming a number of exogenous chemicals and environmental contaminants. Understanding the role of bioactivation and detoxification of these potentially dangerous compounds is important in preventing fetal toxicity, developmental defects, and adult-onset diseases. The purpose of our current research is to develop an ex-vivo bioassay, derived from healthy tissue, to assess the role of the metabolic bioactivation in placental genotoxicity. Conditions for inducing the differentiation of committed murine trophoblasts from extramembranous tissue towards synctiotrophoblast-like cells in culture have been developed. The temporal expression patterns of several components of the PHASE I and II biotransformation systems in differentiated and undifferentiated trophoblast stem-like cells have been determined with RT-PCR. Our studies show differential expression of NAD(P)H dehydrogenase, quinone 1 (Nqo1), Aryl hydrocarbon receptor (AhR), ATP-binding cassette, sub-family B (MDR/TAP), member 1A (Abcb1a), and glutathione S-transferase, mu 1 (Gstm1) in differentiated versus undifferentiated cell populations. Expression of cytochrome P450, family 1, subfamily a, polypeptide 4 (Cyp1a2), epoxide hydrolase 1 (Ephx1), and glutathione S-transferase theta 1 (Gstt1) was detected in both differentiated and undifferentiated cells. These observations will be used as a foundation to guide appropriate time frames for exposure studies involving environmentally relevant genotoxic chemicals and their metabolites.

**1555 PERSISTENT INDUCTION OF CYTOCHROME P450A1 BY 3-METHYLCAROLANTHRENE (MC) IN HEPA-1 CELLS: ROLE OF CYP1A2.**

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Humans are constantly exposed to environmental carcinogenic polycyclic aromatic hydrocarbons (PAHs) through cigarette smoke, diesel exhausts, charcoal-broiled meats, etc. Cytochrome P450A1 (CYP1A1) enzymes play important roles in the activation of PAHs such as 3-methylcholanthrene (MC) to carcinogenic DNA-binding metabolites. We reported earlier that MC causes persistent induction of hepatic and pulmonary CYP1A1 in mice for several weeks after MC withdrawal, and that the phenomenon of sustained hepatic CYP1A1 induction is lost in Cyp1A2-null mice. In this study, we tested the hypothesis that MC elicits persistent CYP1A1 induction on hep-1 cells, and that CYP1A2 contributes to this phenomenon. Hepa-1 cells were treated with the MC (2.5 μM), or dimethylsulfoxide (DMSO) as control, and at selected time points, CYP1A1 promoter activity, CYP1A1 enzyme activities, contents, and CYP1A1 mRNA levels were determined. We found that MC markedly and persistently induced Cyp1a1 promoter activity, transcription, apoprotein expression, and the CYP1A1 associated ethoxyresorufin O-deethylase (EROD) activities for up to 5 days. Transfection of cells with CYP1A2 siRNA resulted in knockdown of CYP1A2 mRNA by 70%, but a statistically significant increase of basal CYP1A1 mRNA by 35-40%. The induction of CYP1A1 promoter activity, CYP1A1 mRNA, and EROD activity by MC were not affected by CYP1A2 siRNA at the 24 h time point, but the CYP1A1 induction was significantly attenuated by CYP1A2 siRNA on day 5, compared to cells that were transfected with control siRNA. These results suggest that CYP1A2, possibly via a metabolite, contributes to the sustained induction of CYP1A1 by MC in hep-1 cells. Further investigations into the mechanisms of persistent induction of CYP1A1 by MC could lead to novel preventative/therapeutic strategies against PAH-mediated carcinogenesis in humans.

**1556 IDENTIFICATION OF PROTEINS INVOLVED IN AHR-MEDIATED CYP1A1 INDUCTION THROUGH RNAI HIGH-THROUGHPUT SCREENINGS.**

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The AhR Hydrocarbon Receptor (AhR) has been shown to have a plethora of physiological and, upon dysregulation, carcinogenicity can occur. This happens by the binding of dioxins, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and certain polycyclic aromatic hydrocarbons (PAHs), such as benzo(a)pyrene, to AhR and the subsequent expression of certain target genes. One target gene of AhR is that encoding Cyp1a1, which can form electrophilic derivatives that bind and mutate DNA. Further characterization of the mechanism(s) of AhR-dependent induction of gene transcription is therefore an important research objective. Using RNAi high-throughput technologies, we have set out to identify proteins that modulate the AhR-dependent induction of Cyp1a1 gene expression. An siRNA library targeted against 6000 proteins in the druggable genome was used to transfect the Hepa-1 murine hepatic cancer cell line, which were treated with TCDD for 24 hours, and then assayed for Cyp1a1 activity using the EROD assay. Following RNAi statistical analysis, we identified 90 hits with a p-value of 0.005 or less and confirmed true positive hits by using a reporter gene assay (firefly luciferase reporter genes). We thus confirmed 9 hits that significantly knockdown both the Cyp1a1 EROD protein activity and the mRNA levels of Cyp1a1. To determine the specificity of these proteins to AhR-mediated genes, we looked at mRNA levels of AhR inducible genes NQO1 and Ahdx3a. In addition, we looked to see if the target hits were affecting Cyp1a1 expression indirectly by affecting the mRNA expression of it’s transcription factors/activators such as AhR. We finally wanted to test the effects from the RNAi, we will co-transfect human cDNAs corresponding to our target hits along with mouse targeted siRNA to see if we can rescue Cyp1a1 expression in Hepa-1 cells. Further characterization of the mechanism(s) of AhR-dependent induction of Cyp1a1 gene expression. An siRNA library targeted against 6000 proteins in the druggable genome was used to transfect the Hepa-1 murine hepatic cancer cell line, which were treated with TCDD for 24 hours, and then assayed for Cyp1a1 activity using the EROD assay. Following RNAi statistical analysis, we identified 90 hits with a p-value of 0.005 or less and confirmed true positive hits by using a reporter gene assay (firefly luciferase reporter genes). We thus confirmed 9 hits that significantly knockdown both the Cyp1a1 EROD protein activity and the mRNA levels of Cyp1a1. To determine the specificity of these proteins to AhR-mediated genes, we looked at mRNA levels of AhR inducible genes NQO1 and Ahdx3a. In addition, we looked to see if the target hits were affecting Cyp1a1 expression indirectly by affecting the mRNA expression of it’s transcription factors/activators such as AhR. We finally wanted to test the effects from the RNAi, we will co-transfect human cDNAs corresponding to our hits along with mouse targeted siRNA to see if we can rescue Cyp1a1 expression in the presence of the siRNA.
1558 EVALUATION OF THE POTENTIAL FOR DRUG-INDUCED LIVER INJURY OF ACYL GLUCRONIDES BASED ON IN VITRO COVALENT BINDING TO HUMAN LIVER PROTEINS.

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Prediction of idiosyncratic drug-induced liver injury (DILI) is difficult, and the underlying mechanisms are not fully understood. DILI is considered to be triggered by formation of reactive metabolites and sequential covalent binding (CB) to cellular macromolecules in the liver. Acyl glucuronides (AGs) have been implicated in DILI. Recently, it has been reported that chemical stability of AGs was a useful key predictor for the idiosyncratic DILI risk (1). However, the relationship between stability of AGs and CB to macromolecules, which is considered as a trigger, is still not fully understood. We have reported that when the CB level for oxidative metabolism was multiplied by the maximum daily dose, which may reflect maximum hepatic exposure, positive compounds leading to idiosyncratic DILI and negative compounds became discriminated. The present study was conducted to clarify whether the risk of idiosyncratic DILI can be estimated by CB of AGs formed in human liver microsomes using 4 positive compounds (benoxaprofen, bromfenac, diclofenac, and zomepirac) leading to DILI and 2 negative compounds (acetylsalicylic acid and ibuprofen). After incubation with microsomes in the presence of NADPH and UDP glucuronic acid (UDPGA) as a cofactor for 60 minutes at 37°C, there was large overlap in the distribution of CB levels between the positive and negative groups. Though benoxaprofen and zomepirac are positive compounds, their respective CB levels were very low. Therefore, after incubation for 60 minutes, the CB levels of compounds generating AGs are not sufficient for risk assessment of DILI. As the half lives of AGs have been considered to be longer than 1 hour, the CB levels of compounds generating AGs can be assessed through optimization of reaction conditions.

(1) R. Sawamura et al., Drug Metab Dispos., 38, 1857-1864 (2010)

1559 GENDER-DEPENDENT PROTECTION BY ALPHA-TOCOPHEROL INVOLVES ALTERED BENZO(A)PYRENE PHARMACOKINETICS AND ANTIOXIDANT MECHANISMS.

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Polycyclic aromatic hydrocarbons (PAHs), including benzo[a]pyrene (BP) are environmental pollutants linked to increased disease susceptibilities. BP metabolism produces both DNA-reactive and non-reactive (3-OH-BP) metabolites. Alpha-Tocopherol (T) supplementation decreases BP-DNA adducts in smokers, particularly females; but the mechanism is unknown. To test the hypothesis that T protection from BP exposure is gender-dependent, male and female rats received 7 daily subcutaneous (SC) injections of T (100 mg T/kg body wt) or vehicle, followed by a single ip injection of BP (20 mg/kg, dissolved in 5% DMSO in corn oil) on day 9. Urine, bile, plasma and tissues were collected pre-BP and 5 and 24 hr post-BP and stored at -80°C. T supplementation increased T levels in females > males (p<0.01). T supplementation increased free and conjugated 3-OH-BP levels >10-fold in urine of females (p<0.05). BP-induced tissue MDA levels were 2-fold higher in non-supplemented females compared to males (p<0.01). SQ T decreased BP-induced liver MDA in female rats (p<0.05). These data are the first to suggest that T protection from BP exposure is gender dependent and occurs by both antioxidant and non-antioxidant mechanisms. Further elucidation of the mechanism(s) of T protection against environmental/occupational toxins may lead to the development of protective strategies for occupational PAH exposures.

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1560 CHARACTERIZATION OF METABOLITES AND CYTOCHROME P450 ISOFORMS INVOLVED IN THE MICROSOMAL METABOLISM OF ACONITINE.


INTRODUCTION: Veratridine is a lipid-soluble alkaloid extracted from Veratrum officinale and other species of the family Liliaceae. Veratridine prevents inactivation of Na+ channel via binding the receptor site 2, causes influx of sodium ion and depolarization and induces apoptosis of neuronal cells. MATERIALS: In the present study, we investigated the metabolism of veratridine and the effects of selective cytochrome P450 (CYP) inhibitors on the metabolism of veratridine in rat liver microsomes. The metabolites were separated and assayed by liquid-chromatography-electro spray ionization-ion trap tandem mass spectrometry ([LC-ESI-QT/MS(n)), and further identified by their mass spectra and chromatographic behaviors.

RESULTS: Result showed that four CYP isoforms (CYP1A, CYP2B, CYP2E1, CYP3A) were involved in the metabolism of veratridine in vitro and seven metabolites of veratridine were detected incubating with rat liver microsomes. CONCLUSION: Some of the metabolites were presumed to be potential mediates of neurotoxicity via protein binging. Further research in vivo needs to link the metabolism of veratridine to its toxicity.

1561 REGULATION OF LIVER CYTOCHROME P450 BY ANTIANDROGENIC FUNGICIDE VINCOLIZOLIN IN ADULT MALE RATS.


Vincolizin (V) is an agricultural fungicide. V administered to rats is hydrolyzed to 2-[[3,5-dichlorophenyl]-carbamoyl]oxy]-2-methyl-3-butanolic acid (M1) and 3’-[[(3,5-dichloro-2-hydroxy-2-methylbut-3-enyl)oxy]-M2. V is efficiently metabolized by cytochrome P450 (CYP) 1A2, 2A, and 2B3 subfamilies. M1 and M2 are antiandrogenic by interacting with the androgen receptor. Data on the regulation of liver CYP by V is limited. Our objective was to determine the effect of V on the regulation of rat liver CYP. Adult male Wistar rats were administered 100 mg/kg/d V in corn oil by gavage. Animals were sacrificed 24 h after last dose and tissues were removed. Liver was processed to obtain microsomal fraction to determine the protein content of different CYP isoforms and some enzyme activities. V exposure significantly decreased relative weight of seminal vesicles, epididymis and prostate. Likewise significantly increased relative weight of kidney, liver and total CYP content. Protein content of CYP1A1, 1A2, 2A, and 2B1 and 3A2 increased 6.5-, 16.0-, 2.3, 6.5- and 1.5-fold, respect to the non-treated group. The protein content of CYP2E1 was not affected by V. Enzyme activities EROD, MROD, PROD and p-nitrophenol hydroxylase associated to CYP1A1, 1A2, 2B and 2E1 increased 3.0-, 6.4-, 6.4- and 1.6-fold, compared to the non-treated animals. The present results suggest that V may regulate hepatic CYP expression by affecting nuclear receptors, transcription factors and hormones involved in liver CYP expression during adult stage. Likewise, induces its own metabolism and consequently its toxikokinetik. The induction of CYP isoforms involved in steroids metabolism may disrupt hormonal homeostasis.

1562 INHIBITION OF CYP3A4 TRANSCRIPTION BY AMIODARONE AND DRONEDARENE.


Pregnane X receptor (PXR) functions as a xenobiotic sensor which recruits transcriptional coactivators such as SRC-1 and HNF4α upon ligand binding. We previously reported that ketoconazole inhibits CYP3A4 expression through disrupting PXR interaction with SRC-1 and HNF4α. To investigate the possible inhibitory role of antirhythmic drugs, amiodarone and dronedarone, on CYP3A4, we used mammalian two-hybrid assay to study the interaction among transcriptional factors. CYP3A4 promoter activity and CYP3A4 mRNA expression in HepG2 cells

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are inhibited by both drugs. Unlike ketoconazole, PXR-SRC-1 interaction is not affected by modafinil, but SRC-1 interaction is inhibited by both drugs, and not dronedarone. At clinical concentrations, none of two drugs showed significant inhibition on CYP3A4 activity. Amiodarone, but not dronedarone, is more likely to decrease CYP3A4 expression after long term use.

1563 A COMPARISON OF CYNOMOLGUS MONKEY AND HUMAN NEUTROPHIL FUNCTION.


Immunotoxicity testing of drug candidates includes the assessment of potential effects on the innate and acquired immune systems. Neutrophils are a key component of innate immunity and play a critical role in host defense against bacterial and fungal infections. Suppression of neutrophil function can lead to serious, life-threatening infections. Cytoskeletal and oxidative burst activities of neutrophils are essential elements of the phagocytic process. The current study compared phagocytosis and oxidative burst by cynomolgus monkey peripheral blood neutrophils to human neutrophils. To measure phagocytosis, peripheral blood was incubated with fluorescein isothiocyanate (FITC)-labeled opsonized E. coli. Neutrophils were stimulated with unlabelled opsonized E. coli to measure oxidative burst. The production of reactive oxygen species in the form of superoxide anions was assessed indirectly by measuring the oxidation of the fluorogenic substrate, dihydrorhodamine. The percent phagocytic cells, and phagocytic and enzymatic (burst) activities were measured by flow cytometry. Cytochalasin D, a chemical that inhibits actin polymerization, was used as a positive control inhibitory agent to test the sensitivity of these assays. Phagocytosis and oxidative burst were concentration-dependent activities. However, inhibition of phagocytosis was demonstrated by treatment with cytochalasin D. The magnitude of suppression of percent phagocytic cells by cytochalasin D in human neutrophils, but not monkey, was highly dependent on the concentration of E. coli, with less inhibition seen at high particle numbers. Consistent with the phagocytosis assay, oxidative burst by cynomolgus monkey and human neutrophils was inhibited by cytochalasin D in a concentration-dependent manner. However, inhibition of oxidative burst by cytochalasin D did not appear to be dependent on the numbers of E. coli particles added to culture. The results of the current study demonstrated robust phagocytic and oxidative burst by both cynomolgus monkey and human neutrophils. Additional experiments are needed to further evaluate the ability of these assays to detect drug-induced inhibition and stimulation of neutrophil function.

1564 DOWNREGULATED IMMUNITY BY SLEEP DEPRIVATION MAY NOT BE COMPENSATED WITH WAKING DRUG, MODAFINIL.


Modafinil is a psychostimulant, which enhances wakefulness and vigilance. Pharmacological profile of modafinil is notably different from other psychostimulants such as the amphetamine, methylphenidate, or cocaine, although exact mechanism of action is not clear. Here, we investigate modafinil effects on neuro-immune interaction in sleep-deprived mice. Splenic monoamines, which are dopamine,norepinephrine, serotonin, were significantly increased by modafinil (p<0.05, p<0.05, p<0.03, respectively), and those were independent to stress by sleep deprivation (SD). However, modafinil compensated down-regulated splenic dopamine/DOPAC turnover rate by SD stress. Modafinil had no influence on serum corticosterone level, however modafinil decreased significantly serum corticosterone level in SD stressed mice (p<0.05). Serum CRP level, which is an inflammation index, was significantly increased by sleep deprivation stress for 24 hr (p<0.003). Elevated serum CRP level in mice with stress was not reduced by modafinil. Mice injected with modafinil were significantly less resistant to Listeria infection (p=0.04). SD stress was also associated with increased bacterial burden, however it was not exacerbated by modafinil administration. This study suggests that modafinil may partly affect decrease of stress responses induced by SD stress, but that modafinil may not compensate down-regulated immunity caused by sleep deprivation.

1565 PESTICIDE EFFECTS ON HUMAN CELL-MEDIATED IMMUNITY AND THEIR RELATIONS WITH VIRAL HEPATITIS.

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Pesticides exposure may play a great role in suspected fragile immune system, & may result in altered disease susceptibility. Aim: Our aim is to illustrate the effects of occupational exposure to pesticides on human cell mediated immunity and to assess this relation with the prevalence of viral hepatitis (HBV&HCV) among the workers. Methods: Study was conducted on 80 subjects in one of the major companies formulating pesticides. All subjects were divided into two sections that were defined by the production sectors were 55 subjects (Group A),their age ranged between 26-55 years (mean +/- SD : 44.26 +/-5.33), they were exposed to different types of organophosphates and carbamates insecticides. They mostly did not use the administered protective equipment during work hours. The control subjects were (Group B) 25 adult men without occupational exposure in production of pesticides. They were chosen from other departments of the company.Investigations done for the examined groups (A & B) were abdominal ultrasonography & blood examination for Hb, WBCs, Lymphocytes, sALT, HbsAg, Anti HCV, CD4, CD8, CD4/CD8, CD56 and IL-2. Results: The most significant results were alteration of the cell mediated immunity among the exposed group and increase in the prevalence of Hepatitis B virus (HBV) & Hepatitis C virus (HCV) which confirmed by ultra-sonographical examination. The relation between different liver diseases as bilharzial liver, liver cirrhosis and mixed liver disease and cell mediated immunity, had been illustrated. However, there was no significant difference between duration of exposure to pesticides and different immunological parameters. Conclusion and Recommendations: It was concluded that occupational exposure to pesticides may cause modulation and/or derangement of the immune system. Modulation of the immune system among the exposed group to pesticides may be due to the high percentage of HBV and HCV. Further studies on a large scale and studying the whole immune system of those subjects is mandatory as well as early detection of such changes are recommended.

1566 RETROSPECTIVE EVALUATION OF THE IMPACT OF US EPA’S FUNCTIONAL IMMUNOTOXICITY TESTING REQUIREMENT ON PESTICIDE RISK ASSESSMENT.

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As a result of recent revisions to 40CFR Part158, functional immunotoxicity testing, as outlined by the EPA Health Effects Test Guideline OPPTS 870.7800, is now required for all food and non-food use pesticides. In complying with this data requirement, pesticide registrants have submitted a substantial number of guideline-compliant T-cell dependent antibody response (TDAR) studies, providing an opportunity to review the results and their impact on pesticide hazard identification and human health risk assessment. This evaluation coordinated by Crop Life America includes a total of 82 TDAR studies conducted by various pesticide registrants on 78 unique chemicals over a diverse range of product types. A retrospective review of this data-set revealed that no effect on the TDAR response was noted at any dose level in 78 studies, while 4 studies demonstrated a reduction of TDAR only at the highest dose level tested. To assess the potential impact of these results on risk assessment, the NOAELs from the TDAR studies were compared to endpoints selected by the EPA for relevant risk assessment scenarios. For all 78 chemicals, including those with TDAR effects, the TDAR NOAEL was greater than the NOAEL selected for each of the key risk-assessment scenarios. The ratios of the TDAR NOAELs to the EPA-selected endpoints for chronic reference dose, short-term, intermediate, and long-term exposure scenarios ranged from 3 - 27000, 3 - 1688, 3 - 1688, and 4.9 - 1688, respectively. Based on these analyses, functional immunotoxicity testing appears to have limited implications for pesticide hazard identification and, more importantly, no impact on human health risk assessment and therefore may not be a justifiable use of animals. Weight of evidence approaches based on existing data should receive strong consideration prior to the conduct of functional immunotoxicity testing.

1567 PROTECTIVE EFFECT OF TWO RECOMBINANT DNA RICIN VACCINES IN THE NEW ZEALAND WHITE RABBIT SUBJECTED TO A LETHAL AEROSOLIZED RICIN CHALLENGE: SURVIVAL, IMMUNOLOGICAL RESPONSE, AND HISTOPATHOLOGICAL FINDINGS.

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Ricin isolated from the castor bean plant Ricinus communis is included on the Centers for Disease Control and Prevention (CDC) Category B list of bioterroism agents, indicating that the toxin is moderately easy to disseminate and could result in moderate morbidity rates. This study evaluated two promising recombinant ricin subunit vaccines, one made using an Escherichia coli codon-optimized gene and the other using a yeast codon-optimized gene in E. coli-based fermentations.

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Rabbits were vaccinated four times over a period of 6 months and challenged with -10 to 30 times the median lethal dose of aerosolized ricin. All unvaccinated control rabbits were found dead or humanely euthanized within 30 h postchallenge while the rabbits vaccinated with either vaccine survived the exposure without adverse clinical signs. When the protective antibody responses were analyzed, no significant difference was seen between the two vaccines. However, there was a significant difference in the immune response over time for both vaccines tested. Although clinical pathology was unremarkable, significant histological lesions in the control animals included fibrinonecrotic pneumonia, acute necrotizing lesions in the upper respiratory tract, and necrotizing lymphadenitis in the lymph nodes draining the upper and lower respiratory tract. Vaccine treated rabbits exhibited resolving lesions associated with ricin exposure, namely chronic inflammatory lesions in the upper respiratory tract and lungs, fibrosis, type II pneumocyte hyperplasia, and bronchiolitis obliteratoris. This study confirmed the safety and efficacy of two recombinant ricin subunit vaccines in rabbits, offering potential protection to warfighters and select populations.

1568 EFFECTS OF ETHANOL AND ROLE OF HYPOTHERMIA IN SUPPRESSION OF HOST RESISTANCE TO SEPSIS BY ADMINISTERING ETHANOL 18-HR AFTER BACTERIAL CHALLENGE.

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Acute exposure to ethanol has been found to decrease resistance to bacterial infections and increase mortality in patients with sepsis. Administration of ethanol 30 minutes before E. coli challenge inhibits production of pro-inflammatory cytokines and chemokines and suppresses phagocytosis by bacteria of macrophages and neutrophils in the peritoneal cavity in our mouse model of sepsis. Bacteria were effectively cleared in control mice but not in ethanol-treated mice, and decreased bacterial clearance correlated with decreased survival. In the present study, E. coli was injected intraperitoneally and ethanol was given orally at 6 g/kg 18 hr later. Interestingly, although the mortality rate was similar, compared with the group treated with ethanol 30 min before E. coli injection, the group with late ethanol administration reached the same mortality rate in a much shorter period of time (20 h vs. 64 h). Moreover, ethanol decreased body temperature of mice in this group -2°C for at least 10 h. To determine if low body temperature contributed to decreased survival time, mice were heated on heating pads after ethanol administration. This did decrease the ethanol-mediated increase in hypothermia (which is normally associated with sepsis in mice). Surprisingly, the mice that were maintained at a warmer temperature died more quickly than mice that were not heated. This suggests that effects other than hypothermia account for the lethal outcome. In a previous study, bacteria were almost cleared -18 hr after E. coli challenge and a difference in the immune response over time for both vaccines tested. Although clinical pathology was unremarkable, significant histological lesions in the control animals included fibrinonecrotic pneumonia, acute necrotizing lesions in the upper respiratory tract, and necrotizing lymphadenitis in the lymph nodes draining the upper and lower respiratory tract. Vaccine treated rabbits exhibited resolving lesions associated with ricin exposure, namely chronic inflammatory lesions in the upper respiratory tract and lungs, fibrosis, type II pneumocyte hyperplasia, and bronchiolitis obliteratoris. This study confirmed the safety and efficacy of two recombinant ricin subunit vaccines in rabbits, offering potential protection to warfighters and select populations.

1569 AH RECEPTOR ACTIVATION INDUCES REGULATORY DENDRITIC CELLS AND ANTIGEN-SPECIFIC REGULATORY T CELLS IN VIVO.

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Environmental pollutants capable of activating the aryl hydrocarbon receptor (AhR) can cause immunosuppression. AhR activation can induce regulatory T cells (Tregs), which may contribute to the observed immunosuppression. However, the mechanisms underlying Treg induction and immunomodulation following AhR activation are unclear. We hypothesized that AhR activation by TCDD generates regulatory dendritic cells (DCs) that directly induce Tregs in vivo. To test this hypothesis, splenic DCs from mice orally exposed to Vehicle or TCDD (150 μg/kg) were isolated and their regulatory phenotype assessed. DCs from TCDD-exposed mice upregulated expression of the inhibitory PD-1, IDO2, and TGFβ3. Increased regulatory gene expression in DCs was seen as early as 48 h and up to 10 days post-TCDD exposure. On day 10 there was an increased frequency of CD11c+ MHC II+ CD103+ regulatory DCs and CD4+ CD25+ FoxP3+ Tregs in TCDD-exposed mice. To determine if AhR-activation induces antigen-specific Tregs in vivo, CD4+ T cells isolated from OTII FoxP3eGFP mice were transferred i.v. into vehicle- or TCDD-exposed mice followed by footpad immunization with ovalbumin (ova) peptide-loaded bone marrow-derived DCs. The antigen-specific immune response in the draining popliteal lymph node was dampened in TCDD-exposed mice as determined by a reduction in the number of ova-specific CD4+ T cells and an increase in ova-specific CD4+ FoxP3+ Tregs. In addition, CD4+ T cells from the draining lymph node produced significantly less IL-2, IFNγ and IL-10 when restimulated with ova peptide. Overall, these results demonstrate that in vivo exposure to the prototypical AhR ligand, TCDD, induces both regulatory DCs and FoxP3+ Tregs, which contribute to the generation of an immunosuppressive environment. Studies are ongoing to determine the direct involvement of AhR-activated regulatory DCs and whether other AhR ligands can induce similar immunomodulatory events. This research was supported by the grants, ES013784 and RR017670.

1570 TIME COURSE FOR HEMATOXICITY OF MNX (HEXAHYDRO-1-NITROSO-3, 5-DINITRO-1, 3, 5-TRIAZINE), RDX ENVIRONMENTAL DEGRADATION PRODUCT, IN RATS.


MNX (hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine), an environmental nitroreduced product of RDX, contaminates military firing ranges and tertiary ammuni tion plants. Our previous studies identified bone marrow and spleen as acute hematological targets of oral MNX in rats. At 14 d after a single MNX exposure, splenic hemosiderosis and loss of blastoid granulocytes and bone marrow myeloid progenitor cells (CFU-GMs) occurred with NOAELs of 47, 47 and 1 mg/kg, resp. Present studies address whether these late effects are due to persistence of early effects or are delayed after a required expression period. Time course studies were conducted with female Sprague-Dawley rats orally gavaged with MNX from 0 to 94 mg/kg and hematology, organ weights, tissue histopathology and bone marrow CFU-GMs were evaluated at 2, 7, 10, 12, 14 d. Blood granulocytes were increased at 2d (NOAEL 47 mg/kg), unchanged at 7d and decreased at 14d. Bone marrow CFU-GMs were unaffected at 2d and 7d, but were decreased by 47 mg/kg MNX from 12 – 14d with maximal effect at 12 d. Relative spleen weight decreased 2d post-exposure and was unaffected at later times. Enhanced immunohistochemical staining with antibody ED1. Blood MNX was 0.2-0.4 μg/mL at 2d after 24-94 mg/kg, but undetectable at 14d. Collectively these data demonstrate that while splenic effects of a single exposure to MNX are early onset and persist for at least 14 days post-exposure, bone marrow toxicity is delayed presumably because of time required for development of preceding events that drive the myelosuppressive outcome. (Support: DoD/CDMRP, US Army Corps of Engineers)

1571 IMMUNOHISTOCHEMICAL CHARACTERIZATION OF GLOMERULOPATHY IN CYNOLOGOUS MONKEYS.


Biotherapeutics often induce immune responses in animals, and the kidney is a known site of immune complex deposition; however, it is often uncertain whether adverse renal events and glomerular lesions are related to immune complex deposition or toxicity. In a 13-cycle toxicity study, a minimal glomerulopathy was observed in one of sixteen cynomologous monkeys that received weekly doses of chimeric monoclonal antibody Y(mAb-Y). The affected animal had developed anti-drug antibodies; thus, this finding was thought to represent immune complex-mediated glomerulopathy. To better understand glomerulopathy in cynomologous monkeys exposed to biotherapeutics, we developed immunohistochemistry protocols for a panel of antibodies to characterize the phenotype and activation state of glomerular cells. These antibodies were applied to kidney tissue sections from 24 cynomologous monkeys that had been dosed with C-mAb-Y (n=16) or vehicle (n=8). We found that the monkey with glomerulopathy (animal 11) had greater numbers of Ki67 positive cells than all others in the study. Additional glomerular lesions in animal 11 included hypertrophy and hyperplasia of podocytes and wrinkling of the glomerular basement membrane, which was embellished by synaptopodin expression. Cumulatively, these findings support a diagnosis of proliferative podocytopathy in animal 11. Further, we found that synaptopodin, WT-1, and ICAM-1 expression were dramatically lower in animal 15 (which had a lethal anaphylactic reaction to the test article) than all other monkeys in the study. The relationship of these findings to the fatal anaphylactic reaction is yet to be determined. Additional studies demonstrate that immunohistochemical evaluation of glomerular antigens can facilitate the diagnosis of glomerulopathy in NHPs exposed to biotherapeutics.
N-acetyltransferase 1 (NAT1)-mediated N-acetylation is an important detoxification pathway for the hair dye ingredient and well-known contact allergen paraphenylenediamine (PPD). Recently, we demonstrated that immune relevant cells like keratinocytes and monocyte derived dendritic cells are able to N-acetylate PPD. However, NAT1 acetylation capacity may be modulated by the microenvironment. In keratinocytes, we demonstrated a substrate-dependent NAT1 degradation after treatment with PPD. In this study we aimed to evaluate the influence of tumor necrosis factor alpha (TNF-α), an important cytokine released after allergen exposure, on the NAT1 activity of keratinocytes and monocytes. We treated THP-1 monocytes and HaCaT keratinocytes with 10 ng/ml and 500 ng/ml TNF-α for 24 h. Afterwards, cells were lysed and NAT1 activities were determined by quantification of coenzyme A via staining with 5,5'-dithio-bis(2-nitrobenzoic acid) after N-acetylation (30 min) of 1 mM PABA by 25 or 50 μg protein in the presence of 1 mM acetyl coenzyme A. TNF-α itself did not influence the NAT1 activity of those cells. However, co-treatment of THP-1 cells with PPD and 10 ng/ml TNF-α reduced the PPD-induced NAT1 inhibition about 30%. To further analyze the influence of TNF-α on the N-acetylation reaction, we measured NAT1 activity of primary keratinocytes (HPLC analysis) from 4 different donors in the presence and absence of TNF-α. We found a 3.7±0.4-fold, 4.9±1.4-fold and 4.2±1.3-fold increase of the N-acetylation of PPD, mono-acetyl-PPD and amino-fluorene.

These data suggest that N-acetylation capacities of keratinocytes and monocytes are highly variable and dependent on the microenvironment. This may influence the detoxification capacity of the skin regarding aromatic amines like PPD and thus modify the risk for PPD induced contact allergy.

Asbestos exposure can cause malignant mesothelioma and lung cancer. In the anti-tumor immunity, cytotoxic T lymphocytes (CTL) play a critical role. Previously, we reported that asbestos exposure suppressed the induction of human CTL during mixed lymphocyte reactions (MLR), accompanied by the decreases in IFN-γ and TNF-α. Therefore, we examined the functional property of CD8+ lymphocytes in PBMCs of asbestos-exposed people with pleural plaque (PL) by flow cytometric analysis, and compared with healthy volunteers (HV) in the present study. Freshly prepared PBMCs were assayed for percentage and cell number of CD3+CD8+ cells and percentage of granyme B+ and perforin+ cells in CD8+ lymphocytes. PBMCs were stimulated with PMA/ionomycin for 4h and assayed for percentage of IFN-γ+ cells, as well as granyme B+ and perforin+ cells in CD8+ lymphocytes. Percentage and cell number of CD3+CD8+ cells had a tendency to be lower in PL-positive people compared with HV. There was no difference in the percentage of IFN-γ+ cells in CD8+ lymphocytes stimulated with PMA/ionomycin between PL and elder HV groups, the age-similar control, although the percentages of both groups were higher than those of young HV. In contrast, both the percentages of granyme B+ and perforin+ cells in PL group were higher compared with elder group of HV. The stimulation induced the decrease in percentages of granyme B+ and perforin+ cells in CD8+ cells of elder HV, whereas PL group showed a mix of increase/decrease or almost no change in those. Contrary to expectation, these results from specimens paradoxically suggest the possibility that most of PL-positive people might have a higher cytotoxic potential of CD8+ T cells. Further investigation about cellularity and functional property of CD8+ cells in people exposed to asbestos will contribute to the resolution of this paradox.
Polycyclic aromatic hydrocarbons (PAHs) are common environmental contami- 
nants that are carcinogenic and immunosuppressive. Previous studies have demon- 
strated that oral or IP administration of 7, 12-dimethylbenz(a)anthracene (DMBA) 
significantly reduces bone marrow (BM) progenitor lymphoid and myeloid cells at 
6 h. This study examined whether feeding mice a diet containing 20 % Spanish 
black radish (SBR) for two weeks prior to DMBA administration could enhance 
metabolism of this carcinogen and inhibit the DMBA-mediated bone marrow 
toxicity. Expression of Phase 1 (Cyp1a1, Cyp1a2, Cyp1b1) and Phase II (Ephx1, 
Gst2, Qr, Tnxed1) detoxification enzymes was significantly greater for mice on 
SBR diet than in mice fed a nutritionally-matched control diet. Six hours after 
DMBA administration (50 mg/kg) the blood levels of DMBA in SBR-fed mice was 
significantly lower than those on control diets, suggesting SBR-enhanced metabo- 
lism of the carcinogen. DMBA significantly reduced BM cells of mice fed control 
diet, whereas mice on the SBR diet showed diminished effect. Colony forming as-
says demonstrated that mice on the SBR diet had significantly: 1) less reduction in 
lymphoid CPU-preB progenitor cells, 2) greater recovery of CPU-preB progenitor 
cells at 168 hours, and 3) less reduction of CPU-GM progenitor cells at 6 hours. 
Therefore, in contrast with mice fed the control diet, mice fed a 20% SBR diet for 
two weeks had higher expression of detoxification enzymes, faster metabolism 
of DMBA and a reduction in DMBA-induced bone marrow toxicity.

Environmental tobacco smoke (ETS) is particularly harmful to children, who are 
not fully developed physically and respires at a higher rate than adults. Many previ- 
ous findings have found that those exposed to ETS have increased incidence of in- 
fec tions of the lower respiratory tract. However, little is known how exposure to 
ETS during the perinatal period affects the susceptibility of infants and young chil- 
dren to infection. The objective of this study was to determine whether perinatal 
exposure to ETS would increase the incidence, morbidity and severity of respira- 
tory influenza infection followed by secondary bacterial infection. Female Balb/c mice 
were exposed to either ETS for 6 h/day, 7 days/week beginning on gestation day 14 
and continuing to 6 weeks of age or to filtered air (FA) only. At the end of exposure, 
mice were intranasally inoculated with a murine-adapted influenza A virus at a dose 
of 50 TCID50 in 40 μL. One week later an intranasal inoculation of S. aureus bac- 
teria at a dose of 108 CFU per 10 μl 7 was administered. Animal behavior and 
body weights were observed and recorded daily following viral infection prior to 
bacterial infection. Mice were necropsied 1-day post-bacterial infection. Bronchoalveolar lavage (BAL) fluid and lung tissue were collected for cell analysis 
and histopathology, respectively. We found ETS exposure increased BAL cell num- 
ber in both virus and bacteria infected mice, while also decreasing body weight 
compared to mice infected only with either virus or bacteria. Increased morbidity in 
animals exposed to ETS, regardless of treatment was also observed, although 
signs of illness appeared later than observed with FA. This data suggests perinatal 
exposure to ETS alters the response of neonates to the timing and severity of infec- 
tion indicating a change in susceptibility to secondary infection due to perinatal 
exposure to ETS.
chloride), a class of small RNAs, act as negative regulators of gene expression, thereby modulating most of the cellular pathways. However, not much is known about the precise role of miRs in immune cell dysregulation. Prenatal exposure to TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin), a potent AhR ligand, is well known to cause thymic atrophy as well as alterations in T cell differentiation, the precise mechanisms of which are unclear. In the current study, therefore, we investigated the role played by miRs in regulating the toxic effects of TCDD on the thymus following prenatal exposure. To this end, pregnant mice on gestational day 14, were injected with TCDD (5 μg/kg) and on postnatal day 4, the thymi were harvested and high-throughput miR arrays were performed. We observed more than 100 miRs out of a total of 610 miRs examined, to be up- or down-regulated greater than 1.5 fold in fetal thymocytes post TCDD exposure compared to controls. We selected few miRs that showed significant levels of alterations by TCDD for further analysis and characterization. Our studies demonstrated that TCDD caused marked changes in miRs involved in important functions including early signaling, apoptosis, toxicity and cancer. Some of the miRs such as miR-18a, -18b, -23a, -23b, -31, -98, -162, -190, 203, -217, -320, -490, -494 were significantly altered in TCDD-exposed thymocytes. Real-Time PCR analysis demonstrated changes in miR profile that affected expression of important genes such as, AhR, CYP1A1, Foxp3, IL-17, Fas, FasL, CYP1A1, Foxp3, IL-17, Fas, FasL, which are known to regulate signaling, toxicity, apoptosis, thymic atrophy, and immunosuppression caused by TCDD. Together, our studies suggest that the prenatal immunotoxicity induced by TCDD may be triggered by dysregulation in miRs (Supported in part by NIH grants P01AT003961, R01AT006888, R01ES019513, R01MH094755).

1582 ROLE OF MICRORNAS IN THE REGULATION OF TCDM-MEDIATED TOXICITY AGAINST THYMOCYTES FOLLOWING PRENATAL EXPOSURE.

P Nagarkatti, N. P. Singh, U. Singh and M. Nagarkatti, Pathology, Microbiology, and Immunology, University of South Carolina School of Medicine, Columbia, SC.

Micro RNAs (miRs), a class of small RNAs, act as negative regulators of gene expression, thereby modulating most of the cellular pathways. However, not much is known about the precise role of miRs in immune cell dysregulation. Prenatal exposure to TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin), a potent AhR ligand, is well known to cause thymic atrophy as well as alterations in T cell differentiation, the precise mechanisms of which are unclear. In the current study, therefore, we investigated the role played by miRs in regulating the toxic effects of TCDD on the thymus following prenatal exposure. To this end, pregnant mice on gestational day 14, were injected with TCDD (5 μg/kg) and on postnatal day 4, the thymi were harvested and high-throughput miR arrays were performed. We observed more than 100 miRs out of a total of 610 miRs examined, to be up- or down-regulated greater than 1.5 fold in fetal thymocytes post TCDD exposure compared to controls. We selected few miRs that showed significant levels of alterations by TCDD for further analysis and characterization. Our studies demonstrated that TCDD caused marked changes in miRs involved in important functions including early signaling, apoptosis, toxicity and cancer. Some of the miRs such as miR-18a, -18b, -23a, -23b, -31, -98, -162, -190, 203, -217, -320, -490, -494 were significantly altered in TCDD-exposed thymocytes. Real-Time PCR analysis demonstrated changes in miR profile that affected expression of important genes such as, AhR, CYP1A1, Foxp3, IL-17, Fas, FasL, which are known to regulate signaling, toxicity, apoptosis, thymic atrophy, and immunosuppression caused by TCDD. Together, our studies suggest that the prenatal immunotoxicity induced by TCDD may be triggered by dysregulation in miRs (Supported in part by NIH grants P01AT003961, R01AT006888, R01ES019513, R01MH094755).

1583 PRENATAL EXPOSURE OF MICE TO DIETHYLSTILBESTROL (DES) DISRUPTS T-CELL DIFFERENTIATION BY REGULATING FAS/FASL EXPRESSION THROUGH ERE AND NF-KB MOTIFS.

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Prenatal exposure to diethylstilbestrol (DES) is known to cause altered immune functions and increased susceptibility to autoimmune disease in humans. In the current study, we investigated the effect of prenatal exposure to DES on thymocyte differentiation involving apoptotic pathways. Prenatal DES exposure significantly upregulated Fas and Fasl expression in thymus and T cells. To examine the mechanism underlying DES-mediated regulation of Fas and Fasl, we performed luciferase assays using EL4 cells or T cells transfected with luciferase reporter constructs containing full length Fas or Fasl promoter. There was significant luciferase induction in the presence of Fas or Fasl promoter following DES exposure. Further analysis demonstrated the presence of several cis-regulatory motifs on both Fas and Fasl promoters. When DES-induced transcription factors were analyzed, ERE, NF-kb, NF-AT, and AP-1 motifs of Fas promoter as well as ERE, NF-kb, and NF-AT motifs of Fasl promoter showed binding affinity with the transcription factors. EMSAs were performed to confirm the functionality of cis-regulatory motifs of Fas or Fasl promoter with transcription factors. There was shift in mobility of probes (ERE or NF-kb2) of both Fas and Fasl in the presence of nuclear proteins from DES-treated cells and the shift was specific to DES as these probes failed to shift their mobility in the presence of nuclear proteins from vehicle-treated cells. NF-kb1 probes of both Fas and Fasl on the other hand, did not show shift in their mobility, demonstrating their lack of participation and confirming the results obtained from earlier analysis. These data demonstrate that DES adversely affects neonatal mice and one of the mechanisms may be by regulating Fas and Fasl expression through their cis-regulatory motifs (Supported in part by NIH grants P01AT003961, R01AT006888, R01ES019513, R01MH094755).

1584 MANIPULATION OF THE STANDARD CFU-GM ASSAY: TARGETED SCREENING ON HEMATOPOIETIC MYELOID PROGENITORS.

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A key feature of hematopoietic stem cells and their progeny is that their proliferation and differentiation can be regulated by external stimulation from various cytokines. Cytokines differentially affect intracellular signaling pathways to direct lineage commitment and differentiation in hematopoietic progenitors which give rise to mature cell types such as granulocytes, monocyte/macrophages and red blood cells. The development of compounds that specifically target key components of these hematopoietic signalling pathways has increased dramatically; most notable are the class of kinase inhibitors. A crucial tool to the continued discovery and characterization of kinase inhibitors is the use and modification of the colony forming cell-granulocyte, monocyte (CFU-GM assay). This in vitro clonogenic assay is the current gold standard for the quantification of myeloid hematopoietic progenitors in clinical samples. By manipulating the type and concentrations of cytokines within the medium formulations, this assay can be customized to support growth of specific myeloid progenitors (CFU-G, CFU-M, or CFU-GM), allowing differential assessment of the effects of compounds on the cell populations of interest. EMSAs have provided various media formulations with the ideal growth conditions for detecting inhibition of myeloid progenitors from human bone marrow samples. We then tested different tyrosine kinase inhibitors to determine IC50 and IC90 values for each progenitor population. Differential effects were observed in the IC50 values of Sunitinib and Imatinib on CFU-M and CFU-G. In addition, IC50 values obtained for these compounds on CFU-G inhibition are similar to what is seen in published literature and more importantly shown to be clinically relevant, with the more potent IC50 values correlating with an increased incidence of clinical neuropenia.
Therapeutics based on RNA interference have the potential to address a wide range of molecular targets, including those considered intractable using traditional small molecule and protein-based approaches. However, several hurdles remain in profiling and mitigating safety concerns for this novel drug class. Efforts are currently under way to characterize toxicity findings associated with the use of small interfering RNA (siRNA)-containing lipid nanoparticles (LNPs) in vitro, and to model these findings using in vivo and ex vivo systems. One ex vivo method that has been used to assess potential immunotoxicity is the murine splenocyte ex vivo proliferation assay involving siRNA-containing LNPs in whole blood and studying the induction of inflammatory cytokines and chemokines. The aim of this work was to analyze the immunostimulatory properties of LNP-formulated siRNAs in whole blood from human, cynomolgus monkey, dog, and rat in order to compare differential sensitivity and particle uptake across pharmacologically and clinically relevant species. Follow-up experiments will help to identify relevant mechanisms of immune stimulation associated with LNP-formulated siRNAs including the contributions of both the lipid and nucleic acid components, and will help to optimize toxicity species selection for supporting first in human studies.

The immune system can be the target of a broad variety of chemicals in the form of environmental contaminants, food additives and preservatives, and day-to-day household products with adverse effects to human health. Immunotoxicants can induce suppressive or stimulatory responses and DNA damage in components of the immune system. In this study, dendritic cells (DC) were used to monitor immunotoxicant induced phenotypic changes (surface marker expression), functional alterations (cytokine release), and DNA damage (comet assay). We evaluated the effect of 5 immunotoxic compounds (ITC) and 2 reproductive hormones, estradiol and progesterone, on DC responses. Finally we checked DNA damage of DC following treatment with the carcinogenic agent, methyl methanesulfonate (MMS). DC were exposed to non-cytotoxic concentrations of the ITC and 2 reproductive hormones for 24 h and PLS for an additional 18 h. FACs analysis of ITC-exposed DC showed effects in the rank order of immunotoxicity (severe to low effect): Tributylin chloride > Cyclosporine A > Benzoylpyrene > Fusemide and Urethane. Dose-dependent decreases in the secretion of immuno-stimulatory cytokines including interleukin (IL)-12, IL-6, and IL-1β were observed. Furthermore, treatment of DC with the reproductive hormones, estradiol and progesterone, showed a decrease in lipopolysaccharide-induced CCR7 expression and IL-12 secretion. Exposure of DC to MMS also showed dose dependent DNA damage. Conclusion: The release of immunostimulatory cytokines and DNA damage may serve as endpoints to predict immunotoxicity. Due to concerns with animal models in terms of cost, ethical issues, and relevance to hazard assessment in humans, the human primary DC-based assay is an attractive in vitro model to predict the immunotoxicity of compounds and formulations.
IVIS® Spectrum imaging system. The pH-sensitive pHrodo™ dye will dramatically increase in fluorescence once the particle is phagocytosed and taken up into low pH phagolysosomes. If the test agent affects the ability of the Kupffer cells to phagocytose the BioParticles®, a change in fluorescence is measured. In the development of this model, we have titrated BioParticles® showing that liver fluorescence increased as the dose of BioParticles® increased. The specificity of our read-out was confirmed by immunohistostaining showing that BioParticles® co-localize with Kupffer cell surface markers, ED1 and ED2. Intravenous administration of clodronate liposomes, which deplete Kupffer cells, prior to the administration of the BioParticles®, decreased bioparticle fluorescence in the liver. This model has the potential to determine if a test agent will affect the ability of the MPS to phagocytose treated with hydrocortisone or levamisole, we found the mouse peritoneal macrophages of phagocytosis treated with hydrocortisone or levamisole, we found the mouse peritoneal macrophages of phagocytosis with pHrodo™ dye to be more sensitive than any other method of evaluating mouse peritoneal macrophages of phagocytosis with pHrodo™ dye to be more sensitive than any other method of evaluating mouse peritoneal macrophages of phagocytosis. Further studies are needed to extrapolate to multiple donors due to the large number of peripheral blood mononuclear cells (PBMCs) traditionally required for such studies. We present here approaches using microcapillary cytometry, small cell sample sizes and simplified approaches to investigate changes in features such as mitochondrial potential changes, annexin V–based apoptosis detection or caspase assays in CD4 and CD8 T cell populations simultaneously. The approaches thus provide information in change in immune cell population and a snapshot of immune cell health. Some of the assays have been optimized to work in a no wash fashion thus preventing loss of precious apoptotic or dead cells. In a screening study with 80 cytotoxic compounds, PBMCs from multiple donors were treated with cytotoxic compounds and then evaluated for their impacts on CD4 and CD8 T cells and their expression of cell health assays followed by flow cytometry on the guava easyCyte cytometry platform. The studies identified several compounds such as gambogenic acid, thimerosal, phenylmercuric acetate, 2,6 dimethoxyquinone and sanguinarine sulfate which caused high levels of apoptosis in both CD4 and CD8 T cells with overnight treatment with some of them having differential effects on individual sub-populations. Study of apoptosis and cell death mechanisms in immune cell subpopulations simultaneously can provide valuable information on identified stressed cells, and understanding the compound’s mode of action especially on immune cell populations.

**1590 SIMPLIFIED CYTOMETRIC METHODS TO EVALUATE IMPACTS ON IMMUNE CELL HEALTH IN CYTOTOXICITY STUDIES.**


Immunotoxicity studies require an understanding of impacts of compounds and conditions on immune cell sub-populations. These studies are complicated and cumbersome to perform since they require parallel identification of immune cells and identification of impact of the treatment condition on immune cell health. Further these studies are harder to extrapolate to multiple donors due to the large number of peripheral blood mononuclear cells (PBMCs) traditionally required for such studies. We present here approaches using microcapillary cytometry, small cell sample sizes and simplified approaches to investigate changes in features such as mitochondrial potential changes, annexin V–based apoptosis detection or caspase assays in CD4 and CD8 T cell populations simultaneously. The approaches thus provide information in change in immune cell population and a snapshot of immune cell health. Some of the assays have been optimized to work in a no wash fashion thus preventing loss of precious apoptotic or dead cells. In a screening study with 80 cytotoxic compounds, PBMCs from multiple donors were treated with cytotoxic compounds and then evaluated for their impacts on CD4 and CD8 T cells and their expression of cell health assays followed by flow cytometry on the guava easyCyte cytometry platform. The studies identified several compounds such as gambogenic acid, thimerosal, phenylmercuric acetate, 2,6 dimethoxyquinone and sanguinarine sulfate which caused high levels of apoptosis in both CD4 and CD8 T cells with overnight treatment with some of them having differential effects on individual sub-populations. Study of apoptosis and cell death mechanisms in immune cell subpopulations simultaneously can provide valuable information on identified stressed cells, and understanding the compound’s mode of action especially on immune cell populations.

**1591 FLOW CYTOMETRY AS A TOOL TO EVALUATE MOUSE PERITONEAL MACROPHAGES OF PHAGOCYTOSIS IN IMMUNOTOXICITY TESTING.**

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Phagocytosis is extremely complex, no single model can fully account for the diverse structures and outcomes associated with particle internalization. Phagocytosis by macrophages is critical for the uptake and degradation of infectious agents and senescent cells, and it participates in development and tissue remodeling. Phagocytosis by macrophages may be important to the role in immune response, and it’s an important indicator in immunotoxicity evaluating. The mouse peritoneal macrophages may be induced to phagocytose particles in vivo and in vitro. Of special interest is the fluorescence beads(2 in vivo), but the method of in vivo is more sensitive and convenient. The overall data suggest it is a sensitive, convenient, accurate and high flux method for evaluating mouse peritoneal macrophages of phagocytosis with flow cytometry, which permits rapid quantitative evaluation of the effects of chemicals on phagocytosis in immunotoxicity testing.

**1592 FLOW CYTOMETRIC NATURAL KILLER CELL ANALYSIS: NEW STRATEGIES FOR TOXICOLOGICAL ASSESSMENT IN NONHUMAN PRIMATES.**

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Natural Killer (NK) cells lack a single immunophenotypic attribute or unique antigen receptor that can universally define them. The large number of receptors that NK cells use to identify their targets makes it difficult to distinguish the entire NK cell population in nonhuman primates with a single marker. Flow cytometric analysis of human lymphocytes typically use CD56 in conjunction with CD16 as a total NK cell marker, however the CD56 antigen is not cross-reactive with cynomolgus monkey NK cells. Traditional flow cytometric approaches for measuring total NK cells in nonhuman primates use CD3-CD16+ or a negative exclusion perspective such as CD3-CD20- to distinguish the cell population. These conventional approaches run the risk of excluding subsets of NK cells that are immunologically relevant and typically yield low intensity staining that causes difficulties in consistent population gating. The fringe populations that are not captured by these assessments may hold evidence of unknown (possibly advantageous) drug effects. Taking the total population into consideration allows for a deeper understanding of the drug’s effects on the immune system. Here we evaluate new immunophenotyping strategies for NK cells using newly available NK cell markers and gating strategies not conventionally used for nonhuman primates. To demonstrate the best approach for total NK cell analysis, testing was conducted on sexually mature cynomolgus monkeys, using isolated leukocytes from whole blood; each sample was stained with cell surface markers against CD3, CD20, CD16, CD159a, CD35, CD37, and NKp60. Data was evaluated individually and in combination with other markers to determine the strategies that would most easily distinguish and capture the complete NK cell population. These strategies were compared and discussed for feasibility and risk management.

**1593 AN IMPROVED METHOD FOR IMMUNOPHENOTYPING IN THE CYNOMOLGUS MONKEY.**


The measurement of the number and activation status of leukocytes in the peripheral blood, lymph nodes and spleen are key indicators of the effects of biopharmaceuticals on the immune system. Here we describe the development and validation of a panel of commercially available antibodies that takes advantage of recent developments in both reagent and instrumentation technology to allow the rapid and reliable quantification of the number and phenotype of key cell populations in the Cynomolgus monkey tissues. Blood was collected from six Cynomolgus monkeys at weekly intervals for 4 weeks and at the end of the study terminal blood samples, bone marrow and lymph nodes were also collected. To measure the cellular distribution in these tissues we have designed panels of NHP specific antibodies to determine T (CD3), B (CD20) and NK cells (CD3-/CD16+), monocytes (CD14), and neutrophils (CD16). The activation status of CD4+/CD8+ was determined using CD25 and CD69 expression, and the activation of B-cells by HLA-DR. Further panels have been defined using cell surface markers to determine subsets of T cells including Th1 (CD195), Th17 (CD196), T naïve (CD45RA/CD184) and Treg (CD45RA/CD25). Initially we have conducted a comparison of lysing method for removal of red blood cells (RBCs) using the ImmunoprepTM reagent system, FACS Lysing solution and VersalyseTM. Following this we have evaluated the optimum titre of each antibody used for nonhuman primates. To demonstrate the best approach for total NK cell analysis, testing was conducted on sexually mature cynomolgus monkeys, using isolated leukocytes from whole blood; each sample was stained with cell surface markers against CD3, CD20, CD16, CD159a, CD35, CD37, and NKp60. Data was evaluated individually and in combination with other markers to determine the strategies that would most easily distinguish and capture the complete NK cell population. These strategies were compared and discussed for feasibility and risk management.
In-life assessment of the immune system is often restricted to blood measurements. Administration of test item via the inhalated route or when the lung is targeted can mean any changes in blood are secondary and sampling from BAL fluid is more appropriate. Sampling BAL fluid is often performed at necropsy and sampling during the study requires additional animals. This study’s objectives were to determine the feasibility of repeat-in-life BAL in the cynomolgus monkey and perform immunophenotyping, by flow cytometry, from the isolated cells. BAL sampling was performed in-life on three, anaesthetised, cynomolgus monkeys at weekly intervals on 3 occasions. BAL samples were also taken at necropsy to provide comparative data. Results from in-life samples were consistent on all 3 occasions. The largest population of cells were CD14+/MHC class II+ (20-55%) followed by CD3+ T cells (18-30%). CD3/CD4+ and CD3/CD8+ T cells were typically ~15% of total cells, whilst MHC class II+/CD14- and MHC class II-/CD14+ cells each constituted 2 to 7.5%. At necropsy, a marked mean decrease in the proportion of CD3+ T cells (~54%) was observed, which was due mainly to the loss of double negative T cells only as no change in CD3+/4- and CD3+/8- proportions was observed. Proportions of MHC class II+/CD14- and MHC class II-/CD14+ cells, at necropsy, were similar to those observed in-life but a marked increase (>5-fold) was noted in CD14+/MHC class II+ cells. Repeated-in-life sampling was generally well-tolerated in all animals. In conclusion, in-life sampling of BAL fluid in monkey provides a method for quantifying immune cells of the lungs, however the differences observed between samples taken in-life and at necropsy warrant further investigation as they may have implications for interpretation of suspected treatment-related changes to the immune system.

Lymphocyte subset analysis is commonly included in immunotoxicity rodent studies, but there is lack of data in the dog. In the first part of this study, analysis of blood lymphocyte subpopulations with a Navios flow cytometer was validated for lack of non-specific binding, linearity and precision using PE-LSM 11.425 antibody for body for B cells, APC-LSM 8.358 antibody for total T cells, PE-LSM 12.145 antibody for CD8. Lymphocyte subsets were quantified in 37 and 48 blood samples taken from male and female control Beagle dogs, respectively. In the second part of this study, groups of 3 male and 3 female Beagle dogs were treated orally with either 2 mg/kg cyclophosphamide on 4 days each week or 25 mg/kg cyclophosphamide or the same volume of drinking water daily, for 4 weeks starting on day 1. Lymphocyte subsets were analyzed 10 days before the start of treatment, and then on days +11, +18 and +28 with the previously validated method, and in addition with PE-VIX030.9 antibody for CD4, Alexafluor 647-YCATE 55.9 antibody for CD8 and FITC-CA.17.2A12 antibody for CD3. Values in control beagles obtained with the validated assays were 0.57 ± 0.17 G/L, 1.86 ± 0.43 G/L, 1.19 ± 0.33 G/L, and 0.39 ± 0.15 G/L for total T, T helper and T cytotoxic lymphocytes, respectively in males, and 0.55 ± 0.20 G/L, 1.99 ± 0.47 G/L, 3.31 ± 0.36 G/L, 0.41 ± 0.10 G/L in females. Bland–Altman correlation plot showed that both markers gave identical results for T helper cell counts, while labeling with AFE647-YCATE 55.9 antibody resulted in a constant mean bias of +0.3 G/L compared to the FITC-LSM 1.140 antibody for T cytotoxic cell counts. Inter-day variation in control dogs was low over the 40-day observation period. No effects of cyclophosphamide on any lymphocyte subset were observed. Cyclophosphamide markedly decreased lymphocyte counts from days +11, correlating with decreases in T helper and B lymphocytes.

Adaptive immunity is a critical component of host defense, protecting organisms against invading pathogens. The adaptive immune repertoire consists of naïve, effector and memory lymphocytes that drive specific and directed attacks against these pathogens. T lymphocytes, in particular, are central for potentiating these immune responses and understanding how their activity can be influenced by pharmacology is an increasingly important component of drug safety evaluation. We have developed a high-throughput method to characterize T cell subsets in human and non-human primate whole blood, as well as assess activation, through intracellular cytokine staining (ICS). Memory and naive T lymphocytes in both species were characterized by CD3, CD4, CD8, CCR7, CD45RA, CD95 and CD28 expression in a designed cross-species flow cytometric antibody staining panel. Peripheral human and non-human primate whole blood culture and subsequent ICS for IFN-gamma, IL-2 and TNF-alpha was also investigated. T lymphocytes were activated in a polyclonal fashion with phorbol 12-myristate 13-acetate and ionomycin in an antigen-specific manner, following restimulation with vaccine antigens. Our data detail the qualitative similarities between human and non-human primate T cell subsets and cytokine profiles following stimulation in whole blood. Non-human primate multi-parametric T cell staining analysis can be used to evaluate ex vivo drug immunotoxicities for compounds that are immuno-modulators or immune-depleting agents which may influence T cell subsets and cytokine expression. Further, this technique is useful for the assessment of in vitro drug toxicity and evaluation of clinical biomarkers. The versatility of the multi-parametric flow cytometry platform makes it an important tool in both pre-clinical and clinical safety assessments of immuno-modulatory agents and investigations of any potential influence on the adaptive immune system.
1598 VALIDATION OF A T-DEPENDENT ANTIBODY RESPONSE TO KLH IN CYMONOLGUS MACAQUE.

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We have utilized the keyhole limpet hemocyanin (KLH) antigen to generate a T lymphocyte dependent antibody response (TDAR) in cynomolgus macaque. Methods were developed and validated for measurement of IgG and IgM antibody responses to KLH in cynomolgus sera by modification of commercial kits using a sandwich enzyme-linked immunosorbent assay (ELISA). Validation tests performed included inter-assay and intra-assay precision and accuracy with low, mid, and high quality control (QC) samples. The range of quantification was determined to be 3.13 to 150 Units/mL for IgG and IgM in cynomolgus serum. Dilutional linearity was determined to be within 20% from 200 to 76,887 fold for IgG and 1,000 to 32,000 for IgM. Stability was determined utilizing tests for 4 freeze thaw cycles and temperatures at ambient, 2-8°C, -20°C, and -80°C. The method is semi-quantitative with results indicative of inverse titer values. In order to assess cynomolagus-derived antibodies to KLH, two animals were administered a single 10 mg dose of KLH without adjuvant subcutaneously between the shoulder blades. A primary antibody response for IgM peaked at Day 7 and fell at Day 21 (48 and 36-fold above background, respectively), whereas IgG responses were detectable at Day 7 and continued to rise by Day 21 (148 and 225-fold above background, respectively). Six additional animals were treated with a sub-optimal dose of 0.75 mg/kg/day for 21 days with FK506 via oral gavage to assess detection of immunosuppression. A primary and secondary boost with KLH occurred on Days 8 and 15, respectively, and antibody responses were lowered but still detectable in dosed animals at multiple timepoints following KLH immunization. In summary, we have validated an assay to detect TDAR responses in Cynomolgus macaque and verified its performance with and without immunosuppressive agents in vivo.

1599 CANDIDA ALBICANS DELAYED-TYPE HYPERSENSITIVITY MODEL IN JUVENILE RATS: EVALUATION OF IMMUNOSUPPRESSION BY PHARMACEUTICAL DRUGS.

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The delayed-type hypersensitivity (DTH) response is used widely to assess immunosuppression by both environmental chemicals and pharmaceuticals. DTH responses (type IV hypersensitivity reactions) are driven by antigen-specific T-cells and do not involve the production of antigen-specific antibodies. However, a number of antigens frequently used to elicit a DTH response (e.g., keyhole limpet hemocyanin) are also used to evaluate humoral immunity. Therefore, even in optimized systems which use these antigens, antibody production may confound the DTH response. Recently a DTH model was established in mouse which used Candida albicans as the sensitizing agent, with subsequent challenge using chitosan. This model produces a robust DTH response and is devoid of antigen-specific antibody production. We evaluated this model for use in juvenile rats as a means to assess the developing immune system for immunosuppression with pharmaceutical drug parameters. Parameters optimized in this DTH footpad swelling assay included the number of Candida organisms used for sensitization, time between sensitization and challenge (challenge interval), and quantity of chitosan used for challenge. The optimized protocol established 2x10^7 formalin-fixed Candida organisms for sensitization, with a peak challenge interval of 10 days. The model was validated in juvenile rats dosed with known immunosuppressive compounds, including dexamethasone (0.003-3.0 mg/kg/day), cyclosporine (1-30 mg/kg/day), and cyclophosphamide (5-30 mg/kg/day).Rats were dosed from PND 23-37, with sensitization on PND 28. Animals were challenged with chitosan by footpad injection on PND 38 and footpad swelling determined 24 and 48 hours later. Juvenile rats generated a robust DTH response which was suppressed in a dose-dependent manner by each of the compounds tested. We believe that this DTH model is a valuable addition to the toolbox for developmental immunotoxicity testing for pharmaceutical drugs, and fills a critical gap identified in recent workshops.

1600 GLUTATHIONE S-TRANSFERASE OMEGA 1 (A140D) POLYMORPHISM IN A TURKISH POPULATION: IS THERE ANY ASSOCIATION WITH SUSCEPTIBILITY TO LUNG CANCER?

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In recent years there is a growing evidence that ethnic differences exist in the frequency of glutathione S-transferase Omega 1 (GSTO1) (A140D) gene polymorphism with which associations have also been reported for various cancers such as breast and liver. However, no data exists concerning lung cancer in this respect. In this study, GST O1 (A140D) gene polymorphism was determined among 214 unrelated healthy individuals of a Turkish population. In addition, 172 non-small cell lung cancer (NSCLC) patients were studied in order find out whether this gene polymorphism is associated with NSCLC susceptibility in a Turkish population. The frequency of GSTO1 (A140D) gene polymorphism was determined by using the polymerase chain reaction-restriction fragment length polymorphism (PCR/RFLP) method. In 214 healthy individuals, the observed frequencies of A140A (wild type), A140D (heterozygous variant) and D140D (homozygous mutant) GSTO1 (A140D) genotypes were % 48.60, % 40.65 and % 10.75 respectively. No significant sex differences were noted in the genotype frequencies. The allele frequencies of GSTO1 (A140D) polymorphism were 0.689 for A140 and 0.311 for D140. The statistical analysis showed no difference in genotypic distribution between overall NSCLC (OR 1.04, CI 95 %: 0.65-1.66, p=0.87) and control or subtypes of NSCLC (adenocarcinoma, OR 1.45, CI 95 %: 0.71-2.96, p=0.307; squamous cell carcinoma, OR 0.87, CI 95 %, 0.47-1.61, P=660) and control. These results show that the frequencies of GST (A140D) gene polymorphism in a Turkish population is similar to Caucasian populations and that this polymorphism is not associated with susceptibility to NSCLC in a Turkish population (Supported by the Research Fund of Ankara University, Grant, 2008-08-03-006HPD).

1601 CATALASE CAN PROTECT CELLS AGAINST THE GENOTOXIC EFFECTS OF MONOMETHYLARSONIC ACID.


Although it is widely known that arsenic-contaminated drinking water causes cancer and other health effects, its exact mode of action (MOA) is not fully understood. Induction of oxidative stress has been proposed as a key event in the MOA of arsenic. Our studies are centered on identifying a reactive oxygen species involved in the genotoxicity of arsenic using a catalase (Cat) knock-out mouse model, which is impaired in its ability to break down hydrogen peroxide (H2O2) that leads to an increase in hydroxyl radicals (OH). We assessed the induction of DNA damage using the comet assay following exposure to monomethylarsonic acid (MMAIII) of Cat+/- and Cat-/- primary lymphocytes to identify the potential role of H2O2 in mediating cellular effects of this metalloid. Our results showed that the Cat-/- lymphocytes are more susceptible to DNA damage following exposure to MMAIII than the Cat+/- lymphocytes by a small (1.49-fold), but statistically significant difference. Cat activity assays demonstrated that liver tissue has ~3 times more Cat activity than lymphocytes. Therefore, comet assays were performed on primary Cat+/-, Cat+/-, and Cat-/- hepatocytes to determine if the Cat-/- hepatocytes were more susceptible to MMAIII than lymphocytes. Results with the comet assay showed that the Cat-/- hepatocytes exhibit higher levels of DNA strand breakage than did Cat+/- and Cat-/- hepatocytes exposed to MMAIII. Our experiments suggest that Cat is involved in protecting cells against MMAIII-generated H2O2, and that OH might be involved in its genotoxicity path. Furthermore, individuals afflicted with genetic polymorphisms in their Cat gene could exhibit altered susceptibility to arsenic toxicity. [This is an abstract or proposed presentation and does not necessarily reflect EPA policy.]

1602 NQO1*2 POLYMORPHISM MODIFIES THE DNA DAMAGE ENVIRONMENT IN MEXICAN CHILDREN EXPOSED TO BENZENE.

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Benzene is an air urban pollutant considered a potent carcinogen, mainly to children, one of the most vulnerable populations. It is deactivated by NAPD(H)-quinone oxidoreductase (NQO1), among other enzymes. NQO1*2(C/T) variant
is considered a null-polymorphism; therefore it is a factor in the susceptibility to benzene toxicity. We evaluated the role of gene-environment interaction on DNA damage in children from Ecatepec, Mexico State, Mexico. This county is one of the most industrialized, populated and polluted in Mexico. A cross-sectional study was conducted in 190 children (7-10 years old) from 3 elementary schools. Blood and urine samples were obtained, a medical examination was done and parents answered a structure questionnaire. The metabolite t,μ-mucronic acid (t,μ-MA) was quantified by reverse phase HPLC as a benzene exposure indicator, DNA damage in mononuclear cells was determined by the Comet assay, DNA oxidation by 8-OHdG levels, and NQO1*2 variant by RT-q-PCR. Fifteen percent of children had t,μ-MA levels higher than the limit for workers (500 ng/g Cr), and we observed genetic and oxidative damage in mononuclear cells. After stratifying benzene exposure in tertiles, TT children in the second tertile had significantly more DNA damage (OTM values) than the reference group (first tertile and wild-type), while CT or TT children had about 2-fold higher (p<0.05) 8-OHdG levels than the reference group. This indicates that children from Ecatepec County are exposed to high benzene concentrations and that NQO1*2 polymorphism modulates DNA damage caused by benzene exposure in children (Supported by CONACYT-México, Grant #106034).

**1603 FUNCTIONAL CHARACTERIZATION OF ALLELIC VARIANTS OF HUMAN CYTOCHROME P450 2A6**


Cytochrome P450 2A6 (CYP2A6) catalyzes important metabolic reactions of many xenobiotic compounds including coumarin, nicotine, cotinine, and clinical drugs. Genetic polymorphism of CYP2A6 can influence on the metabolic activities of xenobiotic compounds including coumarin, nicotine, cotinine, and clinical drugs. In this study, we have analyzed the functional activities of six CYP2A6 alleles (CYP2A6*5, *7, *8, *18, *19, and *35) containing nonsynonymous SNPs, which were found in Asian populations with high frequencies. Recombinant variant enzymes of CYP2A6*5, *7, *18, *19, and *35 were successfully expressed in Escherichia coli and purified. However, no P450 holoenzyme spectrum was detected for CYP2A6*5 allele variant (G479V). Structural analysis showed that G479V mutation may make the interacting site of both A helix and C helix regions. Enzyme kinetic analyses indicated that the effects of mutations in CYP2A6 allele variants on drug metabolism are very dependent on substrates. Functional characterization of these allelic variants of CYP2A6 can be expected to define the importance of CYP2A6 polymorphism in the metabolism of many clinical drugs.

**1604 GENE-ENVIRONMENT INTERACTIONS ON POLYCYCLIC AROMATIC HYDROCARBONS (PAH) METABOLISM AND RELATED DNA DAMAGE IN CHILDREN.**

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Polycyclic aromatic hydrocarbons (PAHs) are carcinogenic pollutants present in urban cities and children are most vulnerable to their toxicity. Enzymes involved in PAH metabolism (activation/deactivation) are CYP1A1, CYP1B1, GSTM and GSTT are polymorphic, which may explain the interindividual differences in PAH excretion and toxicity. PAH metabolism determines their toxicity; therefore we evaluated the modulation of genetic polymorphisms of some enzymes on PAH excretion and genotoxicity in children living in the vicinity of a petrochemical complex area. Children 6-10 years old attending school located near the industrial area were included. Urinary 1-hydroxypyrene (1-OPH, as biomarker of PAH exposure) was determined by reverse-phase-HPLC, DNA damage in blood through the Comet assay (OTM parameter), CYP1A1*2C and CYP1B1*3 polymorphisms by real time-PCR while GSTM1*0 and GSTT1*0 by multiplex PCR. Median 1-OPH value was 0.37 ng/ml in children and children with higher genotoxicity than this value had increased DNA damage. Significantly higher 1-OPH concentration was observed in homozogous children to CYP1A1*2C or CYP1B1*3, as well as in children carrying the combination of CYP1A1*2C or CYP1B1*3 with GSTM1*0 or GSTT1*0 polymorphisms compared to corresponding wild-type individuals. In addition, we found a positive interaction (p<0.05) between CYP1A1*2C polymorphism and OTM values in heterozygous children with the highest PAH exposure. Our data indicated that children living in the surroundings of the petrochemical area have DNA damage due to high PAH exposure and that CYP1A1*2C polymorphism may modulate PAH metabolism and toxicity, suggesting an increased health risk for children exposed to high PAH environmental levels. (Supported by CONACYT-México Grant #87234).

**1605 RISK HAPLOTYPES FOR PAH METABOLISM IN A MEXICAN POPULATION AND THEIR ASSOCIATION WITH DNA ADDUCT LEVELS IN LEUKOCYTES.**

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We investigated 13 single nucleotide polymorphisms (SNP) in genes related to PAH metabolism (AhR, CYP1A1*2C, CYP1A1*2A, CYP1A1*4, mEH3, mEH4, GSTM1, GST1T1 and GSTP1). DNA repair (XRCC1, ERCC2/XP and MGMT) and cell cycle (p53) and the PAH-DNA adducts levels in blood leukocytes from 101 healthy volunteers: 50 smokers and 51 non-smokers living in the Mexico City. DNA was obtained from the buffy coat of blood samples using a standard phenol-chloroform extraction. Genotyping was determined according to the different protocols described by Perez Morales et al. (2011; in press). 3P-Postlabeling analysis was performed according to a standardized procedure as previously described (Phillips, 2007). Multivariate analyses were performed using the logistic regression method. All the statistical analyses were run in STATA SE.10. The mean ± SD Ln levels of adducts measured in white blood cells DNA were 2.09±0.45 adducts/108 nt in smokers and 2.04±0.41 adducts/108 nt in non-smokers. A multiple regression analysis model was generated using the allelic variants associated with higher risk for adduct formation among smokers and non-smokers. For the haplotype: AhR arg/arg, GSTM1*0, MGMT #4 phe/phe, XRCC1 arg/gln, and ERCC2 lys/gln with interaction GSTM1*0, MGMT #4 phe/phe, we found a significant association between this haplotype and the formation of DNA adducts among non-smokers (R2 =0.1821, p=0.0363) and smokers (R2 =0.2202, p=0.0136), the strongest association was observed with the alleles GSTM1*0 and the repair enzyme MGMT#4 phe/phe. The presence of both variants conferred a greater risk for higher levels of adducts in this Mexican population. Partially supported by CONACYT46451-M.

**1606 NEW STATISTICAL METHODS USED FOR LYMPHBLASTOID CELL LINES DRUG RESPONSE SHOWS PROMISE IN PHARMACOGENOMICS GENE DISCOVERY.**

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Cytotoxicity assays of immortalized lymphoblastoid cell lines (LCLs) represent a promising new approach in pharmacogenomics discovery research. Previous studies employing LCLs in gene mapping have used simple statistical methods, which may not adequately capture the true differences in nonlinear response profiles between genotypes. The current study investigates two commonly used association methods, as well as four novel methods, and compares their power to detect differences between the response profiles of genotypes under a variety of alternatives. The most powerful method was found to depend not only on the choice of alternative, but also on the choice for the dosages used. A new method, based on an analysis of variance (ANOVA) design, was found to be the most robust for power in detecting differences between genotypes. The ANOVA method was applied to a genome-wide association study (GWAS) of 520 LCLs of Caucasian descent obtained from the Children’s Hospital Oakland Research Institute (CHORI). Cells from each line were exposed to four replicates at each of six concentrations of the drug Temozolomide. Viability measurements were applied to a seven-stage quality control process and were used as the response for association analysis. Significance was assessed using a sequential sampling permutation procedure. A number of SNPs within the gene for coding for the enzyme methylated-DNA-protein-cysteine methyltransferase (MGMT) showed significant association (P<10e-8) with response. Methylation of the promoter region of MGMT has shown increased efficacy in combination with Temozolomide. This result is a proof of concept that LCLs can be used to identify genes that are valuable in the prediction of clinical therapeutic effectiveness.
1607 GENETIC VARIANTS IN HLA GENES ARE ASSOCIATED WITH DISOCYANATE-INDUCED ASTHMA IN EXPOSED WORKERS.

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Disocyanates, low-molecular weight reactive chemicals extensively used in a variety of industrial processes, are one of the most common causes of occupational asthma. Both immunological and inflammatory mechanisms have been implicated in the development of disocyanate-induced asthma (DA). A case-control study was conducted to investigate whether genetic variations in genes located within the major histocompatibility complex may play a role in susceptibility to DA using a high density SNP map. The study population consisted of 140 workers exposed to disocyanates (hexamethylene diisocyanate (HDI), methylene diphenyl diisocyanate, and toluene diisocyanate) of which 73 were diagnosed with DA based on a positive specific inhalation challenge and 67 were asymptomatic workers exposed to HDI. Genotype analysis was performed on genomic DNA, using Illumina GoldenGate Genotyping technology. The microarray platform consisted of 2,360 loci with an average spacing of 2 kb. After adjusting for potential confounders, single nucleotide polymorphisms in HLA-E (rs1573294), HLA-B (rs1811157), HLA-DOA showed association with DA. After adjusting for potential confounders, single nucleotide polymorphisms in HLA-DQA2 (rs7773955) and HLA-DPB1 (rs928976) showed association with DA. The most strongly associated polymorphism was that at HLA-DQA2 (rs7773955) with a p-value of 2.1x10^-8. This association remained significant after correcting for multiple testing. The study suggests that genetic variations in HLA genes may be associated with susceptibility to disocyanate-induced asthma.

1608 IS WHOLE GENOME AMPLIFIED (WGA) DNA SUITABLE FOR ACCURATE GENOTYPING?

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Advances in genomic technologies have created novel molecular genetic methods with applications in the understanding, diagnosis, and management of genetic diseases and cancer. One of the challenges is acquiring sufficient DNA from clinical samples for analysis. WGA methods have been developed to overcome this problem. The most commonly used WGA approach is the multiple displacement amplification (MDA) method because of its high processivity and low error rate. However, the uniformity of amplification across the genome has not been well-characterized. Here, we compared two MDA kits: GenomiPhi (GE Healthcare) and Repli-G (Qiagen) using array-base comparative genomic hybridization (CGH) to evaluate DNA copy number variations (CNVs) in amplified DNA. Amplified and unamplified DNA samples from a normal individual and two patients with cystic fibrosis were evaluated by Agilent Human 1 million feature CGH arrays. Analyses of Komarov distances and Phi correlations showed high consistency within each amplified sample pool. Both Repli-G and GenomiPhi generated very similar amplified DNA samples. Less than 2% of the genome showed more than 2-fold CNV after amplification. The majority of the CNVs were under-amplified regions located in the telomeric regions. No CNVs or mutations were detected in the CFTR gene region due to WGA. This was confirmed by quantitative PCR copy number assays at 10 locations within the CFTR gene and sequencing of a 2-kb region within the CFTR gene. These results indicate that WGA DNA is generally suitable for accurate genotyping. However, because there are consistent differences between the WGA DNA and the native DNA, characterization of the genomic region of interest would be necessary to ensure the reliability of genotyping results from WGA DNA.

1609 POLYMORPHISMS IN DNA METABOLIZING AND REPAIR GENES GSTM1, GSTT1, GSTP1, AND OGG1 AND TYPE 2 DIABETES MELLITUS RISK: A CASE-CONTROL STUDY IN A TURKISH POPULATION.

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We investigated impact of polymorphisms in GSTs (GSTM1, GSTT1 and GSTP1) which are very important protective mechanism against oxidative stress and in OGG1 gene which has important role in DNA repair, to risk of Type 2 Diabetes Mellitus (T2DM) with obesity and hypertension in our study. We examined 127 T2DM and 127 control subjects. DNA was extracted from whole blood. Analysis of GSTM1 and GSTT1 gene polymorphisms was performed by allele specific PCR and GSTP1 Ile105Val and OGG1 Ser326Cys by PCR-RFLP. Our data showed that GSTM1 null genotype frequency had 2.6 times statistically significant increase in patient group (OR 3.841 [95% CI 2.288-6.469], p<0.001) but not observed any significant difference between GSTT1 null and GSTP1 Ile105Val genotypes. When T2DM patients with OGG1 Ser326Cys polymorphism was compared with patients with wild genotype, statistically 2-3 times increase has been observed (OR 1.858 [95% CI 1.099-3.141], p<0.021). The joint effect of GSTM1 null and OGG1 variant genotype frequencies have shown statistically significant. Similarly, the risk of T2DM was statistically increased with GSTM1 null (OR 3.841 [95% CI 2.288-6.469]), GSTT1 null-GSTP1 (H+M) (OR 4.118 [95% CI 1.327-12.778]) and GSTM1 null-OGG1 Ser326Cys (H+M) (OR 3.322 [95% CI 1.898-5.816]) and GSTT1 null-OGG1 Ser326Cys (H+M) (OR 2.179 [95% CI 1.083-4.386]) compared to control. However, when patients with T2 diabetic hypertension and obesity are compared with the control group, no significance relationship was observed. According to our study results, it has been seen that combination of especially GSTM1-GSTT1-GSTP1 and OGG1 Ser326Cys gene polymorphisms can be used as candidate gene in etiology of T2DM, especially in development of T2DM.

1610 EVALUATION OF THELIN USING A MOUSE DIVERSITY PANEL DEMONSTRATES GENETIC DIFFERENCES IN LIVER RESPONSE.

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Sitaxsentan sodium (Thelin) is an endothelin receptor antagonist developed for the treatment of pulmonary arterial hypertension. In clinical studies, Thelin was associated with liver injury. Previous studies have shown that genetically diverse inbred mouse strains comprising a mouse diversity panel (MDP) have utility for identifying genetic markers associated with drug toxicity. In this study, female mice from 34 inbred strains were treated orally by gavage once daily for 7 consecutive days with 300 mg/kg Thelin or vehicle (water; N=4/strain). At necropsy, a significant increase in the liver to body weight ratio in all strains treated with Thelin was observed (two-way ANOVA, p<0.05), ranging from a 28.7% increase in KK/HIJ mice to a 154% increase in P/J mice. Preliminary clinical chemistry analysis revealed large inter-strain variations in serum cholesterol levels ranging from a 19% decrease in Mrl/MpJ mice to an 86% increase in C57BLKS/J mice in Thelin versus vehicle-treated groups. Plasma concentration of Thelin was determined 2 hours after the last dose administered, and despite large variations in concentration observed across strains, there was no statistical correlation between Thelin plasma concentration and the changes in liver to body weight ratio or cholesterol levels (Spearman correlation, p=0.05). Taken together, these data suggest that genetically diverse mouse strains differ in hepatic response to Thelin, and this response is not attributable to variable drug exposure in these strains. Thus, the MDP can be used to investigate the biochemical pathways and genetic markers associated with the variable hepatic response.

1611 P-GLYCOPROTEIN TRANSPORT OF PESTICIDES ASSOCIATED WITH PARKINSON’S DISEASE.

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P-glycoprotein (P-gp), encoded by the ABCB1 gene, is an efflux transporter expressed in many tissues important in xenobiotic disposition. P-gp is highly expressed at the blood-brain-barrier (BBB) and protects the brain from substances circulating in the blood; as a result P-gp substrates do not accumulate in the brain.
1612 DIFFERENT SENSITIVITY OF CARBOXYLESTERASE 1 GENETIC VARIANTS TO IRREVERSIBLE INHIBITION BY OXON METABOLITES OF ORGANOPHOSPHATE PESTICIDES.

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The carboxylesterases (CES) are a class of phase 1 enzyme important for lipid metabolism and in the biotransformation of numerous xenobiotics. Like the acetylcholinesterase, CES are irreversibly inhibited by covalent binding of the active oxon metabolites of organophosphate pesticides (OP). Although deleterious to the metabolic function, this steric inhibition provides a buffer against the neurotoxic effects of OPs. The CES are polymorphic and the goal of the present study was to investigate the effect of a non-synonymous polymorphism on the human CES1 gene on the sensitivity to inhibition by oxons. HEK293 cells transfected with an empty vector, the wild-type (WT) CES1 or the CES1 variant p.Gly143Glu were cultured in DMEM with 10% FBS, 2mM L-glutamine and antibiotics. At 95% confluence, cells were harvested, homogenized and centrifuged to obtain S9 fractions. CES activity was determined by measuring the rate of formation of the colorimetric hydrolysis product of the prototypical substrate p-nitrophenyl vatepectrophotometrically at 405nm during a 5 minute period. The IC50 were determined for the WT and SB forms using 0-1000nM of the following OP o-analogs: diazoxon, chlorpyrifos-oxon, chlorpyrifos methyl-oxon, paraoxon, methyl paraoxon and omethoate. There were no statistically significant difference in affinity of chlorpyrifos methyl-oxon or chlorpyrifos oxon for the the WT or variant CES1. Omeothate did not inhibit the WT or variant CES1. However, higher concentrations of paraoxon, methyl paraoxon, and diazoxon were necessary to achieve 50% inhibition of the activity of the variant CES1 relative to the WT enzyme (p<0.05). The current results suggest that this substitution lowers the affinity of the enzyme for some of the oxon metabolites of organophosphate pesticides which may lower the neuroprotective potential of the enzyme. Thus, individuals bearing the p.Gly143Glu variant of the carboxylesterase 1 gene may be more susceptible to OP toxicity.

1613 EFFECT OF CYP2B6 VARIANTS ON CHLORPYRIFOS METABOLISM: IMPLICATIONS FOR HUMAN RISK.

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Chlorpyrifos (CPF), a widely used organophosphorus (OP) pesticide must be bioactivated by cytochrome P450s (CYPs) to the active chloropyrifos oxon (CPF-O). CYP2B6 has the lowest reported Km and highest Vmax for bioactivation of CPF. CYP2B6 is a polymorphic enzyme and variants of this enzyme may impact human susceptibility to this OP. In this study, CYP2B6*1, *4, *5, *6, *7, and *18 were over-expressed in mammalian COS-1 cells to assess the impact of CYP2B6 variants on the Km and Vmax for bioactivation of CPF. Cell lysates were incubated with CPF (0-100uM) and the production of CPF-O was measured via HPLC analysis. Analysis was performed in at least triplicate. CYP2B6 content was determined by western blot. Previous studies had no activity and CYP2B6 protein could not be detected. The Km value for each of the four other variants was neither significantly different from wild-type CYP2B6 nor from each other while the Vmax value for each variant was significantly higher than wild-type. The Kms for CPF-O formation by variants and wild-type CYP2B6 ranged from 0.30-1.97uM while Vmax values exhibited more variability with genotype (4.13x103 – 4.52x105pmol/min/mmol CYP2B6). In addition, human liver microsomes (N=22) genotyped for CYP2B6*1 and CYP2B6*6 were assayed for ability to metabolize CPF at 10uM and at 0.5uM, concentrations straddling the predicted Km. *6 specimens had both reduced protein expression and CPF-O metabolite formation. Together, these data support the conclusion that variants of CYP2B6 may have altered capacity to metabolize CPF and affect individual susceptibility by altering hepatic expression of CYP2B6 protein and/or Vmax for CPF-O formation. In addition, the kinetic parameters generated here may be used to assess the impact of CYP2B6 genotype on current human risk assessment efforts for CPF which currently primarily rely on rat kinetic data and may under represent human variability. (NIH R01 ES016308 and EPA STAR grant R833454).

1614 HUMAN ALDH1B1 POLYMORPHISMS MAY AFFECT THE METABOLISM OF NITROGLYCERIN AND ALL-TRANS RETINALDEHYDE—IN VITRO STUDIES AND MOLECULAR MODELING.

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A substrate profile for ALDH1B1 determined previously, that includes acetaldelyde and other short-chain aldehydes, but not lipid peroxidation products. There is evidence that additional substrates for ALDH1B1 may include nitroglycerin and retinaldehyde. Humans that are deficient in ALDH2, a key enzyme in nitroglycerin metabolism, still retain as much as 30% efficacy for reduction of nitroglycerin. In addition, ALDH1B1 may be implicated in stem cell biology raising the possibility that this protein may mediate retinaldehyde signaling. ALDH1B1 polymorphisms have been reported. Based on epidemiological studies, one of them (ALDH1B1*2) is associated with ethanol avoidance, increased systolic blood pressure, and ethanol hypersensitivity reactions. In this study we have investigated the role of ALDH1B1 in nitroglycerin metabolism and have determined the kinetic parameters of ALDH1B1 for all-trans retinaldehyde using human recombinant ALDH1B1. Our results indicate that ALDH1B1 metabolizes and appears to be inhibited by nitroglycerin, but has favorable kinetics for all-trans retinaldehyde. Using computationally-based molecular modeling, we have examined structural differences among ALDH1B1 variants that may rationalize differences in ALDH1B1-mediated metabolism of acetaldelyde, nitroglycerin, and all-trans retinaldehyde based on poor binding profiles. In conclusion, ALDH1B1 metabolizes nitroglycerin and all-trans-retinaldehyde and the reported polymorphisms of ALDH1B1 may affect stem cells and ethanol metabolism. This work was supported by NIH grants R01 AA 017754 and F31 AA 20728.

1615 A MOUSE DIVERSITY PANEL APPROACH PREDICTS DB289-RELATED RENAL TOXICITY.

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Pafuramidine maleate (DB289) is an oral diamidine prodrug developed to treat first stage human African trypanosomiasis (sleeping sickness). Phase 1-3 clinical studies at worldwide sites demonstrated reduced toxicity, resulting in the recommendation of first stage human African trypanosomiasis (sleeping sickness). Phase 1-3 clinical studies at worldwide sites demonstrated reduced toxicity, resulting in the recommendation of Phase 1-3 clinical studies at worldwide sites. In this study, we determined that this protein may mediate retinaldehyde signaling. ALDH1B1 polymorphisms have been reported. Based on epidemiological studies, one of them (ALDH1B1*2) is associated with ethanol avoidance, increased systolic blood pressure, and ethanol hypersensitivity reactions. In this study we have investigated the role of ALDH1B1 in nitroglycerin metabolism and have determined the kinetic parameters of ALDH1B1 for all-trans retinaldehyde using human recombinant ALDH1B1. Our results indicate that ALDH1B1 metabolizes and appears to be inhibited by nitroglycerin, but has favorable kinetics for all-trans retinaldehyde. Using computationally-based molecular modeling, we have examined structural differences among ALDH1B1 variants that may rationalize differences in ALDH1B1-mediated metabolism of acetaldelyde, nitroglycerin, and all-trans retinaldehyde based on poor binding profiles. In conclusion, ALDH1B1 metabolizes nitroglycerin and all-trans-retinaldehyde and the reported polymorphisms of ALDH1B1 may affect stem cells and ethanol metabolism. This work was supported by NIH grants R01 AA 017754 and F31 AA 20728.

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Nuclear translocation of PPAR-alpha was not modulated by PFOS treatment.

In vivo PFOS exposure resulted in significant increases in IL-6 production from CH12.LX cells as compared to unchallenged mice. Although the mode of action on IgM suppression is still unclear, these new data shed additional light on immunological effects of PFOS.

**1616 METABOLOMIC PROFILING OF THE MOUSE MODEL OF HUMAN POPULATION (MMHP) IDENTIFIES CYP46A1 AS A REGULATOR OF BILE ACID SYNTHESIS.**

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The mouse model of human population (MMHP), a collection of 35 inbred strains with a genetic and phenotypic diversity that approximates that of the human population, has been increasingly recognized as a valuable model system for studying drug safety. Yet much is unknown about the underlying genomic and metabolic mechanisms that drive the differential drug responses seen in these mice. In this study, we conducted metabolomic profiling for 210 known endogenous metabolites in the serum of MMHP. A total of 40 unique metabolites were identified with a 2-fold change in at least 30% of the strains tested. The bile acid homeostasis and purine metabolism pathways showed particular enrichment for highly differentiated metabolites. Focusing on the bile acid variation, a genome-wide association study was performed to identify candidate genomic loci associated with levels of primary bile acids [taurocholic acid (TCA), glycocholate (GCA), glycochensodeoxycholic acid (GCDCA), and taurochenodeoxycholic acid (TCDDA)]. A candidate genomic locus was identified on Chr 12 in a region spanning 8 genes; the region was found to contain a polymorphism that disrupted the start codon of Cyp46a1, a member of the bile acid synthesis pathway. To confirm functional involvement of Cyp46a1 in bile acid homeostasis in vivo, we compared bile acid levels in wild-type and Cyp46a1 knockout mice. In the null mice, serum levels of GCA and TCA were found to be significantly increased by ~30% compared to wild-type mice. In contrast, hepatic levels of TCA, and other bile acids including TCDCA, taumuricholate, and muricholate, were significantly decreased by 40-80% in the knockout mice. This finding confirmed functional involvement of Cyp46a1 in bile acid homeostasis. This study provides baseline values for serum metabolites in the MMHP that will aid biomarker discovery and validation of the MMHP as a tool for identifying genetic loci associated with phenotypic variations.

**1617 EVALUATION OF POSSIBLE MODES OF ACTION FOR PFOS-INDUCED HUMORAL IMMUNOSUPPRESSION.**

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Perfluorinated hydrocarbons have been manufactured for over 40 years and have numerous applications in industry. This group of compounds has recently attracted much interest as some of these compounds (i.e., perfluorocarboxylic acid) are persistent in the environment and are detectable in blood samples of both wildlife and humans. Studies show that these perfluorinated compounds cause various toxicological effects; however, effects on immune function have not been addressed at length. We have previously shown decreased SRBC-specific IgM production in adult B6C3F1 mice following exposure to PFOS. The current study assessed possible modes of action for this suppression and an expanded survey of potential effects from exposure. Adult female B6C3F1 mice were exposed orally to PFOS for 28 days. Numbers of follicular T-cells (Tfh), cells were not significantly suppressed following in vitro stimulation with soluble CD154 in mice that were challenged with SRBC as compared to unchallenged mice. In vivo PFOS exposure resulted in significant increases in ex vivo basal IL-1 production in peritoneal macrophages collected from SRBC-challenged mice. Ex vivo production of IL-2 by CD4+ cells stimulated with both anti-CD3 and anti-CD28 was not altered. Nuclear translocation of PPAR-alpha was not modulated by PFOS treatment. Genomic analysis of SRBC challenged mice revealed no alteration in expression of genes for PPAR-alpha, PPAR-gamma, NF-κB or AP-1, but an increase in CD83, MAPK6 (ERK3), BLNK, and Traf3 expression and a decrease in SLAMF1, Ly9, CD79a, CD79b, and VAV1 expression. Although the mode of action on IgM suppression is still unclear, these new data shed additional light on immunological effects of PFOS.

**1618 H51A/H51, 2 OR H53B/H54 IS SUFFICIENT TO MEDIATE TCDD-INDUCED INHIBITION OF THE 3'Igh IN A TRANSGENIC B-CELL LINE.**

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Immunoglobulin (Ig) gene expression is inhibited by AhR ligands including TCDD in both in vivo and in vitro animal models and human cellular models. In mouse models, IgG inhibition correlates with AhR expression and function. Ig heavy chain (Igh) gene expression involves a complex interaction between several regulatory elements including the 3'Igh regulatory region (3'IghRR), which is typically associated with four enhancers (hs3A, hs1.2; hs3B, hs4). We have demonstrated in a mouse B-cell line CH12.LX that TCDD inhibits LPS activation of luciferase reporter regulated by the 3'IghRR or the hs1.2 enhancer alone. Surprisingly, a luciferase reporter regulated by the hs4 enhancer was synergistically activated by LPS and TCDD. The objective of this study was to determine in the context of chromatin the inhibitory effect of TCDD is mediated through the hs1.2 enhancer. CH12.LX cells were stably transfected with constructs containing an LPS-inducible 2b reporter regulated by the 3'IghRR with LoxP sites flanking either the hs3B/h4 or the hs3A/hs1.2 enhancer pairs. Transfection with CRE-recombinase induced LoxP recombination generating cell lines either expressing 2b reporter regulated by hs3A/hs4 or by hs3B/h4. We have demonstrated in a mouse B-cell line CH12.LX that TCDD inhibits LPS activation of luciferase reporter regulated by the 3'IghRR or the hs1.2 enhancer alone. Surprisingly, a luciferase reporter regulated by the hs4 enhancer was synergistically activated by LPS and TCDD. The objective of this study was to determine in the context of chromatin the inhibitory effect of TCDD is mediated through the hs1.2 enhancer. CH12.LX cells were stably transfected with constructs containing an LPS-inducible 2b reporter regulated by the 3'IghRR with LoxP sites flanking either the hs3B/h4 or the hs3A/hs1.2 enhancer pairs. Transfection with CRE-recombinase induced LoxP recombination generating cell lines either expressing 2b reporter regulated by hs3A/hs4 or by hs3B/h4.

**1619 BINARY INHIBITION OF A LYMHPOTOCYTE TERMINAL DIFFERENTIATION BY 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD).**

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The molecular mechanisms underlying suppression of humoral immunity, specifically B cell function, by TCDD, are poorly understood. Here we show that TCDD suppresses the IgM response of individual LPS-activated mouse B cells in a binary rather than graded manner; (i.e., it reduces the number of IgM-secreting plasma cells produced without affecting the IgM content in individual plasma cells). This binary mode of suppression was further investigated with a mathematical model of the B cell transcriptional regulatory circuit, incorporating the key transcription factors Bcl6, Pdml, Pax5 and Bach2. Simulations of the model indicated that two previously identified TCDD-modulated pathways, AP-1 repression and Bach2 activation, could mediate the binary mode of suppression. Both pathways disrupt the bistable switch underlying differentiation of LPS-activated B cells to plasma cell. Acting through these pathways, TCDD increases the LPS threshold concentration required to trigger the differentiation process, thus reducing the probability of bistable switching and thereby the number of resting B cells that differentiate into IgM-producing cells. The model further predicted that TCDD-induced upregulation of Bach2 might delay B cell differentiation and increase the likelihood of isotype switching, thus providing novel insights into the mechanisms and signaling pathways by which TCDD suppresses IgM response. (Supported by NIH ES04911. This work may not reflect the official policies of the US EPA.)
1620 SUPPRESSION OF THE PRIMARY IMMUNOGLOBULIN M (IGM) ANTIBODY RESPONSE BY DELTA-9-TETRAHYDROCANNABINOL (Δ9-THC) IN HUMAN PRIMARY B CELLS.

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We have previously shown that Δ9-THC, a plant-derived cannabinoid, significantly attenuated the primary IgM antibody response induced by ligation of CD40 in mouse splenic B cells. However, the effect of Δ9-THC on humoral immune responses in humans is uncertain. Thus, the objective of this study was to investigate the influence of Δ9-THC on in vitro T cell-dependent antibody response in human B cells using an in vitro activation model, which employs cell surface-expressed CD40 ligand (CD40L) and recombinant cytokines (interleukin (IL)-2, IL-6, and IL-10). Similar to what we observed in mice, pretreatment with Δ9-THC suppressed the number of IgM antibody forming cells induced by CD40L, plus cytokines as determined by ELISPOT. Furthermore, Δ9-THC suppressed B cell activation induced by CD40L plus cytokines as measured by suppressing the upregulation of the B cell activation markers, CD80, CD86, and CD69, as assessed by flow cytometry. Impairment in B cell activation correlated with suppression of B cell proliferation. Collectively, these studies suggest that Δ9-THC-mediated suppression of the primary IgM response is due, in part, to impairment of B cell activation and proliferation. (Supported in part by DA07908 and Royal Thai Government Scholarships)

1621 INDUCTION OF MYELOID-DERIVED SUPPRESSOR CELLS BY ENDOCANNABINOIDS REQUIRES MAST 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 on the development of immune responses in a wide range of inflammatory diseases. Recently, we showed that endocannabinoids can trigger large numbers of a subset of monocyte precursors called Myeloid-Derived Suppressor Cells (MDSCs) that are highly immunosuppressive and prevent T cells from proliferating in response to antigens. In this study, we investigated the mechanism by which endocannabinoids are able to induce MDSCs. Cytokine analysis of mice treated with endocannabinoids showed that several cytokines were secreted in response to endocannabinoid treatment, including G-CSF and GM-CSF. To investigate the source of these cytokines, we attempted to induce MDSCs in mice that were deficient in mast cells. These studies revealed that mast cell-deficient mice were unable to induce MDSCs at a level consistent with wild type mice. When mast cells were adoptively transferred into deficient mice, the ability to induce MDSCs was restored. Taken together, these studies point to a significant role played by mast cells in the induction of MDSCs by endocannabinoids. (Supported in part by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755).

1622 DIFFERENTIAL MODULATION OF NF-κB SUBUNITS BY ACROLEIN.


Cigarette smoking impairs pulmonary immunity by suppressing T cell responses, resulting in compromised immune surveillance and high risk of respiratory infections. In previous studies, we have identified that the ω-3-unsaturated aldehyde acrolein is the major immunosuppressive agent present in cigarette smoke, which reproduces the effects of cigarette smoking on pulmonary immunity. Acrolein inhibits the production of a variety of cytokines involved in T cell response. Mass Spectrometry analysis revealed that acrolein alkylates Cys63 and Arg307 residues in the DNA-binding domain of the upstream transcription factor NF-κB p50 subunit, which results with inhibition of p50 binding to the IL-1 promoter by >99%. In contrast, our preliminary study showed that acrolein elicits the production of IL-8, macrophage chemotractant protein-1 (MCP-1) and cyclooxygenase 2 (COX 2). Furthermore, acrolein has minimal effect on DNA binding of p65 subunit of NF-κB. The purpose of current study is therefore to test the hypothesis that acrolein activates NF-κB p65 subunit, leading to the upregulation of its target genes, such as IL-8, MCP-1 and COX 2. U937 cell line was used to study the effects of acrolein on: (1) the production of cytokines IL-8 and IL-10, (2) the binding capacity of p50 to IL-10 promoter, and (3) the expression of key NF-κB signaling molecules. Results show that acrolein increases IL-8 production, but inhibits LPS-induced IL-10 production. Acrolein promotes the binding of p65 and phosphorylated p65 to the IL-8 promoter. As expected, acrolein inhibits constitutive binding of p50 to IL-8 promoter. Acrolein also induces nuclear translocation of p65 and phosphorylated p65 and increases p38 phosphorylation. Acrolein addition on the DNA binding domain of p65 is currently under investigation. Conclusion: Collectively our data suggest that acrolein promotes IL-8 production by activating NF-κB p65 subunit and thereby alters the activity of NF-κB pathway through differential modulation of its p50 and p65 subunits. This work is partially sponsored by NIEHS grant ES005673.

1623 Δ9-TETRAHYDROCANNABINOL (THC) AMELIORATES STAPHYLOCOCCAL ENTEROTOXIN B (SEB)-INDUCED ACUTE LUNG INJURY THROUGH REGULATION OF EPIGENETIC PATHWAYS.

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Acute Lung Injury (ALI), commonly caused by sepsis, leads to respiratory and multiple organ failure and subsequently, Acute Respiratory Distress Syndrome (ARDS). It is characterized by infiltration of inflammatory lymphocytes in the lung that causes damage to the alveolar epithelial cells, pulmonary edema and fibrosis. In this study, Staphylococcal Enterotoxin B (SEB) was used to induce ALI in mice. SEB is a superantigen that activates T cells expressing Vβ8, which leads to activation of ~20% of T-cells and massive release of pro-inflammatory cytokines, leading to induction of ALI/ARDS. In the current study, we tested the hypothesis that Δ9-Tetrahydrocannabinol (THC), a cannabinoid, known for its anti-inflammatory properties, can ameliorate the toxicity of SEB. Intranasal administration of SEB caused infiltration of lymphocytes into the lung, which was reduced after THC treatment. While SEB caused an increase in absolute numbers of NK, NKT, Macrophages, and Vβ8+ T cells, THC treatment caused a decrease in their absolute numbers. Cytokine analysis of bronchoalveolar fluid (BALF) showed that SEB induced high expression of Th1 cytokine, IFN-γ. Interestingly, THC treatment led to a switch from Th1 to Th2 phenotype (IL-10, IL-4 and IL-6). Epigenetic studies revealed that SEB caused an up-regulation of miRNA(mir)-155 and THC reduced its expression by half indicating that THC may mediate its effect through downregulation of mir-155 and consequent suppression of inflammation. Additionally, methylation studies of the IFN-γ, IL-4 and IL-10 gene promoters indicated that the THC-induced switch in cytokine profiles can be explained in part by modifications at the epigenetic level. Together, our data demonstrated that THC can ameliorate SEB-induced ALI through regulation of epigenetic pathways. (Supported in part by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755).

1624 OXYGENATED FATTY ACIDS IN PLASMA OF TUMOR-BEARING ANIMALS STIMULATE THEIR SCAVENGER RECEPTOR A1-MEDIATED UPTAKE BY DENDRITIC CELLS: MASS-SPECTROMETRIC EVIDENCE.

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Dendritic cells (DC) are the most potent antigen presenting cells responsible for the development of immune responses in cancer. The function of DC in tumor-bearing hosts is severely compromised. To a large extent, the defects in DC function in tumor-bearing mice and patients with cancer are due to the accumulation of high amounts of lipids. To identify possible sources of lipids taken-up by the DC, we performed oxidative lipidomics analysis of plasma and DC of tumor-bearing animals. It is characterized by infiltration of inflammatory lymphocytes in the lung that causes damage to the alveolar epithelial cells, pulmonary edema and fibrosis. In this study, Staphylococcal Enterotoxin B (SEB) was used to induce ALI/ARDS. In the current study, we tested the hypothesis that Δ9-Tetrahydrocannabinol (THC), a cannabinoid, known for its anti-inflammatory properties, can ameliorate the toxicity of SEB. Intranasal administration of SEB caused infiltration of lymphocytes into the lung, which was reduced after THC treatment. While SEB caused an increase in absolute numbers of NK, NKT, Macrophages, and Vβ8+ T cells, THC treatment caused a decrease in their absolute numbers. Cytokine analysis of bronchoalveolar fluid (BALF) showed that SEB induced high expression of Th1 cytokine, IFN-γ. Interestingly, THC treatment led to a switch from Th1 to Th2 phenotype (IL-10, IL-4 and IL-6). Epigenetic studies revealed that SEB caused an up-regulation of miRNA(mir)-155 and THC reduced its expression by half indicating that THC may mediate its effect through downregulation of mir-155 and consequent suppression of inflammation. Additionally, methylation studies of the IFN-γ, IL-4 and IL-10 gene promoters indicated that the THC-induced switch in cytokine profiles can be explained in part by modifications at the epigenetic level. Together, our data demonstrated that THC can ameliorate SEB-induced ALI through regulation of epigenetic pathways. (Supported in part by NIH grants P01AT003961, R01AT006888, R01ES019313, R01MH094755).

1625 TETRAHYDROCANNABINOL (THC) IN HUMAN PRIMARY IMMUNOCYTOPLASMIC RAW MATERIALS: MASS-SPECTROMETRIC STUDIES OF TUMOR-BEARING ANIMALS THROUGH REGULATION OF EPIDERMAL PATHWAYS.
were found in DC from k/o mice vs those detected in wt animals. Further, we esti-
mated whether oxFIA in DC were enteriﬁed into the most abundant class of neu-
tral lipids accumulating in DC of EL-4 tumor bearing animals, triglycerides (TG).
We found that oxTG species containing HODE and corresponding to C16:1/C18:2-OOH/C15:0 was present only in DC from tumor-bearing mice. Thus, we suggest that the presence of oxygenated species of lipids in plasma of EL-4 tumor-bearing mice may be responsible for their uptake by DC, possibly result-
ing in the loss of their immuno-surveillance function. Supported by NIOSH
OH008282; NIH U19 AI068021, HL70755, H094488.

1625 COMPARING THE IMMUNOSUPPRESSIVE EFFECTS OF CYCLOSPORIN A ON MOUSE SPLENOCYTES IN VIVO WITH MOUSE AND HUMAN T-CELLS IN VITRO BY TRANSCRIPTOME PROFILING.

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The immunosuppressive drug Cyclosporin A (CsA) is widely used to prevent graft-
versus-host disease in humans. In a parallelogram approach we compared the effects
of CsA on the transcriptomes of (i) mouse (CTLL-2) and human (Jurkat) T-cell
lines (ATCC), in order to determine the degree of interspecies overlap, (ii) spleno-
cytes of C57BL6 mice exposed in vivo with CTL2 cells exposed in vitro, to verify
whether CTL2 cells are a suitable in vitro model for toxicogenomics. Methods:
The mice were exposed for 11 days to CsA (3; low, 9; mid, and 27; high mg/kg bw)
or to olive oil (Ctrl), respectively. The CTLL-2 and Jurkat cells were exposed for 6
hours to CsA, Na-4 biological replicates. CTL2 cells were exposed to 7.5 μM
(low) or 15 μM (high) CsA, and Jurkat cells to 8 μM CsA or 13 μM DMSO (car-
rier). Equal amounts of total RNA molecules (800ng/sample) were hybridized on
Affymetrix mouse GeneTitan HT430PM arrays, or on human U133A plus 2.0 ar-
rays. These transcriptomes were analysed at the levels of individual genes and at
functional pathway level. Results and Conclusions: We found that the CsA target
genes overlapped by 5% between CTLL-2 and Jurkat cells, and by 2% between the
mouse in vivo and in vitro data, respectively. At the pathway level CsA affected (i)
metabolism, protein synthesis, and apoptosis in the Jurkat and CTL2 cells in vitro,
and (iii) metabolism, cellular processes, and apoptosis/cell death in the mouse
ells, both in vivo (splenocytes), and in vitro (CTL2-2), respectively (FDR<0.10).
In conclusion, at pathway level the immunosuppressive effects of CsA overlap be-
tween mouse immune cells in vivo, and mouse (CTL2-2) and human T-cells in vitro
(Jurkat). At the individual gene level these overlaps are more limited. Based on
our results CTL2-2 cells are a suitable model for toxicogenomics.

1626 INHIBITORY EFFECTS OF AZOLE-TYPE FUNGICIDES ON INTERLEUKIN-17 GENE EXPRESSION VIA RETINOIC ACID RECEPTOR-RELATED ORPHAN RECEPTORS ALPHA AND GAMMA.

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The retinoic acid receptor-related orphan receptors α and γ (ROαRT and ROγRT),
are key regulators of helper T (Th) 17 cell differentiation, which is involved in
the innate immune system and autoimmune disorders. However, it remains unclear
whether environmental chemicals, including pesticides, have agonistic and/or an-
tagonic activity against ROαRT. In this study, we investigated the ROαRT ac-
tivity of several azole-type fungicides, and the effects of these fungicides on the gene
expression of interleukin (IL)-17, which mediates the function of Th17 cells. In the
RO-reporter gene assays, five azole fungicides (imidconazole, hexaconazole, tri-
flumizole, tetraconazole and imazalil) suppressed ROαRT- and/or ROγRT-mediated
transcriptional activity as the benzenesulphonamide T0901317, a known ROαRT
inverse agonist and a potent liver X receptor (LXR) agonist. In particular, imiben-
conazole showed ROγRT inverse agonistic activity at concentrations of 10-6 M
order. However, unlike T0901317, these fungicides failed to show any LXRT/β
agonistic activity. Next, five azole fungicides, showing ROαRT inverse agonist activity,
were tested on IL-17 mRNA expression in mouse T lymphoma EL4 cells treated
with phorbol myristate acetate and ionomycin. The qPCR analysis revealed that these five fungicides suppressed the expression of IL-17 mRNA with-
out affecting ROαRT and ROγRT mRNA levels. In addition, the inhibitory effect of
imidconazole, as well as that of T0901317, was attenuated in RORγT-knockout
mice. Taken together, these results suggest that some azole-type fungi-
cides inhibit IL-17 production via RORγT. This also provides the first evidence
that environmental chemicals can act as modulators of IL-17 expression in immune
cells.

1627 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN INDUCES TRANSCRIPTIONAL ACTIVITY OF THE HUMAN POLYMORPHIC HS1, 2 ENHANCER OF THE 3'Igh REGULATORY REGION.

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2,3,7,8-tetrachlorodibenzop-dioxin (TCDD) is an environmental toxicant known
to inhibit antibody secretion and Ig expression. Inhibition of Ig expression may be
partially mediated through repression of the 3'Igh regulatory region (3'IghRR).
TCDD inhibits mouse 3'IghRR activation and induces aryl hydrocarbon receptor
(AhR) binding to dioxin response elements (DREa) within the 3'IghRR enhancers
hs1,2 and hs4. The human hs1,2 enhancer (hsu-hs1,2) is polymorphic due to the
presence of one to four invariant sequences (IS), which have been correlated with
several autoimmune diseases. The IS also contains a DRE-like site. Therefore, the
objective was to determine if hhu-hs1,2 activity is sensitive to TCDD. Utilizing a
mouse B-cell line (CH12.LX), we compared the effects of TCDD on mouse (mo-
hs1,2) versus hhu-hs1,2 enhancer activity. TCDD inhibited mo-hs1,2 similar to the
inhibitory effect on mouse 3'IghRR activation. In contrast, hhu-hs1,2 was activated
by TCDD and antagonists studies supported an AhR-dependent activation. TCDD
also induced hhu-hs1,2 activity in a human B-cell line (IM-9). Absence of a Pax5
binding site is a major difference between the human and mouse hs1,2 sequences.
Insertion of a Pax5 site in hhu-hs1,2 markedly blunted basal reporter activity but did
not alter TCDD's effect. Additionally, deletion analysis demonstrated a signifi-
cant IS contribution to hhu-hs1,2 basal activity but TCDD-induced activity was not
strictly IS number-dependent. Taken together our results suggest that hhu-hs1,2 is a
significant target of TCDD and support species differences in hhu-hs1,2 regulation.
Therefore, sensitivity of hhu-hs1,2 to chemical-induced modulation may influence
the occurrence and/or severity of human diseases associated with hhu-hs1,2.

1628 EFFECT OF LINDANE ON NITRIC OXIDE AND CYTOKINE RESPONSES IN RAW 264.7 MURINE MACROPHAGES.

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Lindane or gamma hexachlorocyclohexane is a persistent organochloride pesticide
that has been banned for agricultural use in the United States but remains an ec-
toparasiticide treatment for lice or scabies. Lindane may be present in potentially
toxic levels in the environment and has been demonstrated to have immunotoxic-
ity, developmental toxicity and genotoxicity. Our aim is to determine if exposure of
RAW 264.7 murine macrophages to lindane has direct effects on their ability to re-
respond to stimulation by bacterial Lipopolysaccharide (LPS) and interferon gamma
(IFN). RAW 264.7 cells are maintained in continuous adherent culture with pas-
sage every 5-6 days. A concentration of 1×10⁶ cells per milliliter are prepared in a
24-well plate and grown overnight. Cells are pretreated with vehicle (DMSO), or a
cose response of lindane (5, 50 or 200 μM) prepared in DMSO (final concentra-
tion less than 1%) for 24 hours. Following this exposure, cells are washed four
times in culture medium and stimulated with a combination of 100 ng/ml LPS and
0.5 μ IFN. After 24 hours of stimulation, supernatants are collected for analysis
of nitric oxide (Greiss reaction) and cytokine (ELISA) production to determine
macrophage functional responses. Cells are then lysed and proteins isolated for later
Western blot analysis. Nitrite concentrations in supernatants were determined for
no pretreatment controls (58.1 ± 2 μM) and DMSO vehicle controls (44.3 ± 3.4
μM) demonstrated no significant difference. Nitrite concentrations for lindane
treatments of 5 μM (33.6 ± 1.7 μM), 50 μM (30 ± 4 μM) and 200 μM (26 ± 1
μM) treatments showed a significant decrease in nitric oxide response (ANOVA
Dunnett's t-Test, P<0.05) in the 50 and 200 μM treatments compared to controls.
Each value represents an average of four wells of treatment. Our initial findings sug-
gest lindane has direct immunotoxic effects on macrophages in a model that could
be used to understand previously demonstrated immunotoxic effects in the litera-
ture.
1629 EFFECT OF ASBESTOS ON FORKHEAD TRANSCRIPTION FACTORS FOXO1 IN MT-2 CELLS.

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Introduction: Asbestos is known to cause mesothelioma and lung cancer. We previously reported that asbestos affects not only mesothelial and lung epithelial cells, but also anti-tumor immune system. Namely, we found that asbestos enhanced regulation of T(Reg) cell function by using MT-2; an HTLV-transformed human FoxP3 positive cell line. In this study, effect of asbestos on gene expression pattern in MT-2 cells was analyzed by micro array analysis. Methods: MT-2 cells were cultured with asbestos for 8 months to establish Treg cell model exposed to asbestos for long term. Resulting sub-lines show resistance to asbestos and high production of IL-10 and TGF-β. Comparison of these designed as MT-2Rst. Total RNA purified from MT-2Rst and control MT-2 cells were analyzed by micro array containing 41,000 human genes. Results & Discussion: Micro array analysis revealed that total 139 genes were significantly altered in MT-2Rst cells (greater than 2 fold changes). Namely, 84 genes were up-regulated and 55 genes were down-regulated. Forkhead transcription factor FoxO1, which is known to regulate Treg development through FoxP3 expression, was found as one of the down-regulated genes. We confirmed decrease of FoxO1 in MT-2Rst by RT-PCR and immunoblot analysis. Furthermore, FoxO3 mRNA repressed in MT-2Rst as other FoxO1 target genes in consistent to the decrease of FoxO1. These results suggest that FoxO1 is implicated in alteration of MT-2 cell function by asbestos. Now, we are analyzing expression of FoxO3 protein by immunoblot analysis and will discuss the role of FoxO1 in Treg cell function.

1630 CONTINUOUS EXPOSURE OF ASBESTOS ALTERS CELL-CYCLE REGULATION IN MT-2 CELL.

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Asbestos is well known as silicate mineral that causes serious illness such as malignant mesothelioma and lung cancer. We previously reported that asbestos affects not only mesothelial or lung tissue, but also impairs antitumor immune system. Regulatory T cells (Treg), subpopulation of T cells, are known as a suppressor of immune activity. We have hypothesized that asbestos causes less immune reaction including tumor immunity through the enhancement of Treg function. Here we used MT-2 cell line, an HTLV-transformed human FoxP3 positive Treg model cell line, and established 7 sub-lines from original MT-2, which exposed to 3 kinds of asbestos (Chrysotile A, Chrysotile B and Crocidolite) at low concentration (25 μg/ml for long term (more than 8 months). Micro array analysis revealed that transcriptional factor FoxO1 was remarkably decreased in these all of sub-cell lines. FoxO1 is implicated in cell cycle regulation, and it is known that FoxO1 upregulates p27Kip1, Cyclin dependent kinase (CDK)-inhibitor, and down-regulates Cyclin D1. In this study, we observed p27Kip1 was decreased and Cyclin D1 was increased in all of sub-lines with real time PCR analysis. Furthermore, gene expression levels of other CDK-inhibitor, p18 (INK4c), p19 (INK4d), p21Gip1 and p57Kip2 were increased. These results suggested that asbestos accelerates cell cycle progression in Treg through the downregulation of FoxO1. Abnormal proliferation of Treg may down regulate tumor immune activity.

1631 EFFECTS OF SODIUM METHYLTHIOCARBAMATE-INDUCED OXIDATIVE STRESS ON NF-kB SIGNALING.

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Sodium methylthiocarbamate (SMDC) is one of the most widely used pesticides in the US, and has been reported to cause health problems in humans. There have been several studies indicating that SMD is involved in cytokine production through TLR-like receptor (TLR) 4, decreasing IL-12 (Interleukin) production and increasing IL-10 production induced by lipopolysaccharide (LPS) in mice. SMD has an effect on oxidative stress, which could cause activation of NF-kB signaling. This study evaluated the effect of SMD on NF-kB activation. Studies were conducted using NF-kB reporter mice with or without BSO (buthionine sulfoximine), which depletes glutathione and increases oxidative stress, or NAC (N-acetyl cysteine), which is a glutathione precursor and decreases oxidative stress. Mice were treated as naive, LPS only, LPS plus SMD, LPS plus SMD and BSO, and LPS plus BSO. On the 16th day, SMD was orally administered at a dosage of 200 mg/kg and LPS by intravenous injection (60 μg/mouse) 30 minutes after SMD. Mice were imaged 2 hr later using the IVIS imager and samples were collected. SMD at 200 mg/kg was given 1 hour after the last dose of NAC (1 g/kg daily for 3 days). Mice were then challenged with LPS and imaged as the same as with BSO treatment. Results indicated that SMD-induced oxidative stress did not play a priori role in the inhibition of NF-kB activation caused by SMD. Specifically, neither BSO + LPS nor NAC + LPS yielded different levels of activation of NF-kB than LPS alone. Conversely, NAC and BSO did alter the production of a few cytokines, indicating they were present in sufficient concentration to be biologically active. An earlier study indicated that reactive oxygen species had a complex bimodal effect on cytokine production in this experimental system. The work here demonstrates that NF-kB is not affected directly by increased reactive oxygen species (caused by SMD or BSO) or decreased reactive oxygen species (caused by NAC). This work was funded by NIEHS grant R01ES013708.

1632 GLOBAL ASSESSMENT OF GLUCOCORTICOID-MEDIATED CHANGES IN EXPRESSION OF GENES RELEVANT TO IMMUNE AND INFLAMMATORY RESPONSES.

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It is generally thought that the neuroendocrine stress response induced by most inflammatory stimuli leads to increase production of glucocorticoids, which then regulate the inflammatory response. However, in a series of studies, we only noted a few cytokines that were down-regulated in increasing concentrations of glucocorticoid. In the present study, a broader survey of glucocorticoid mediated effects was done using microarray analysis of the spleen from adrenalec-tomized (ADX) or sham ADX mice that were treated with poly I:C. Expression of a number of genes relevant to inflammatory responses were unexpectedly decreased in ADX mice, suggesting that corticosterone is necessary for their expression. Examples include: thrombospondin 1; prostaglandin E receptor 4; dual specificity phosphatase 1; CCL6; CXCL2; C/EBP-beta; CCL9; CD8; CD86; CD80; serum/glucocorticoid regulated kinase 3; Interleukin 10. On the other hand, expression of a number of other genes was increased in ADX mice, indicating that corticosterone normally plays a regulatory role by decreasing expression: lipocalin 2; LPS binding protein; annexin 1; annexin 3; cathepsin G; CD117; catheclicin antimicrobial peptide; neutrophil granule protein; myeloperoxidase; integrin β2 like; eosiophil peroxidase; pepptidoglycan recognition protein; Interleukin 12 b; interferon induced transmembrane protein 2; neutrophil elastase; mitogen activated protein kinase 13; S100 calcium binding protein A9 (calgranulin B). Several of these are consistent with our results in other studies and are consistent with changes in protein expression. Ingenuity pathway analysis revealed expected changes in glucocorticoid receptor activated pathways as well as changes in immune and inflammatory responses, cell migration, and cell death pathways. In summary, corticosterone is needed for expression of some relevant genes, but expression of many others was decreased in the presence of stress-induced glucocorticoids. This work was supported by R01ES013708 and R01AA009505.

1633 OVEREXPRESSION OF THE CALCIUM CHANNEL TRPA1 IN LYMPHOCYTES DERIVED FROM CANNABINOID RECEPTOR KNOCKOUT MICE.

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Cannabinoid receptors (CBRs) are G protein-coupled receptors that bind cannabinoids, compounds derived from marijuana that exhibit immunosuppressive properties. Consistent with the observation that cannabinoids suppress immune function, immune responses are enhanced in mice lacking the two best-characterized CBRs, CB1 and CB2 (CB1(-/-)/CB2(-/-)). For example, CB1(-/-)CB2(-/-) mice exhibit enhanced immune responses to influenza. In the course of administering influenza virus to wild type and CB1(-/-)CB2(-/-) mice, it was observed that CB1(-/-)CB2(-/-) mice do not recover from isoflurane anesthesia as rapidly as wild type mice. Interestingly, one receptor for isoflurane is the transient receptor potential cation channel (trp) a1, a non-selective cation channel, which also binds the psychoactive plant-derived cannabinoid, Δ9-tetrahydrocannabinol (Δ9-THC). Since calcium is a critical regulator of immune function, we hypothesized that overexpression of trp1 in immune cells might contribute to the mechanism by which CB1(-/-)CB2(-/-) mice exhibit enhanced immune function. Results from these experiments demonstrated that trp1
The success of RNA interference (RNAi) therapeutics in treating human diseases depends on safe and efficacious delivery of siRNA to target tissues. Formulation of siRNA in lipid nanoparticles (LNPs) is one of the most widely used strategies for systemic delivery to target tissues. Our current LNPs are comprised of an ionicizable lipid, a phosphatidylcholine, cholesterol, and PEG-lipid. When coupled with siRNA, these LNPs silence therapeutically relevant targets in rodents and non-human primates. siRNA-LNPs can stimulate the innate immune system, resulting in complement activation, cytokine release, and other acute inflammatory responses. In a series of studies, we explored the potential mechanisms of immune stimulation after systemic siRNA-LNP administration. Serum cytokine profiling in splenectomized CD-1 mice indicated that the spleen is an important early source of IL-6. In intact CD-1 mice, chemical modifications to the siRNA component resulted in reduced serum IL-6 and TNFα concentrations 2 and/or 4 hours post-dose and less hepatotoxicity compared to unmodified siRNA. In mice and rats, siRNA-LNP-induced increases in several serum cytokines were mitigated following pretreatment with clodronate (a monocyte/macrophage depleting agent). In Sprague-Dawley rats, a hepatic transcriptome study demonstrated that systemic administration of siRNA-LNPs significantly altered the expression of genes associated with lipid metabolism, acute inflammation, complement, and pathogen-associated molecular pattern (PAMP) recognizing elements. At 24 hours post-dose, gene expression modulation was associated with changes in serum chemistry and liver histopathology. These data suggest the involvement of the liver, spleen, and monocytes/macrophages in the acute immune response to siRNA-LNPs through lipid and siRNA-mediated pathways.

TCDD is known to suppress primary humoral immune responses in virtually all species tested. In humans, epidemiological studies suggested an association between increased incidence of non-Hodgkin's lymphoma as well as decreased antibody titers and exposure to TCDD and dioxin-like compounds. The molecular basis for these effects is poorly understood. Here, human peripheral blood B cells from healthy donors were activated in vitro using CD40 ligand to mimic T-cell B-cell interactions. Using this method, impaired B cell activation was observed in the presence of TCDD as evidenced by marked decreases in B cell activation markers CD80, CD86 and CD69 at the mRNA and protein level. To further examine the consequences of decreased activation by TCDD of human B cells, the role of the transcriptional repressor B cell lymphoma 6 (BCL-6) was investigated using flow cytometry. Typically, BCL-6 binds to regulatory regions in genes to impair B cell activation and differentiation thus maintaining B cells in a resting state. Mutations and deregulation of BCL-6 is associated with B cell oncogenesis. In this study, an increase in the proportion of live, high BCL-6 (BCL-6+) expressing B cells was observed in the presence of TCDD on day 3, when compared to vehicle-treated cells. A corresponding decrease in expression of activation markers CD80 and CD69 was seen in the same population of cells. Furthermore, on day 3, enhanced DNA binding activity of BCL-6 in BCL-6+ cells was observed in untreated cells using nuclear extracts from TCDD-treated human B cells, when compared to the VH controls. Collectively, these results suggest that the deregulation of BCL-6 by TCDD might represent a critical mechanism for impairment of B cell activation and differentiation processes resulting in altered humoral immunity. (Supported by NIH ES04911 and ES002520)
1638 EPIGENETIC MODIFICATIONS OF HISTONES PLAY A CRITICAL ROLE IN ETHANOL-MEDIATED ENHANCEMENT OF FASL GENE EXPRESSION AND CELL DEATH IN CD4+ T LYMPHOCYTES.

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Alcohol abuse is known to induce immunosuppression involving the depletion of CD4+ T cells; however the mechanisms underlying this alcohol effect are not clearly understood. Recent work from our laboratory showed that ethanol decreases S-adenosylmethionine levels and enhances activation induced apoptotic cell death (AICD) in CD4+ T cells. The Fas-FasL system plays a major role in AICD and resultant depletion of CD4+ T lymphocytes. Increased FasL gene expression has been seen in various disease conditions such as HIV infection, autoimmune disease states and cancer. Epigenetic modifications particularly histone acetylation mediated by histone acetyltransferases (HATs) and deacetylation mediated by histone deacetylases (HDACs) play a critical role in the regulation of the transcriptional activation. Accordingly, the present work was carried out to investigate the immunotoxic effects of ethanol by examining FasL promoter histone modifications in the regulation of FasL gene expression in CD4+ T lymphocytes exposed to alcohol. Examination of the FasL promoter in CD4+ T cells exposed to ethanol showed increase in histone H3 acetylation which is associated with active transcription. In correspondence with increased histone acetylation ethanol was observed to enhance the recruitment of histone acetyltransferase and transcriptional co-activator p300. Further, histone acetylation induced by ethanol resulted in increased promoter occupancy by the relevant transcription factors and correlated with increased FasL expression. Notably, genistein, a specific p300HAT inhibitor markedly reduced p300 targeting, histone acetylation and FasL gene expression. Overall these data identify the role of ethanol-mediated p300 targeting at FasL promoter and resultant epigenetic modifications are critical for ethanol induced FasL expression and AICD in CD4+ T cells and ensuing immunosuppression.

1639 DES AND METHOXYPHENYLISOTHIOCHLOR METABOLITE, HPTE, INDUCTION OF CELL DEATH AND ALTERATION OF THYMCYTE DEVELOPMENT DOSE AND POTENTIAL MECHANISM.


Endocrine disrupting chemicals such as diethylstilbestrol (DES) and methoxychlor have been shown to induce thymic atrophy and to potentially alter T cell development. However, the dose and the mechanism by which these effects occur remain unclear, in part because of the varied model systems, modes of exposure, age of animals tested, and doses of exposure used to study the phenomenon in the past. The current studies were undertaken to elucidate and to compare the dosage and mechanism of action of DES and hydroxyphenyl-trichloroethane (HPTE), the primary physiological metabolite of methoxychlor, that result in the alteration of the development of T cells. Because the developing immune system during gestation is the stage most vulnerable to perturbation, embryos at GD16-18 were used for the studies. The effects of exposure of developing thymocytes to DES and HPTE were examined using an in vitro differentiation assay that mimics the early stages of T cell development in the thymus. Doses in the nanomolar to micromolar range of each EDC were employed. Phenotypic markers of thymocyte maturation (CD4 and CD8), signaling status (TCR and CD5), and apoptosis (Annexin V and PI staining) were analyzed using flow cytometry. Doses of 12.5 micromolar and above (HPTE) and 25 micromolar and above (DES) were found to significantly reduce cell viability and differentiation in culture. Results indicate that death by apoptosis was induced early, at 8 hrs in culture, suggesting that DES and HPTE may be utilizing nongenomic pathways to mediate their effects on embryonic thymocytes. *

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1640 PULMONARY HYPERTENSION-INDUCED BY EXPOSURE TO ANTIGEN AND URBAN PARTICULATE MATTER, ROLE OF B CELLS AND ANTIBODIES.


Many studies have suggested a contribution of air pollution for the development of pulmonary arterial hypertension (PAH). Auto-antibody responses have long been associated with the severity of PAH. Previous studies in our lab have shown that a prolonged T helper 2 (Th2) response to inhaled antigen induces severe pulmonary arterial remodeling. We have also shown that urban particulate matter (PM) from air pollution exacerbates antigen induced pulmonary arterial remodeling and pulmonary hypertension. Our study was designed to identify the role of B cells (anti-body producing lymphocytes) for pulmonary hypertension induced by antigen and urban PM.2.5. Urban PM2.5 was collected in New York City. Th2 primed mice were intranasally challenged with soluble antigen (Ovalbumin) combined with urban PM intranasally. Pulmonary arterial remodeling was determined as well as right heart weights. Right ventricular systolic pressures were measured by heart catheterization in anesthetized, spontaneously breathing mice. In contrast to wild type mice, Th2 primed B cell KO mice did not show significantly increased right heart weights, or right heart systolic pressures in response to intranasal challenge with antigen and urban particulate matter. Reconstitution with anti-antigen antibody restored the development of pulmonary hypertension in antigen-urban PM challenged B cell KO mice. Like wild type mice, B cell KO mice had significant pulmonary arterial remodeling that was slightly increased by reconstitution with antibody. Our studies indicate that antigen-specific antibody is necessary for the development of pulmonary hypertension induced by the exposure to a Th2 antigen combined with urban PM2.5.

1641 CHARACTERIZING THE TOXIC MODE OF ACTION OF MERCURY ON THE B CELL.

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Inorganic mercury (Hg2+) is a potent immunomodulator, but an overall understanding of the mechanism by which Hg2+ disrupts cellular function has remained elusive. Chelated Hg2+ is well known to bind to reduced sulphydryl groups. Because most protein phosphatases contain sulphydryls at their active sites, it has been suggested that much like okadaic acid (OA), Hg2+ primarily disrupts cell metabolism by globally inhibiting protein phosphatases. In this study, WEHI-231B cells were exposed to HgCl2, OA, or as a control to RPMI media then harvested for analysis of protein phosphorylation state by mass spectrometry. Proteins were trypsin digested and phosphopeptides isolated by TiO2, affinity selection prior to nano-LC-MS/MS analysis with an LTQ-XL. The identity of the phosphoproteins was determined using the Mascot algorithm in Proteome Discoverer 1.1 with quantitation by spectral counting. The Hg2+ and OA treated cells displayed significantly different phosphoprotein profiles from controls. More importantly, the Hg profile differed dramatically from the OA profile, indicating that Hg2+ and OA dependent toxicity are mechanistically distinct. Aiding a bioinformatics approach, we performed pathway and key node analysis of the Hg2+ data. It was determined that the set of phosphoproteins was enriched with proteins associated with the B Cell Receptor (BCR) pathway and that within that pathway the protein tyrosine kinase Lyn is the most significant node. Utilizing multicolor phosphoflow cytometry we then looked specifically at the effect of Hg2+ on the (anti-Ig stimulated) BCR signaling pathway by focusing on ERK, Syk, Btk and Blink 65; important phosphoprotein elements of the BCR pathway which are downstream of Lyn. Normally, signaling through the BCR pathway is associated with rapid transients in the tyrosine phosphorylation state of these proteins. Exposure to Hg2+ decreased the phosphorylation state of these proteins. This decrease in the phosphorylation state was associated with an increase in the degradation of Lyn. The data suggest that the Hg2+ exposure may be inducing apoptosis in the cells. Further studies are in progress to determine if this is the case.

1642 INVOLVEMENT OF IL-17 AND T-HELPER 17(TH17) CELLS IN DRUG-INDUCED LIVER INJURY (DILI).


Rationale: The mechanisms of many DILI remain largely unknown, especially for the idiosyncratic type (IDILI). Recently, it has been suggested that TH17 cells may play an active role in inflammatory liver diseases. We also found that IL-17 was detectable in ~ 60% of acetaminophen (APAP)-induced liver injury and IDILI patients. We thought that the APAP toxicity would be too acute to result in an expansion of TH17 cells and it would be more likely that the IL-17 came from cells of the innate immune system. The aim of this study was to further characterize the source of IL-17 and role of TH17 cells in DILI. Procedures and Results: Male Balb/c mice were given saline or APAP at a dose of 300 mg/kg (p) after an 18 hr fast. Flow cytometry with intracellular staining was performed to identify the cellular sources of IL-17 in the liver. It was found that both ALT and IL-17 levels started to increase at 2 hours after APAP treatment. The percentage of CD3+CD4+IL-17+ cells significantly increased in the animals treated with APAP (Student’s t-test, P<0.03). In another study, blood samples were collected at various time points in patients being treated with isoniazid (INH) because of a positive TB skin test. Lymphocytes were isolated by differential centrifugation with Ficol. Three patients

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developed mild liver injury after INH treatment. The percentage of Th17 cells greatly increased when the ALT was elevated (ALT >40 U/L). The percentage of regulatory T cell (Treg) appeared to decline after the development of liver injury. An immune imbalance (represented by the ratio of Th17/Treg) has been observed during the abnormal state (Student's t test, P <0.03). Conclusions: These studies demonstrate that CD17 cells are involved in both "toxic" and idiosyncratic liver toxicity. CD4⁺ cells are usually considered to be part of the adaptive immune response but these studies indicate that Th17 cells can respond rapidly, presumably without antigen specificity, and therefore part of the innate response. This pathway could be a new target for the therapeutic interventions to treat DILI. This work was supported by Canadian Institutes of Health Research.

1643 COVALENT BINDING OF NEVIRAPINE IN VITRO AND IN VIVO.

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Rationale: Nevirapine (NVP) treatment is associated with a significant incidence of idiosyncratic immune-mediated skin rash and hepatotoxicity. The female Brown Norway (BN) rat animal model of NVP-induced skin rash was used to test the hypothesis that development of skin rash is mediated by covalent binding of a NVP metabolite to proteins in skin. Methods: An anti-NVP antibody was produced in New Zealand White Rabbits and used in immunoblotting studies to detect covalent binding of NVP to skin and liver proteins. Samples were obtained from BN rats given NVP (150 mg/day) with or without concurrent application of 1-phenyl-1-hexanol onto the skin at ~20 mg/kg/day. In vitro incubation of NVP or metabolites with hepatic microsomes. Conclusions: Covalent binding was observed in isolated epidermal fractions from NVP-treated BN rats after primary treatment and after re-challenge. Major modified bands appeared between 40K and 60K. Application of 1-phenyl-1-hexanol locally inhibited the skin rash and no covalent binding was detected in whole skin homogenate from inhibitor-treated areas. No effects on blood levels of 12-NVP-sulfate were observed. Histology of inhibitor versus vehicle areas displayed a decreased inflammatory infiltrate in the upper dermis and decreased blood vessel involvement. As expected, hepatic covalent binding in vivo was significantly lower for 12-OH-NVP than NVP. Covalent binding was also greater for NVP than for deuterated NVP or 12-OH-NVP in vivo in human CYP 3A4, mouse, and rat hepatic microsomes. Conclusions: The reactive metabolite that led to hepatic covalent binding is likely a quinone methide formed by oxidation of the methyl group; however, it does not appear that this occurs in skin. In contrast, it appears that covalent binding in the skin is due to 12-NVP-sulfate. Based on the effect of a topical inhibitor, it appears that the rash is due to formation of this sulfate in the skin. This research was funded by the Canadian Institutes of Health Research.

1645 MAGNITUDE OF STIMULATION DICTATES THE CANNABINOID-MEDIATED DIFFERENTIAL T-CELL RESPONSE TO HIV-GP120.

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Cannabinoids have immunosuppressive properties, but it is unknown whether cannabinoids further improve the immune status of immunocompromised HIV patients, as approximately 25% of HIV patients smoke marijuana for its putative therapeutic benefit. A surrogate in vitro mouse model to induce polyclonal T cell responses to HIV is used to study the effects of tetrahydrocannabinol (THC) on T cell responses. The objective of this study was to determine if THC differentially modulates T cell responses, PMA/ionomycin (Io) or anti-CD3/CD28 were used for activation. THC suppressed or enhanced IFNγ production by mouse splenocytes (SPLC) under optimal or suboptimal activation, respectively. However, THC elevated intracellular calcium, regardless of the stimulation level with PMA/Io, suggesting that the cannabinoid-induced calcium increase provides an appropriate signal for activation in suboptimally stimulated T cells, but an anergic-like signal due to excessive calcium in optimally stimulated T cells. Overall, these data identify a mechanism by which THC differentially modulates T cell function and suggest possible enhancement of immune function by marijuana use among HIV patients (Supported by NIH DA07908).

1646 IMMUNOTOXICITY OF POLYCYCLIC AROMATIC HYDROCARBONS AND ARSENIC FOLLOWING COMBINED EXPOSURES IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS.

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Studies in our laboratory have shown that in vitro exposure of mouse spleen cells to sodium arsenite (As+3) and polycyclic aromatic hydrocarbons (PAHs) results in synergistic immune suppression of T-dependent antibody responses (TDAR). Kozul et al. (EHP, 2009) have demonstrated that mice exposed to 100 ppb As+3 in drinking water for 5 weeks have a decreased resistance to influenza A virus. We have previously shown that human peripheral blood mononuclear cells (HPBMC) exposed to PAHs display pronounced inhibition of T-cell proliferation. Soto-Pena et al. (FASEB, 2006) have shown that children exposed to arsenic in drinking water had diminished T-cell proliferation responses. Thus, the purpose of our present study was to determine if As+3 interacts with PAHs resulting in suppression of T-cell mitogenesis in HPBMC. In this study, we exposed HPBMC from different donors to environmentally relevant concentrations of As+3 in vitro alone and in combination to PAHs and measured phytohemagglutinin (PHA)-induced T-cell proliferation. Our results demonstrated significant differences between individuals in both their sensitivity to As+3 as well as their PAH sensitivity. We found that extremely low concentrations of As+3 (<1 nM) inhibited T-cell proliferation in some people and that a PAH (BaP-Diol) caused further suppression. We further showed that As+3 exposure resulted in a “J” shaped dose response curve with low concentrations (<1 nM) stimulating these cells. The presence of Δ9-THC for 24 h. Without LPS stimulation, expression of CD86 and MHC class II was greater in As+3-treated cells compared to WT mice. LPS induced the expression of MHC class I, MHC class II, and CD86 in WT and these markers of maturation were significantly suppressed by Δ9-THC. These studies suggest that As+3 is sensitive to Δ9-THC treatment via a CB1 and/or CB2-dependent mechanism. CB1 and/or CB2 might also contribute to regulation of maturation of As+3 as evidenced by the differences observed in AM cell surface markers between WT and CB1/-/-CB2/-/- mice (Supported by NIH DA07908).

Δ9-TETRAHYDROCANNABINOL (Δ9-THC) IMPAIRS ALVEOLAR MACROPHAGE RESPONSES TO INFLUENZA VIRUS IN VITRO AND TO TOLL-LIKE RECEPTOR STIMULATION IN VITRO IN A CANNABINOID RECEPTOR (CB1) AND/OR 2-DEPENDENT MANNER.

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Alveolar macrophages (AM) protect the respiratory tract against pathogens entering the lungs. The role of the CB1 and CB2 in AM function and sensitivity to the effects of exogenous plant-derived compounds, including Δ9-THC, within the context of an anti-influenza response is not known. The present study examined the effects of Δ9-THC on AM responses after A/PR/8/34 influenza virus (PR8) infection in vivo and lipopolysaccharide (LPS) stimulation in vitro in C57Bl/6 (WT) and CB1/CB2 null (CB1/-/-CB2/-/-) mice. Three days after PR8 infection, CD11b+CD11c+Gr-1- AM isolated from lungs were evaluated using flow cytometry. The presence of AM was increased in the lungs after PR8 infection in WT and even to a greater extent in CB1-/-CB2-/- mice. Administration of Δ9-THC (75 mg/kg) decreased the influx of AM into the lungs after PR8 infection only in WT mice. In contrast to MHC I and MHC II, CD86 was induced by PR8 infection in WT and more robustly in CB1-/-CB2-/- mice. Furthermore, modest suppression of PR8-induced CD86 expression by Δ9-THC was observed in WT mice only. AM were isolated from broncho-alveolar lavage fluid and stimulated in vitro with LPS in the presence of Δ9-THC for 24 h. Without LPS stimulation, expression of CD86 and MHC I was greater in AM isolated from CB1-/-CB2-/- compared to WT mice. LPS induced the expression of MHC I, MHC II, and CD86 in WT and these markers of maturation were significantly suppressed by Δ9-THC. These studies suggest that AM are sensitive to Δ9-THC treatment via a CB1 and/or CB2-dependent mechanism. CB1 and/or CB2 might also contribute to regulation of maturation of AM as evidenced by the differences observed in AM cell surface markers between WT and CB1-/-CB2-/- mice (Supported by NIH DA07908).
EXPOSURE TO METAL-RICH PARTICULATE MATTER GENERATED FROM WELDING HAS BEEN LINKED TO CARDIOVASCULAR DYSFUNCTION AND REDUCED IMMUNE COMPETENCE. THE AIM OF THIS STUDY WAS TO INVESTIGATE THE MOLECULAR CHANGES AND RESPONSIVENESS OF CIRCULATING LEUKOCYTES FOLLOWING WELDING FUME EXPOSURE. RATS WERE EXPOSED TO MANUAL METAL ARC STEEL WELDING FUME (MMAW-SS) AT 2 mg/rat by intratracheal instillation and harvested 4 and 24 hr post-exposure. BLOOD WAS COLLECTED AND ANALYZED FOR DIFFERENTIAL CHANGES BY FLOW CYTOMETRY AND GENE EXPRESSION CHANGES BY MICROARRAY AND SUBSEQUENT PATHWAY ANALYSIS. IN ADDITION, ANTAGOCOAGULATED BLOOD WAS INCUBATED FOR 24 hr WITH AND WITHOUT LPS STIMULATION UTILIZING THE TRUCULTURE® WHOLE BLOOD COLLECTION SYSTEM. AFTER INCUBATION, SUPERNATANTS WERE COLLECTED FOR PROTEIN ANALYSIS AND THE CELLULAR FRACTION WAS COLLECTED FOR GENE EXPRESSION CHANGES. ANALYSIS OF MICROWAVE DATA FROM 4HR POST-EXPOSURE SHOWED 254 NETWORK ELIGIBLE GENES (137-UP AND 117-DOWN). THE TOP BIOLOGICAL CATEGORY “INFLAMMATORY RESPONSE” HAD 70 MOLECULES OF WHICH 75% WERE SIGNIFICANTLY REDUCED. BY 24 HR THERE WERE 75% FEWER NETWORK ELIGIBLE AND ALTERED “INFLAMMATORY RESPONSE” GENES. THESE RESULTS INDICATE A RAPID EFFECT ON THE CIRCULATING BLOOD CELL POPULATION AFTER PULMONARY EXPOSURE THAT WAS LESS APPARENT WITH TIME. EX VIVO STIMULATION WITH LPS OF CIRCULATING LEUKOCYTES SHOWED REDUCED PRODUCTION OF CC14, CC12, CCL10 AND TNF alpha protein in MMAW-SS treated rats. Cellular gene expression changes from MMAW-SS and PBS rats were similar after ex vivo LPS stimulation indicating effects were not at the transcriptional level. These results showed a reduced capacity of circulating leukocytes to produce inflammatory proteins in response to a secondary stimulus following a metal-rich particulate matter pulmonary exposure and provide mechanistic insight into epidemiological and experimental evidence illustrating immune-suppression following welding fume exposure.

1650 PULMONARY HYPERTENSION-INDUCED BY EXPOSURE TO ANTIGEN AND URBAN PARTICULATE MATTER, ROLE OF IL-33.


Previous studies in our lab have shown that a prolonged T helper 2 (Th2) response to inhaled antigen induces severe pulmonary arterial remodeling. Urban particulate matter (PM) from air pollution is known to exacerbate lung and cardiovascular conditions. The first goal of our study was to test the hypothesis that PM would also exacerbate pulmonary arterial remodeling and cause pulmonary hypertension. The second goal was to identify the molecular mechanisms. We focused on IL-33, which can induce Th2 responses, lung inflammation and remodeling. On the other hand, IL-33 signaling via its receptor, ST2, is cardio-protective for the left heart. Urban PM2.5 was collected in New York City. Th2 primed wild type or IL-33 deficient (KO) mice were intranasally challenged with soluble antigen combined with urban PM2.5. Scores for pulmonary arterial remodeling were determined as well as right ventricular systolic pressure by heart catheterization of anesthetized, spontaneously breathing mice; IL-33 and ST2 gene expression by qPCR. Results and Conclusions: 1) Urban PM2.5 exacerbated antigen-induced pulmonary arterial remodeling. 2) Combined exposure with urban PM2.5 and antigen induced pulmonary arterial hypertension as shown by increased right ventricular systolic pressure and right ventricular hypertrophy. 3) IL-33KO mice were no different from wild type with respect to pulmonary hypertension or right heart hypertrophy induced by combined exposure to antigen and urban PM2.5. IL-33KO mice had similar airway inflammation but reduced dendritic cells in the bronchoalveolar lavage relative to wild type in response to antigen and urban PM. 4) Initial data suggest that IL-33 is down- (not up-) regulated in the right hearts of animals exposed to an exaggerated Th2 response. This finding could explain the lack of a difference between IL-33KO and wild type mice in our experiments. In the future, we plan to test the idea expressing IL-33 at control levels is beneficial for the right heart during a Th2 response.
Th1 and Th2 cytokine responses by DNCB and TMA, respectively. Under these conditions, only DNCB provoked IL-17 expression. Interestingly, priming of the mice followed by a single challenge completely abrogated TMA-induced IL-17 expression whereas DNCB treatment provoked a similar pattern of IL-17 production (transient peak at 6h) to that observed in the absence of priming, albeit at much lower levels. The pattern and kinetics of IL-17 production is consistent with a single acute exposure to allergens being more immunoregulatory for both allergens stimulating IL-17 production by γδ T cells. During the development of the adaptive immune response, however, exposure to TMA down-regulates γδ T cell IL-17 production. Whereas not only is the γδ T cell response maintained following DNCB priming, but there is also evidence of Th17 cell activation.

**1652 DENDRITIC CELL-MEDIATED T CELL PROLIFERATION—A FUNCTIONAL BIOINDICATOR OF INFLAMMATORY SOURCE-SPECIFIC PARTICULATE MATTER.**

M. A. Williams, M. J. Daniels, T. J. Smith and L. Gilmour, NHEERL-EPHD, US EPA, Research Triangle Park, NC.

Previously we found that dendritic cells (DC) were sensitive functional bioindicators of ambient PM (APM) exposure mediating Th2-allergic inflammation in the draining lymph nodes. Here, the ability of bone-marrow-derived DC (DC) and putative BM-derived basophil (Ba) to present antigen and activate autologous T cells following exposure to source-specific PM was assessed. DC and Ba propagated from female OVA-specific C57Bl/6 OT-II mice whose T cell receptors were transgenic for the chicken ovalbumin peptide OVA323-339. OVA-specific CD62L+ CD4+ T cells were purified from lymph nodes by immunomagnetic selection. DC or Ba were stimulated with APM, environmental diesel PM (DEP) or emission-source diesel PM (EPAs) for 24h, washed then loaded with or without endotoxin-free OVA (50μg/ml;4h) prior to co-culture for 5d with T cells and assessed for T cell activation by both bromodeoxyuridine uptake and Th1/Th2 cytokine responses. PM and DEP exposure resulted in functional maturation of DC and BA and provoked a Th2-skewed response. DC but not Ba promoted enhanced T cell activation with exposure to APM or DEP alone compared to “resting” DC. Moreover, exposure of DC to APM or DEP followed by OVA, enhanced T cell activation compared to control DC or DC stimulated with LPS or CD40L (positive controls). While Ba could stimulate T cells, DC responses were more sensitive to PM exposure. We conclude that DC are activated on exposure to APM and stimulate enhanced CD4+ T cell activation in the absence of OVA antigen loading. In DC primed with APM or DEP and subsequently loaded with OVA, T cell activation was markedly enhanced as compared non-OVA pulsed DC or DC not exposed to PM. The autologous OTL12 DC/T cell antigen presentation assay is a sensitive bioindicator of the immunotoxic effects of environmental pollutant particles in vitro. This assay could be applied to hazard identification and risk assessment of previously untested PM species. (This abstract does not reflect EPA policy).

**1653 THE ENHANCEMENT OF CD8-POSITIVE T-CELL PROLIFERATION CAUSED BY TRICHLOROETHYLENE.**


The prevalence rate of allergic disorders is increasing in industrial areas and countries. Recent reports suggest that some environmental pollutants are related to the increase in allergic diseases. Trichloroethylene (TCE) is widely used in many industries, and commonly detected as an environmental contaminant. TCE is known to have toxicity for the nervous system, liver, and kidneys, and also to show immunotoxicities like Th1 cells activation and inhibition of CD4 positive (CD4+) T cells apoptosis. We previously reported that TCE is a candidate chemical for causing the increase of allergic diseases, because TCE ingestion enhanced Th1 responses and antigen stimulated lymphocytes proliferation. However it has been unclear that TCE ingestion affects T cell responses. In the present study, we focused on the direct effect of TCE treatment on T cell proliferation in vitro. The splenocyte and lymphocyte proliferation with T cell receptor (TCR) stimulation were significantly increased by TCE treatment. The enhancement of proliferative response was remarkable in CD8 positive (CD8+) cells. In addition, the phosphorylation of Lck in the CD8+ cells was also increased by TCE treatment. The ratio of CD8+ cells was also significantly increased by TCE ingestion with immunization. These results show that TCE affects TCR stimulated cell proliferation and Lck phosphorylation that may cause the enhancement of sensitivity of CD8+ T cells to antigen. In conclusion, TCE exposure enhances TCR induced proliferation of CD8+ T cells to facilitate Lck phosphorylation and may cause disrupting in peripheral T cell activities.

**1654 INTERACTION OF ARYL HYDROCARBON RECEPTOR (AHR) WITH NF-κB-MEDIATED RESPONSES IN DENDRITIC CELLS (DC) AND TISSUES FROM MICE.**

C. Vogel1, 2, D. Wu1, S. Guth1, G. Yang2, P. Leung1, E. Gershwin1,2, I. Abel1, J. Herrmann-Stempn1,3, A. Hess1,3 and E. Matsunami1,4, 1Environmental Toxicology, University of California Davis, Davis, CA, 2Allergy and Clinical Immunology, University of California Davis, Davis, CA, 3Institute for Environmental Research, University of Dusseldorf, Dusseldorf, Germany, 4Department of Molecular Biosciences, University of California Davis, Davis, CA and 5Center for Health and the Environment, University of California Davis, Davis, CA.

The aryl hydrocarbon receptor (AHR) has been identified as an important transcription factor regulating the responses of immune cells including T cell and dendritic cells (DC). Most recent data indicate that ligand-dependent activation of AHR may drastically alter the classical LPS- and NF-κB-mediated inflammatory responses. Depending on the target gene the LPS-induced expression of CC or CXC chemokines as well as DC-specific cytokines and surface markers can be synergized or antagonized by activation of AHR with TCDD based on the interaction of AHR with NF-κB Rel proteins. Ligand specific effects of 6-formylindolo[3,2-b]carbazole (FICZ) and TCDD on inflammatory marker genes were found in human derived DC and BMDC from mice. Dose-response studies show that even a low-dose of TCDD or other AHR ligands (ω/Ω-showing any CYP1A1 induction) can drastically change the inflammatory response such as the induction of IL-8 elicited by a low dose of a pathogen like LPS. The role of AHR in LPS-mediated inflammatory responses has also shown been in AHR null mice. Results from a transgenic mouse model indicate that expression of a transgenic AHR Repressor (AHRR) construct may suppress inflammatory marker genes as well as constitutive expression of AHR regulated genes and cytokines. A possible role of AHRR in LPS- and NF-κB-dependent signaling will be discussed. In summary the data suggest that the AHR in crosstalk with NF-κB becomes especially critical under inflammatory conditions and that classical inflammatory signals may be regulated through TCR or NF-κB are dysregulated which may cause immunotoxic effects and the development of chronic inflammatory conditions like asthma, allergy or autoimmune diseases.

**1655 CIRCULATING MICRORNAS: A NEW CLASS OF BIOMARKERS FOR TISSUE-SPECIFIC TOXICITY.**

W. Hu1 and G. J. Fallas1, 1Drug Safety R&D, Pfizer, Inc., San Diego, CA and 2Safety Assessment, GlaxoSmithKline, Research Triangle Park, NC.

MicroRNAs (miRNAs) are endogenous, small noncoding RNAs that down-regulate gene expression. Some miRNAs are produced at high concentrations within cells in a tissue-specific manner, and such miRNAs have recently been reported to be remarkably stable in plasma or other accessible body fluids. More importantly, differential increases in circulating miRNA populations have been shown by numerous studies to be associated with different disease or toxicity phenotypes. For example, tumor-derived miRNAs have been shown to distinguish patients with cancer from healthy individuals. Plasma concentrations of miR-122, miR-133a, and miR-124 have recently been shown to correspond to injuries in liver, muscle, and brain, respectively. Elevation in cardiac-specific miR-208a in plasma has been implicated as a potential biomarker for early diagnosis of acute myocardial infarction in humans. Taken together, because of their size, abundance, tissue specificity, and relative stability in body fluids, miRNAs hold promise as a new class of accessible biomarkers to monitor tissue-specific injuries. Our panel will explore the importance of this topic by providing perspectives from both industry and government sectors on the application of miRNAs as potential tissue injury biomarkers, the promises and pitfalls of miRNAs, and technical issues related to miRNA profiling in tissue and biofluids. Finally, our discussion will end with the impact of miRNA biomarkers on drug development in nonclinical studies, and the potential translatability to safety assessment in clinical settings.

**1656 MICRORNACHANGES IN RAT MESENTERY AND SERUM ASSOCIATED WITH DRUG-INDUCED VASCULAR INJURY.**

M. Seichihno and R. Thomas, Safety Assessment, GlaxoSmithKline, Kingsville, PA.

Identification of a pathogenic mechanism of drug-induced vascular injury (DIVI) and acquisition of validated clinical and nonclinical noninvasive methods for monitoring vascular integrity are significant hurdles in drug development. Recently, miRNA have been analyzed from mesentery and serum taken from rats given vascular toxic drugs to study DIVI. Fenoldopam (FM), dopamine (DM), and yohimbine (YH) were selected as they alter hemodynamics due to agonism of DA1,
MicroRNAs (miRNAs), a recently discovered class of non-coding RNAs, play important regulatory roles in many cellular processes. Some miRNAs have also been found in various body fluids. The extracellular miRNAs are stable, the expression of some miRNAs is specific to tissues or biological stages, and the level of miRNAs can be easily assessed by various methods, which are some of the requisite features of good biomarkers. Using acetaminophen overdose as a model, we have demonstrated the possibility of using specific circulating miRNA levels in plasma to detect drug-induced liver injury. Despite many desirable properties for circulating miRNAs based biomarker, the most fundamental challenge to use such biomarker is the ability of reliably and accurately measure the level of specific miRNA. Some of the issues associated with miRNA measurement will be discussed.

For new drugs to be tested in male volunteers, it is necessary to assess their effects on male fertility. This is currently based primarily on testicular histopathology, which is invasive and time-consuming, doesn't enable longitudinal monitoring in preclinical species, and isn't appropriate in the clinic. Ideal biomarkers of testicular toxicity would reflect damage to seminiferous tubules, easily detectable in the blood, and show a robust response to damage. MicroRNAs (miRNAs), involved in regulation of gonadogenesis and spermatogenesis, have emerged as novel biomarkers of testicular toxicity and potential therapeutic targets. Expression levels of a panel of miRNAs were assessed in tests and plasma from immature and mature rats using Dynamic Array™ Integrated Fluidic Circuits (Fluidigm). miRNA expression levels in plasma from mature rats following castration were also monitored during an 8 week recovery period to confirm testicular specificity of miRNAs. In addition, testicular specific miRNAs were profiled in rat major tissues using ABI custom TaqMan low density array (TLDA). In a study we focus on dysregulated miRNAs in serum of rats following a single dose of 20mg/kg of Cisplatin (CP). Serum miRNA concentrations were measured with the TLDA method. The expressions of miRNAs in serum of CP treated rats were significantly dysregulated. In particular, a number of elevated circulating miRNAs in serum collected from CP-treated animals and previously demonstrated to be preferentially expressed in the testis. Additionally, their changes can be detected significantly earlier before histopathology was observed. These results provide further support for the utility of miRNAs as promising candidates as sensitive biomarkers of testicular injury.

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High-throughput screening (HTS) studies are providing a rich source of data that can be applied to in vitro profiling of chemical compounds for biological activity and potential toxicity. Chemical profiling in ToxCast covered 965 drugs-chemicals in over 500 diverse assays testing for biochemical activities, receptor binding activities, reporter gene activation and gene expression profiles, stress-response indicators, and perturbation in cell state and cellular function. Also included were assays to monitor effects in zebrafish embryos and pathways of differentiation in mouse embryonic stem cells. In vitro profiles (AC50 in μM) are compared with reference and in vivo data using machine-learning algorithms to identify patterns of biological activity and optimal feature selection for predictive modeling. Findings from Phase-I chemical library (309 compounds) reflect that developmental toxicity does not emerge from a simple molecular stream. Computer models are needed to capture the complexity of multicellular networks and the key events underlying dysmorphogenesis. EPAs Virtual Embryo project is building a framework for incorporating knowledge into computational systems models that integrate cellular and molecular dynamics with adverse outcome pathways across life stage and species. Cell-agent-based computer models that run a morphogenetic series of events with cell signaling networks and gene expression can be used to analyze complex relationships and enhance predictive models relevant to key developmental key pathways and processes. Progress has been demonstrated for systems such as limb-bud morphogenesis and angiogenesis. This abstract does not necessarily reflect US EPA policy.
targeted delivery of cytotoxic drugs (ADC, immunoliposomes), by engineering antibodies with dual specificity, or by enhancing antibody effector function by engaging T cells and effector cells using bispecific T cell engagers (BiTE®) or glycoengineered antibodies. Due to their smaller size (e.g., nanobodies), format (e.g., ADC, immunoliposome), or pharmacology (e.g., bispecific), the development of these novel biotherapeutics presents an unusual toxicological challenges such as off-target toxicity (e.g., ADC, immunoliposome), immunotoxicity (e.g., BiTE®), and preclinical immunogenicity. This workshop will discuss general considerations for how to conduct nonclinical pharmacology and toxicology studies for these novel unconventional biotherapeutics with special focus on immunosafety assessment and the impact of nonclinical findings on clinical development.

### 1668 A NOVEL ANTITHROMBOTIC NANOBODY® TARGETING VON WILLEBRAND FACTOR: PRECLINICAL EXPERIENCE AND TRANSLATION TO CLINICAL PHARMACOLOGY.


Nanobodies are therapeutic proteins based on the smallest functional fragments of heavy chain-only antibodies, which occur naturally in the Camelidae family. Ablynx’s first clinical candidate is a bivalent Nanobody targeting the platelet adhesive von Willebrand Factor (vWF). vWF is critically involved in a variety of thrombotic pathologies, such as platelet adhesion to areas of vascular damage in ACS/PCI and platelet string formation in TTP. The anti-vWF Nanobody entered two distinct clinical development pathways: as adjunctive treatment in the acute intervention and platelet string formation in TTP. The anti-vWF Nanobody shows a unique pharmacology in that (i) the availability of the target largely determines the systemic exposure, thus limiting over-dosing potential and (ii) the target concentration itself is modulated by the binding to the drug molecule. Preclinical safety and biomarker results have been predictive and scalable to clinical development. This presentation will focus on the safety evaluation strategies and challenges of this complex molecule, as well as insights into the toxicities observed in nonclinical animal models and their clinical implications.

### 1669 SAFETY EVALUATION OF ANTIBODY-DRUG CONJUGATES.

S. Willy, Roche Genentech, South San Francisco, CA. Sponsor: V. Veleta.

Antibody-drug conjugates (ADCs) constitute an expanding class of therapeutic molecules in preclinical and clinical development for oncology indications. ADCs are comprised of monoclonal antibodies conjugated to highly potent cytotoxic molecules through specialized linkers. These biologic-based drugs are designed to increase delivery of cytotoxic payloads to tumor targets in order to improve efficacy and minimize toxicity. This presentation will focus on the safety evaluation strategies and challenges of these complex molecules, as well as insights into the toxicities observed in nonclinical animal models and their clinical implications.

### 1670 ALTERNATIVE APPROACH FOR NONCLINICAL SAFETY ASSESSMENT OF MEDI-565 (MT111)—A NOVEL BISPECIFIC SINGLE-CHAIN BITE® ANTIBODY.

P.C. Ryan¹, S.A. Hammond¹, S. Renpine , P. Lutterbuese¹, M. Amann², M.D. Oberste¹, K. Mulgrew², R. Criste¹, S. Fuhrmann¹, N. Lee¹, R. Gross¹, M. Liang¹, A. Schneider¹, R. Dikit¹, P.A. Baumerle¹, B. Ranted¹, S. Coats¹, L. Roskos¹, B. Jallal¹ and L. Richmann¹, ¹Medimmune LLC, Gaithersburg, MD and ²Micromet AG, Munich, Germany.

MEDI-565 (MT111) is a novel bispecific single-chain antibody of the BiTE® (bispecific T-cell engage) class that transiently links carcinoembryonic antigen (CEA; also called CEACAM5, CD66e) on cancer cells with CD3 on T-cells. Binding of MEDI-565 to CEA and CD3 results in T-cell-mediated killing of cancer cells expressing CEA. MEDI-565 specifically binds to human and cynomolgus monkey CEA with high affinity but not to any other member of the CEACAM family; rodents do not express CEA. MEDI-565 binds to human CD3, but does not bind to CD3 of cynomolgus monkey or mouse. Consequently, no pharmacologically relevant animal species exists for testing the toxicity of MEDI-565. In an effort to introduce a pharmacologically relevant model, two surrogate antibodies were made, cyS111 and hyS111, with specificity to monkey or mouse CD3, respectively. However, the characteristics of these two antibodies were different from those of MEDI-565 to an extent that it was determined that hyS111 and cyS111 would not be used for nonclinical studies. Therefore, it was not possible to conduct in vivo toxicity studies in a relevant animal model with either MEDI-565 or with the two surrogate antibodies. Rather, MedImmune implemented a strategy that utilized an in vitro approach to assess nonclinical safety instead of performing in vivo toxicity studies. Results from these studies were successfully used to select an appropriate starting dose for Phase 1 clinical studies of MEDI-565 for the treatment of patients with cancers expressing CEA.

### 1671 NONCLINICAL SAFETY APPROACHES IN DEVELOPING TARGETED IMMUNOLIPOMES.

K. J. Olivier, Merrimack Pharmaceuticals, Cambridge, MA.

Encapsulated small molecules has been shown to increase efficacy and decrease serious adverse events in patients. Doxil®, an approved liposomal encapsulated formulation of doxorubicin (pegylated liposomal doxorubicin; PLD), reduces the risk of heart damage while remaining effective against various cancers. Immunoliposomes take this innovation one step further by directing liposomes to bind to targets overexpressed by tumor cells and deliver an active pharmaceutical ingredient. MM-302 is a liposomal encapsulated formulation of doxorubicin with attached anti-HER2 antibodies. MM-302 is designed to bind to cancer cells that overexpress HER2, thereby releasing doxorubicin at the site of the tumor. In vitro studies have confirmed a threshold level of HER2 receptor expression to enable MM-302 docking and internalization requiring ≥200,000 HER2 receptors per cell. Interaction of MM-302 with normal cells, which express ≤200,000 HER2 receptors per cell, is limited, further reducing interaction with non-target cells. To support Phase I clinical trials, two single dose studies, one rodent and one non-rodent, were conducted. The dose levels for these studies represent multiples (up to 3 times higher) of anticipated dose levels in the clinic (~30 mg/m2). Toxicity was assessed by evaluating mortality, morbidity, clinical observations, body weights, food consumption, physical and ophthalmic examination observations, ECGs, clinical pathology (hematology and serum chemistry), urinalysis, local injection site tolerance, organ weights, and macroscopic and microscopic anatomic pathology. Based on lower body weights (18.7%) and lower testes weights with corresponding microscopically observed germ cell depletion, the no observed adverse effect level (NOAEL) was 1 mg/kg for male and 8 mg/kg for female rodents. Based on dermal and gastrointestinal findings, the no observed adverse effect level (NOAEL) was 4 mg/kg in non-rodents. Using immunoliposomes to alter the ADME kinetics of highly effective small molecule APIs can better meet the needs of our patients. The strategy and challenges in the discovery and development of MM-302 will be described.
ENVIRONMENTAL EXPOSURES AND DISEASE PREVALENCE IN HISPANIC ALONG THE TEXAS-MEXICO BORDER.

K. S. Ramos1 and I. N. Ramos2, 1Biochemistry and Molecular Biology, University of Louisville, Louisville, KY and 2School of Public Health and Information Sciences, University of Louisville, Louisville, KY.

The United States-Mexico border region comprises an area that stretches 2,000 miles from San Isidro California to Brownsville, Texas. Texas has the largest number of colonias relative to other states along the shared U.S. border with Mexico, with approximately 1,800 colonias, and more than 500,000 residents along the U.S. side of the Texas-Mexico border. The presence of health problems along the border is complicated by social, economic, and cultural factors. Data from a cross-sectional study examining the impact of pesticide exposures on disease prevalence among Hispanic residents of Cameron Park, a community along the Texas-Mexico border, revealed that asthma and allergies are among the most prevalent respiratory diseases reported in both adults and children. Other diseases affecting the community in higher numbers include breast cancer, diabetes, heart disease and high blood pressure. Among children, the most prevalent health conditions are asthma, followed by lung diseases, allergies, and to a lesser degree, skin rashes. These findings are consistent with known genetic susceptibilities to disease among Hispanics and emphasize the importance of gene-environment interactions in the incidence and severity of disease among Hispanics. The data have been used in the development of education and public health intervention programs to address the medical and social needs of Hispanics in this community.

GENOMIC INSTABILITY IN MEXICAN-AMERICAN CHILDREN EXPOSED TO ENVIRONMENTAL TOXINS.

M. A. Hernández-Valero. Department of Health Disparities, University of Texas MD Anderson Cancer Center, Houston, TX. Sponsor: A. Cuevas.

Associations between chronic exposure to environmental toxins such as, organochlorines (OC), organophosphates (OP) and polychlorinated biphenyls (PCBs) and genomic instability were studied in a population of thirty Mexican-American children (5-18 yrs old) living in rural Lower Rio Grande Valley (LRGV), Texas near various Superfund sites and thirty age-matched control children. Preliminary data from serum and urine samples indicate Mexican-American children living in close proximity to the Superfund sites and agricultural fields have higher frequencies of chromosomal aberrations than Mexican-American children of the matched age and gender living further from these sites. The exposure to OCPs, OPs, and PCBs was determined by quantitative telomeric DNA analysis via fluorescence in situ hybridization (FISH) to determine if exposure to the aforementioned toxins is potentially associated with shortening of telomeres. Short dysfunctional telomeres have been known to induce genetic instability, which may lead to the development of cancer. Chromosomal damage revealed mutagenic impact, suggesting a genotoxic effect from a complex occupational pesticide exposure. In general, these findings indicate the importance of personal protection, during high-exposure re-entry activities, in preventing pesticide uptake and genetic damage.

PRENATAL EXPOSURE TO ORGANOPHOSPHATE PESTICIDES AND IQ IN 7-YEAR-OLD LATINO CHILDREN.

B. Eskenazi1, M. F. Bouchard1 2, J. Chevrier1, K. G. Harley1, K. Kogut1, N. Ved1, N. Calderon1, C. Trijillo1, C. Johnson1, A. Bradman1 and D. Boyd Barr1. 1School of Public Health Division of Epidemiology, University of California Berkeley, Berkeley, CA, 2Environmental and Occupational Health Sciences, Sainte-Justine Hospital Research Center & Université de Montréal, Montréal, QC, Canada, 3Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS), Clínica de Salud del Valle de Salinas, Salinas, CA and 4Rollins School of Public Health, Emory University, Atlanta, GA. Sponsor: A. Cuevas.

This study examined associations between prenatal and postnatal exposure to OPs and cognitive abilities in school-age children. By utilizing a birth-cohort study (CHAMACOS) among predominantly Latino farmworker families from an agricultural community in California, exposure to OPs was measured by measuring di-alkyl phosphotriester (DAP) metabolites in urine collected during pregnancy and from children at 6 months and 12, 2, 3½, and 5 years. Administration of the Wechsler Intelligence Scale for Children-IV to 329 seven-year old children indicated that averaged maternal DAP concentrations were associated with poorer scores for Working Memory, Processing Speed, Verbal Comprehension, Perceptual Reasoning, and Full Scale IQ. Children in the highest quintile of maternal DAP concentrations had an average deficit of 7.0 IQ points compared with those in the lowest quintile. Prenatal urinary DAP concentrations were associated with poorer intellectual development in 7-year-old children.

INHALATION EXPOSURE OF PESTICIDES AMONG HISPANIC MOTHERS AT THE US-MEXICO BORDER.

C. Miller. Family & Community Medicine, University of Texas Health Science Center, San Antonio, TX. Sponsor: A. Cuevas.

Air sampling and analysis for 45 pesticides in the homes of 25 pregnant Hispanic women at the US-Mexico Border revealed low level exposures to multiple classes of pesticides. Further, using a validated instrument—the Quick Environmental Exposure and Sensitivity Inventory (QUEST), it was demonstrated that mothers of children with autism and ADHD report more intolerances for everyday, low level chemical exposure, compared to control mothers. Such intolerances have been linked to differences in genetic polymorphisms and detoxification capacity. Such polymorphisms implicated are paraoxonase 1 and glutathione S-transferase 1 gene interaction, which have also been linked to pre-term delivery.

PESTICIDE EXPOSURE AND HEALTH EFFECTS OF CHILDREN LIVING IN AN AGRICULTURAL COMMUNITY.

D. Rohman, Center for Research on Occupational and Environmental Toxicology, Oregon Health & Science University, Portland, OR.

There is increasing concern regarding the widespread use of pesticides in agricultural communities and potential impact on public health. Children of agricultural workers have a higher risk of exposure to pesticides than the general population because of take-home exposures and, often, proximity of their homes to fields where pesticides are applied. This study examines health effects of children living in an agricultural community. Potential exposure to pesticides was characterized by parent’s occupation, pesticide concentrations in home dust samples and a qualitative measure of lifetime exposure. Neurobehavioral performance was examined to characterize developmental progress and the impact of agricultural exposures on health.

HOW USEFUL ARE LIVER IN VITRO MODELS FOR TOXICITY AND MODE-OF-ACTION PREDICTION?

C. Corton1 and W. Dekant2. 1US EPA, Research Triangle Park, NC and 2University of Würzburg, Würzburg, Germany.

The liver is a common target for chemicals and drugs and is frequently the most sensitive tissue target in two-year biosays. Given the number of chemicals that need to be assessed for hepatotoxicity by the pharmaceutical and chemical industries, investigators currently use many types of in vitro rodent and human models, from simple primary hepatocytes and hepatocyte-derived cell lines to more complex three dimensional cocultures containing many liver cell types. These in vitro models have been used to predict different types of liver toxicities including cancer in rodents and cytotoxicity in humans. Additionally, transcript profile information is routinely used to identify altered pathways and to predict mode of action. As advances are rapidly being made in this area, we must assess the current strengths and limitations of these in vitro models and methods to predict chemical toxicity in the intact liver. To begin, we will explore the European perspectives partly, driven by REACH, which will include a discussion of the value of stem cell-derived human hepatocyte models. We will then turn our attention to the US EPA and industry perspectives, focusing on pathway prediction and next generation coculture models. The final overview will provide a platform to discuss the bioinformatic tools to mine databases used to predict drug hepatotoxicity from the perspective of the US FDA. At the conclusion of this session, our panel of experts will engage participants in an interactive discussion about the topic. The diversity of speakers will allow perspectives from a number of groups whose work is driven by various regulatory pressures. This session is sure to be of interest to those interested in high-throughput screening, hepatotoxicity, toxicogenomics, and mode of action research.

FROM PRIMARY CELLS TO HUMAN STEM CELL-DERIVED MODELS: TOWARDS MORE PREDICTIVE IN VITRO MODELS OF LIVER TOXICITY.

S. Mueller. Merck, Darmstadt, Germany.

Today’s solution of non-clinical in vitro and in vivo safety testing is hampered by use of insufficient surrogate models resulting in limited prediction and high attrition rate in drug development mainly due to poor prediction of safety and lack of efficacy in humans. In recent years we have seen increased availability of human model systems based on stem cell derived models and/or 3D-co-cultures. The rapid improvement of generating and analyzing multiplexed data sets covering a broad

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range of safety markers has also made significant advances. In our current projects we combine multiple endpoints derived from chemically- and genetically-engineered cell and other human derived model systems, generating a large reference compound database that can be used to improve human safety prediction. The initial focus of our work has been on genotoxicity, mitochondrial toxicity and steatosis/phospholipidosis using high content imaging as well as multiplexed protein quantification. We have established a panel of predictive markers for genotoxicity using Illumina whole genome arrays and are applying those to generate a subset of sensitive and specific protein markers predictive for human effects. Looking at specific toxicity markers for perturbation of signaling pathways in relevant human cells proved to be instructive for de-selecting compounds in drug discovery as well as guiding compound design.

**1680 INTERINDIVIDUAL VARIABILITY IN GENOMIC RESPONSES IN HUMAN PRIMARY HEPATOCYTES AND COMPARISON WITH HUMAN CELL LINES.**

J. van Delft, M. Jetten and J. Kleijnjas. Maastricht University, Maastricht, Netherlands.

Within the human population, susceptibility towards hazardous effects caused by chemicals is highly variable. In risk assessment procedures, uncertainty factors are taken into account for adjusting for these potential inter-individual differences. Genetic and life-style factors are considered to heavily contribute to this variability. It is unknown if inter-individual epigenetic differences, e.g., in DNA methylation patterns, affect susceptibility. Therefore, within the European Union project carcinoGENOMICS we examined the dose-dependent genomic responses in primary human hepatocytes (PHH) from 13 human donors exposed to toxic compounds (i.e. AhR, CAR, PXR (1AhR), and Retinoid X Receptor (RBP)) in relation to phenotypic markers of toxicity and baseline DNA-methylation profiles. A benchmark dose approach was applied to study responses at gene and canonical pathway level, which was correlated to DNA-methylation patterns. Thus, this study provides information about the variation in genomic responses within the human population. A comparison of the responses in PHH to those in human hepatocyte derived cell lines, may provide information on the representativeness of these cell lines. Historic data from the Netherlands Toxicogenomics Centre on HepG2 and HepaRG cell lines, show that baseline gene expression levels are very different from PHH, both for whole genome profiles, as well as for specific functional gene sets like phase 1/2/3 genes. Genomic responses to BaP exposure differ profoundly between all three human liver models, for genome-wide effects as well as in specific sub-sets of genes including those involved in DNA damage response, apoptosis and biotransformation. In contrast, the cell line responses to BaP were very similar to those of PHH. In summary, our studies show that the toxicogenomic and phenotypic responses to AhR and BaP in vitro show major inter-individual variations. Also, depending on the compound, gene-responses in PHH are different from those in HepaRG or HepG2 human liver cell lines.

**1681 GENETIC IDENTIFICATION OF PATHWAY SIGNATURES AND ASSESSMENT OF EFFECTS IN MOUSE AND HUMAN IN VITRO LIVER MODELS.**

C. Corton, EPA, Research Triangle Park, NC.

Assessing pathway activation after chemical exposure using toxicogenomics techniques is widely used in toxicity studies. Pathway perturbation is sometimes predicted using software programs that suffer weaknesses in their predictive capabilities, and need improvement. Genetic and toxicogenomic methods, toxicogenomics and in silico approaches. The goal of the project is to integrate diverse data derived from different experimental platforms for identification of DILI biomarkers for FDA-approved drugs. The primary goal is to provide a wealth of mechanistic information and biomarker models for the study of liver toxicity with a potential to be a resource for FDA to utilize and reference when liver toxicity issues arise during various stages of the regulatory review process. Several topics will be discussed in this presentation: (1) Can genomic biomarkers from rat liver discriminate drug candidates that have the potential to cause DILI in susceptible patients from drugs that do not despite the lack of conventional indicators of liver toxicity in preclinical studies; (2) Can the FDA drug labeling system be used to assess DILI potential of a drug?; (3) Can clinical findings from the post-marketing stage be used to remove the drug candidates with high DILI risk in the preclinical setting; (4) Can the application boundary be defined for in vitro assays to discriminate drugs that cause severe DILI in humans from those that don’t.

**1682 PREDICTION OF COMPLEX TOXICITIES USING 3D LIVER COCULTURE AND TOXICOGENOMICS.**


The two-year rodent bioassay is the traditional method for evaluating carcinogenic activity and chronic toxicity of chemicals. However, many chemicals of concern have not been tested due to the high costs and low throughput associated with this assay. To increase throughput primary hepatocyte cultures are often used but they have serious limitations of comparison of transcriptional profiles of primary hepatocytes to toxicity signatures and pathways obtained from profiling rodents dosed with over 660 compounds (DrugMatrix, Entelos, Inc, Foster City, CA) demonstrated their limited predictive power. The monolayer rodent hepatocytes were able to match in vitro directional changes of basic toxicity responses but lacked the tissue architecture required to model in vivo responses. False scores for the in vitro DrugMatrix signatures as well as in toxicity pathway analysis (GeneGo, Inc., St. Joseph, MI) resulted. A more physiologically relevant in vitro hepatic model is obtained by coculturing hepatocytes and microenvironmental liver cells on 3D scaffolds (3DLC) to form liver tissue that maintains hepatic function for months, providing an in vitro alternative to predict the chronic toxicity potential of compounds. Transcriptional profiles of rat 3DLC and 2D hepatocytes treated with 26 of the 660 DrugMatrix chemicals giving rise to complex toxicity pathways demonstrated the 3DLC provide in vitro predictive responses as opposed to 2D hepatocytes which provide partial or no prediction. The compound set included inflammatory and DNA damaging agents, cholesterol biosynthesis inhibitors, estrogen receptor agonists and CAR/PXR, AhR, and PPAR-alpha agonists. As examples, 3DLC treatments match DrugMatrix gene signatures indicative of hepatic inflammatory infiltrate, whereas primary hepatocyte treatments do not. P450 gene expression is repressed both in vivo and in 3DLC by LPS, TNFα, and IL-6, but not in primary hepatocytes. Overall, 3DLC are more similar to in vivo with regard to toxicity response than are primary hepatocytes.

**1683 TRANSLATIONAL BIOMARKERS FOR DRUG-INDUCED LIVER INJURY.**

W. Tong, US FDA, Jefferson, AR.

Although conventional preclinical animal testing identifies many types of toxicity preventing human exposure to dangerous compounds, none are completely effective at assessing examples of drug-induced liver injury (DILI), evidenced by the high percentage of drugs that fail in clinical trials or are withdrawn from the market due to liver toxicity. A large effort has been made in the FDA's Liver Toxicity Knowledge Base (LTKB) that explores various translational biomarkers for DILI with in silico methods, toxicogenomics and in vitro approaches. The goal of the project is to integrate diverse data derived from different experimental platforms for identification of DILI biomarkers for FDA-approved drugs. The primary goal is to provide a wealth of mechanistic information and biomarker models for study of liver toxicity with a potential to be a resource for FDA to utilize and reference when liver toxicity issues arise during various stages of the regulatory review process. Several topics will be discussed in this presentation: (1) Can genomic biomarkers from rat liver discriminate drug candidates that have the potential to cause DILI in susceptible patients from drugs that do not despite the lack of conventional indicators of liver toxicity in preclinical studies; (2) Can the FDA drug labeling system be used to assess DILI potential of a drug?; (3) Can clinical findings from the post-marketing stage be used to remove the drug candidates with high DILI risk in the preclinical setting; (4) Can the application boundary be defined for in vitro assays to discriminate drugs that cause severe DILI in humans from those that don’t.

**1684 NONCLINICAL AND CLINICAL APPLICATIONS OF TRANSLATIONAL ORGAN-BASED IMAGING.**

S. D. Pettit, HESI, Washington, DC.

Multimodal imaging is a widely applied and accepted standard of care in many medical settings. Innovations in imaging capabilities have developed rapidly, allowing noninvasive collection of an ever increasing quantity and quality of morphologic, functional, and even molecular data from humans and animals. Accordingly, imaging is becoming an important component of the clinical biomarker toolbox. However, advances in imaging strategies that have allowed for use in animals, including rodents, have not driven a large-scale integration of these capabilities into modern toxicology assessment or environmental hazard identification. Although a number of imaging and biomarker “opportunities” are outlined in the FDA’s “Critical Path Opportunities List,” an organized effort to explore integration of imaging into nonclinical safety assessment and hazard evaluation paradigms is just beginning. We will provide an overview of an organ-based approach to novel imaging
methodologies in nonclinical safety assessment and translational toxicology. The presentations will describe how preclinical imaging can be an innovative tool for toxicity assessment, and how translational imaging can be used to bridge the gap between nonclinical safety assessment and clinical testing.

**1685 CARDIOVASCULAR IMAGING IN NONCLINICAL SAFETY STUDIES: INCREASING ACCEPTANCE AND APPLICATION.**
Cardiac safety issues are a leading cause of both drug candidate attrition and approved drug withdrawal from market. Echocardiography (EchO) and cardiac magnetic resonance imaging (CMR) are frequently used to determine the effects of drug candidates on cardiac structure and function in efficacy and basic science studies, and further improve the benefit of translation to human studies. However, these imaging platforms are used infrequently to determine cardiac structure and function in drug candidate safety studies. The HESI Imaging committee determined in order for these imaging platforms to gain broader acceptance and use in safety studies their ability to accurately and repeatedly quantify drug-induced changes in cardiac structure and function needs to be demonstrated. This presentation will discuss the issues of variability in imaging studies and the value that Echo and CMR can provide. It will describe the HESI Imaging committee initiative to determine the ability of CMR and Echo to accurately and repeatedly determine drug-induced changes in cardiac structure and function at multi-sites.

**1686 MULTIMODAL IMAGING IN DEVELOPMENTAL AND REPRODUCTIVE TOXICOLOGY.**
High-resolution preclinical versions of commercial imaging modalities, such as micro-computed tomography (micro-CT), high-field magnetic resonance imaging (MRI) and ultrasound bio-microscopy (USM), provide unique imaging approaches in developmental and reproductive toxicology (DART). In preclinical imaging studies, examples include: micro-CT volumetric visualization and measurement for evaluation of murine skeletal development, quantitative high-resolution micro-CT measurements for bone microarchitecture biomarkers, and three-dimensional digital tomography imaging-based virtual Wilson slicing analysis. High-resolution MRI may enable whole-body “virtual morphologic analysis” of embryos, fetuses, and the reproductive organs of the mouse, rat or rabbit. With real-time noninvasive in vivo imaging capability, USM could provide imaging approaches for early “live morphologic analysis” of embryos and reproductive organs of the mouse, rat or rabbit, and measurement of some embryonic cardiovascular functions by utilizing multiple ultrasonic Doppler methods to evaluate normal and abnormal cardiovascular development. Other imaging modalities, such as whole-body optical imaging, can also provide innovative solutions for investigating developmental and reproductive toxicity. For example, development of a highly sensitive optical imaging method to investigate potential mouse embryo and fetus exposure to a compound via the intra-vaginal route. Although there are still practical issues and technical limitations, with advancement of multimodal imaging technologies and the modernization of DART workflow, these imaging applications demonstrate that a high-resolution preclinical imaging approach may eventually provide innovative solutions and novel imaging data for developmental and reproductive toxicology.

**1687 PRECLINICAL ASSESSMENT OF NEUROTOXICITY WITH IMAGING.**
Although the use of imaging in the clinical setting is well established, the use of imaging for pre-clinical assessments is infrequent. Even though the advantages such as using the animal as its own control, longitudinal study design and minimal invasive-ness are now well recognized, standardization of approach, resolution and quantita-tion have, until recently, slowed acceptance of imaging as a routine assessment tool. MicroPET now offers functional resolution of 1.5 mm and application of imaging to assessment of any target organ can deliver quantitative information in a mini-mally invasive manner and in parallel with other endpoint requirements. Recent re-port indicate that combined administration of isoflurane (ISO) and N2O triggers neuronal apoptosis in postnatal day (PND) 7 rats. Previously published in vitro, ex vivo and confocal fluorescent imaging studies suggest that the danusyl compounds can accumulate within the cytoplasm of the apoptotic cell. In this study, the effect of ISO and N2O on the uptake and retention of [18F]-DPENSH in the brains of different age rats were investigated using microPET. On PND 7, rat pups in the experimental group were exposed to a mixture of 70% N2O/30% oxygen and 1% ISO for 8 hours and control rat pups were exposed to room air only. On PNDs 14, 21 and 28, [18F]-DPENSH (18.5 MBq) was injected i.p. and thirty minutes later microPET images were obtained over 90 minutes. In PND 14 rats the uptake of [18F]-DPENSH was significantly increased in the ROIs in gene set hypothesis-treated rats. No significant difference was found in radiotracer uptake in the frontal cortex of the brains of PND 21 and 28 rats compared with same aged controls. This result, which is consistent with our previous TUNEL studies, demonstrates that enhanced apoptotic effects are apparent within a week of anesthetic exposure (PND 7-14) and that no significant neuronal apoptosis remains obvious on or after 2 weeks of exposure. Thus microPET imaging can determine the time course of neuronal apoptosis in a minimally-invasive manner.

**1688 IMAGING THE KIDNEY AND LIVER: METHODOLOGIES AND CURRENT PRACTICES.**
The kidney and the liver are the two organs of greatest toxicological concern in safety assessment. Historically, these organs have been the basic of the development of many modern biomarkers. Because of their relative size and location, they have been amenable to nonclinical and clinical multimodal imaging applications. The basic tenants of translational imaging of these organs in nonclinical species and humans will be discussed.

**1689 TRANSLATIONAL CLINICAL IMAGING.**
E. Hoffman. University of Iowa, Iowa City, IA. Sponsor: S. Pettit.
The ultimate goal of nonclinical assessment and biomarker development is to translate early toxicity knowledge into non-invasive means to monitor potential changes in humans during clinical trials. Novel imaging techniques used in non-clinical assessment can be used to bridge the gap to clinical testing. Likewise, imaging modalities developed for use in humans can be translated to approaches in non-clinical species, further expanding the toolbox available for early testing. The presentation will describe translational imaging approaches that have successfully been used to bridge the gap to clinical research.

**1690 NOVEL TOPICS IN ENVIRONMENTAL POLYCYCLIC AROMATIC HYDROCARBON METABOLISM LEADING TO CARCINOGENESIS.**
D. Carlin1 and B. Moorthy, 1NIEHS, Research Triangle Park, NC and 2Pediatrics, Baylor College of Medicine, Research Triangle Park, TX.
Epidemiological evidence indicates that exposure to complete environmental polycyclic aromatic hydrocarbon (PAH) mixtures increases the risk of lung cancer. However, most animal studies have focused on single PAH components. Moreover, little is known regarding the interactions between different PAH metabolites that lead to carcinogenesis. Thus, our panel of experts will provide information on this subject matter that will explore the mechanisms of toxicity mediated by PAH mixtures. Investigations on binary mixtures of PAHs, as well as PAH-natural product mixtures, will be described with regard to their inhibition of cytochrome P450 re-actions of the P4501 family. The roles of critical cell signaling and growth pathways, and metabolic enzymes involved in benzo[a]pyrene (B[a]P) activation pathways in lung tumorigenesis in mice and humans will be examined. Strategies to differentiate how PAHs may affect the ontogeny of enzymes during fetal development and result in epigenetic alterations will also be discussed. Specifically, there will be discussion of how target organ doses of PAHs and ontogeny of PAH-metabolizing enzymes in the fetus impact PAH-dependent alterations in the fetal transcrip-tome and epigenome. It will be important to address how risk assessment ap-proaches can use data from in vitro, detection, exposure, and ‘omics studies to predict the carcinogenicity and relative potency of environmental PAHs and the global perspectives of PAH exposures in human populations. For example, while exposures to PAHs are generally well controlled in the developed world, this is not the case in some areas of Eastern Europe and China, where exposure levels can be extremely high, with predictable consequences for human health. Collectively, it is expected that the presentation of these topics will engage attendees in understanding the need for new research on PAHs to understand the mechanisms and modes of action of PAHs in disease etiology; to better establish the risk associated with PAH exposure; and to develop novel strategies for the prevention and/or treatment of cancers and other diseases caused by environmental PAHs.
1691 INTERACTIONS OF POLYCYCLIC AROMATIC HYDROCARBONS.
F. P. Guengerich1, T. Shimada2 and H. Yamazaki3. 1Biochemistry, Vanderbilt University School of Medicine, Nashville, TN, 2Cellular and Molecular Biology, Okayama Prefecture University, Okayama, Japan and 3Laboratory of Drug Metabolism and Pharmacokinetics, Showa Pharmaceutical University, Tokyo, Japan.

Polycyclic aromatic hydrocarbons (PAHs) are of interest in light of their historical and documented roles in cancer etiology, both in experimental animal models and in humans. However, almost all studies have focused on single PAH components, e.g., benzo[a]pyrene. We have investigated binary mixtures of PAHs, as well as PAH-natural product mixtures, in particular regarding their inhibition of cytochrome P450 (P450) reactions, especially in the P450 Family 1 enzymes, which are the main catalysts of relevance in both bioactivation and detoxication reactions (i.e., 1A1, 1A2, 1B1). Individual PAHs inhibit P450s by competitive and mechanism-based inactivation modes and in some case the inhibition constants are in the sub-μM range, suggesting relevance at PAH concentrations that would be relevant in vivo in practical settings. The inhibition affects the activation and detoxication of other PAHs and is thus relevant to the complex PAH mixtures of most interest, e.g. tobacco smoke, engine exhaust. The exact mechanisms of mechanism-based inhibition are under investigation. Other research involves the ability of natural products and synthetic chemicals to inhibit these P450s involved in the activation of PAHs and other carcinogens. Mixtures can show synergistically higher activation than single PAHs or lower toxicity, depending on the P450s and PAHs involved. These findings, along with the phenomenon of induction of the P450s involved in the oxidations, are important in understanding the toxicity of mixtures of PAHs and ultimately risk assessment, in that studies with single PAHs may not accurately reflect the risks of mixtures. (Supported in part by U01 R01 ES000267).

1692 COMBINED USE OF TRANSCRIPTIONAL AND TOXICOLOGICAL DATA TO AUGMENT THE METABOLIC AND MECHANISTIC UNDERSTANDING OF BENZO(A)PYRENE-INDUCED MOUSE LUNG CANCER AND ITS RELEVANCE TO HUMAN LUNG CARCINOGENESIS.

There is sufficient epidemiological evidence supported by experimental data that exposure to some PAH-containing complex environmental mixtures increase the risk to human lung cancer. The International Agency for Research on Cancer (IARC) has determined that human respiratory cancer has been strongly associated with cigarette smoking. Moreover, benzo(a)pyrene (BaP), a mutagenic (and non-epigenetic) mechanism of PAH-dependent transplacental carcinogenesis and in prevention by maternal dietary supplementation. (Supported by NIH grants P01 CA90890 and P42 ES016465).

1693 TRANSCPLANTAL CARCINOGENESIS DUE TO MATERNAL EXPOSURE TO INDIVIDUAL PAHS AND ENVIRONMENTAL MIXTURES.
D. E. Williams. Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, OR.

PAHs are documented transplantal carcinogens in rodent models, and epidemiological evidence associates exposures of pregnant women to PAHs with enhanced susceptibility to chronic disease later in life. Individual PAHs, such as benzo[a]pyrene (BaP, IARC-1) and dibenzo[def,p]chrysen (DBC, IARC-2A), as well as environmental PAH mixtures, can be transplantal carcinogens, and the target organ and potency is a function of not only dose, but also timing of exposure. We have developed a model of transplantal chemoprotection against PAHs by supplementation of the maternal diet with indole-3-carbinol (I3C) or 3,3'-diindolylymethane (DIM) from cruciferous vegetables. We present results with this model and further mechanistic questions associated with chemoprotection, both in the in vivo mouse model and in an vitro model employing a human T-cell lymphoblastic leukemia cell line. Our hypothesis was that both the PAH-dependent transplacental carcinogenesis and the chemoprotection provided by I3C and DIM were due, at least in part, to epigenetic mechanisms. We employed a Nimbomodel representation array to assess the impact of exposure of human T-ALL cells to I3C or DIM. These inducts tended to increase the degree of hypomethylation compared to untreated cells, consistent with a decrease in DNA Methytransferase 1 (DNMT1). One of the genes which exhibited treatment-related decrease promoter methylation was Cyp1b1. A number of the genes identified to exhibit a treatment-dependent increase or decrease in methylation were consistent with genes known to be important in human leukemias and lymphomas. Studies underway are examining complex environmental PAH mixtures, including those collected from water by passive sampling devices (a Superfund site at Portland Harbor and the Gulf of Mexico), as well as personal monitors. We are continuing to assess epigenetic (and non-epigenetic) mechanisms of PAH-dependent transplantal carcinogenesis and its prevention by maternal dietary supplementation. (Supported by US EPA).

1694 CURRENT EFFORTS IN THE ESTIMATION OF HUMAN HEALTH CANCER RISK OF POLYCYCLIC AROMATIC HYDROCARBON (PAH) MIXTURES.

PAHs primarily occur in complex mixtures generated from the combustion or pyrolysis of substances containing carbon and hydrogen and have most recently been of interest due to concerns about exposure with respect to the Gulf oil spill and runoff from parking lot sealants. Assessment of the cancer risk from long-term human exposure to PAH mixtures would best be conducted with quantitative information on the dose-response relationship for cancer from exposure to the mixture of concern. There are very few toxicity data available for whole PAH mixtures and, in most cases, chemical analyses of the composition of mixtures are limited. In addition, PAH-containing mixtures tend to be very complex; the composition of these mixtures appears to vary across sources releasing these mixtures to the environment and in various environmental media in which they occur. Component-based approaches, involving an analysis of the toxicity of components of the mixture, are recommended when appropriate toxicity data on a complex mixture of concern are unavailable. In this approach, cancer risks from environmental PAH mixtures are estimated using relative potency factors (RFPs) for individual PAHs and a slope factor for benzo[a]pyrene is used to estimate the contribution from PAHs. USEPA's Integrated Risk Information System (IRIS) Program is undertaking an effort to update the available RPF approach for PAH Mixtures. A number of issues have been identified for discussion including: 1) choice of datasets, including the use of in vitro data, and criteria for use in deriving RFPs; 2) assessing the composition of PAH mixtures, including the % of the total PAH mixture not accounted for by the RPF approach; 3) extrapolating across exposure routes and estimating multiple-route risks; and 4) use of existing “omics” data to identify biological pathways and new biomarkers of exposure, effects, and susceptibility are under investigation. This abstract does not reflect EPA policy.

1695 A GLOBAL PERSPECTIVE ON PAHs: WHAT ARE THE ISSUES?
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The history of PAH toxicity and risk assessment is largely a success story in the developed world; once the problem was identified and understood then measures were taken to reduce human exposure and risk. Photographs of Pittsburgh in the 1930s illustrated the huge challenge we faced at that time and elsewhere in the developed world strict standards exist on emissions from fossil fuel burning. Unfortunately that was not the case in some areas of communist era East Europe and in China where generation and exposure levels both domestically and industrially were or can be extremely high, with predictable consequences for human health. For example, when biomarkers of exposure, effects, and susceptibility were measured in PAH-exposed populations from Prague (Czech Republic), Sofia (Bulgaria) and Kosice (Slovakia), personal exposures were described at levels that are associated with genotoxic effects such as DNA adducts, chromosome aberrations and ras oncogene overexpression, although personal exposures did vary markedly between countries and between the occupationally exposed (policeman, bus drivers) and the control populations in each country. In addition to exposure through traffic emissions, studies have shown a significant threat to health by exposure via commercial and domestic kitchens due to traditional Chinese cooking.
Methods that generate hot oil fumes. More recently, the 2008 Beijing Olympics have provided a unique opportunity to study the effect of control measures on the reduction in air pollution and subsequent health effects. Stringent controls on PAH source resulted in a marked reduction in the 17 carcinogenic PAHs with an estimated >40% reduction in estimated inhale cancer risk, if these measures were sustained over time. These observations provide momentum for intervention at source in the developing world to prevent further escalation of PAH exposure. In summary, it is key to highlight the marked differences in global perspective when examining exposure levels, body burdens and potential health consequences of PAHs and learn from the experience of the developed world.

**1696 CAREER ALTERNATIVES AND TRANSITIONS: NEW CHALLENGES AND OPPORTUNITIES IN TODAY’S JOB MARKET FOR TOXICOLOGISTS.**

R. D. Storer1 and J. A. Popp2. 1Safety Assessment, Merck Research Laboratories, West Point, PA and 2Stratxon LLC, Lancaster, PA.

New toxicology graduates have traditionally pursued a diverse spectrum of career opportunities in teaching and research, in industrial or contract toxicology laboratories, or in regulatory agencies and affiliated institutes. Downturns in the global economy, together with a wave of consolidation and downsizing in industry, particularly in the pharmaceutical sector, has created a challenging environment for job seekers. This has compelled new graduates, as well as toxicologists at all phases of their careers, to confront new challenges in securing initial or continuing employment in the area of specialization, consistent with their career goals. This session will explore alternatives available to new graduates as well as to established toxicologists facing career transitions. To begin this important dialogue targeted to new graduates, postdocs, and nontenured faculty, we will examine the challenges facing toxicologists pursuing career paths in research and teaching and will touch on alternative career paths for which the skills developed in completing a doctorate and toxicologists pursuing career paths in research and teaching and will touch on alternative career paths for which the skills developed in completing a doctorate and postdoctoral research in toxicology are transferable. Our panel will review the options and challenges facing the mid- to late-career industry toxicologist confronted with the prospect or actuality of layoffs or early retirement due to corporate downsizing. The focus of the talk will be on the current landscape for toxicology consultants or other independent scientists, emerging consulting companies, or individuals pooling resources to form new consulting groups. If you are considering transitioning your career in a regulatory agency, a review of the opportunities and challenges for toxicologists will be provided. The final talk will provide insight on the impact of cutbacks in the pharmaceutical industry and trends favoring outsourcing of toxicology testing on career development opportunities in contract research. At the conclusion of the talks, a significant amount of time has been set aside by the panelists to allow time for questions from participants.

**1697 CURRENT CHALLENGES IN PURSUIT OF CAREERS IN ACADEMIA.**

B. L. Kaplan, Center for Integrative Toxicology, Michigan State University, East Lansing, MI.

Academic research and teaching in toxicology is vital to the future of toxicology and can be very rewarding. However, this career choice is not without challenges, including the daunting tasks of securing funding and a tenure faculty position in today’s competitive environment. This presentation will examine the current landscape for academic positions in toxicology, opportunities and challenges facing recent graduates in securing academic employment, and other unique challenges in the academic environment that toxicologists face regardless of rank. The last part of the presentation will examine possible alternative paths for which graduate training in toxicology may be excellent preparation.

**1698 CAREER TRANSITION TO TOXICOLOGY CONSULTING.**

J. A. Popp, Stratxon LLC, Lancaster, PA.

Consulting is an exciting and rewarding career option but should be pursued only after careful consideration. First, the personality and personal needs of the potential consultant must be fully and honestly assessed. Such points as ability to work independently and the desire for frequent direct interaction with others must be considered. Second, a potential consultant must consider logistics of establishing a consulting practice including a decision on group versus independent activity, developing a legal entity such as an LLC, determining a focus for the consulting activity, assessing the value of liability insurance and developing a client base. Third, the successful consultant should have a realistic understanding of the diversity of expectations in the consulting marketplace. Future clients may have expectations that the consultant has expertise in basic medical science, in depth knowledge of toxicology in a vast array of toxicology sub disciplines and an understanding of the regulatory environment including in various areas of the world. Unrealistic expectations will be encountered. The fourth point for consideration is the changing workplace environment in toxicology. Although globalization of toxicology is now well advanced, this trend will continue unabated and probably at an enhanced rate in the future. Therefore, a potential consultant must be thinking in terms of career direction in the future not just based on current conditions. The changing future environment will provide opportunities for toxicology consultants with the proper attributes and expertise but also constitute a major pitfall for those who are less adaptable.

**1699 CAREER TRANSITIONS FROM INDUSTRY TO GOVERNMENT.**

H. N. Ghantous, Division of Antiviral Products, CDER, US FDA, Silver Spring, MD.

The speaker will describe her personal experiences and career transition from a Study Director in the toxicology laboratory of a large chemical company, to pesticide registration with EPA, to a reviewer/supervisor at FDA. The presentation will highlight the unique nature and challenges of each job with focus on the pros and cons of transition to different careers, and the importance of remaining active in the scientific community as your career progresses. The personal (job security, work/life balance, and job satisfaction) and professional (high-level scientific interactions, training and experience in regulatory science, likelihood of post-government employment) benefits of a career in the Federal Government will be presented. The value of scientific certification beyond an academic degree will also be discussed in the context of each career, including the benefits of a Diplomate of the American Board of Toxicology certification, or other Toxicology certifications that can open multiple doors for an advancing toxicology career.

**1700 CHALLENGES FACING TOXICOLOGISTS IN TODAY’S JOB MARKET: THE CRO ENVIRONMENT.**

S. K. Durham, Toxicology, Charles River Laboratories, Reno, NV.

The pharmaceutical and contract research organization (CRO) industry has witnessed dramatic changes in the recent past. Globalization has forced changes in the drug development paradigm. This situation coupled with a resource and cost constraint environment has led to a substantial increase in outsourcing to CROs. This presentation will focus on job opportunities for toxicologists in the CRO industry, and compare the CRO environment to the pharmaceutical industry from a business, career path, and scientific perspective. It will also include assessment of challenges and competition from developing nations regarding growth opportunities for toxicologists.

**1701 ADVANCEMENT IN DEVELOPMENTAL NEUROTOXICITY TESTING IN VITRO: DIFFERENTIATING MOUSE EMBRYONIC STEM CELLS INTO FUNCTIONAL NEURONAL NETWORKS.**

A. E. Seiler1, K. Hayes1, G. Podrygajlo2, B. Slawik1, U. Eger1 and A. Luch1. 1ZEBET, Federal Institute for Risk Assessment, Berlin, Germany and 2Biomicrotechnology, Department of Microsystems Engineering – IMTEK, University of Freiburg and Bernstein Center Freiburg, University of Freiburg, Freiburg, Germany. Sponsors: E. Frischknecht.

Increasing public concern on the potential adversity of chemicals, environmental pollutants and pharmaceuticals has spurred renewed efforts to assess deleterious effects on the developing nervous system. Developmental neurotoxicity (DNT) testing has thus become an important component in toxicological testing strategies and triggered DNT studies are recommended under REACH. In response to the growing needs for toxicity testing the establishment of alternative methods providing a
higher throughput became a main goal in DNT test development. Synaptogenesis and the formation of complex neuronal networks are essential processes in brain development. Cellular models that are able to recapitulate the development of active neuronal networks in vitro would therefore be a valuable addition to DNT testing arsenals. Recently, we developed an in vitro assay using mouse embryonic stem cells (mESC) to assess adverse effects of chemicals and other compounds on neuronal development. Molecular and mechanistic endpoints for proliferation and differentiation were successfully established. Here, we show that neurons originating from mESC express presynaptic as well as postsynaptic markers indicating the formation of functionally active synapses. Moreover, neural activity was demonstrated by testing for the response of these cells to stimulation of neurotransmitter receptors, and electrophysiological measurements using microelectrode arrays (MEA) proved that the differentiated cells are able to generate functional neuronal networks. Thus, the mESC model introduced here might represent a useful tool for assessing adverse health effects of xenobiotic agents that affect synaptogenesis and neuronal network formation.

EFFECTS OF DEVELOPMENTAL TOXICANTS ON MICRORNA EXPRESSION DURING NEURAL DIFFERENTIATION OF MURINE EMBRYONIC STEM CELLS (mESC).


Studying chemical disturbances during neural differentiation of mES cells has been established as ZEBET as an alternative in vitro testing approach for the identification of developmental toxicants. miRNAs represent a class of small regulatory RNA molecules, which bind to target mRNAs thereby repressing their translation. Many studies have shown an essential role of miRNAs in regulation of gene expression during neural development and ESC differentiation. Thus, neural differentiation of ESC in vitro allows investigating the role of miRNAs in chemical-mediated developmental toxicity. The main goal of this project was to analyze the expression of neural-specific miRNAs during neural differentiation of mESC while being exposed to the developmental neurotoxicants, valproate (NaVPA) and arsenite (NaAsO2). Analyzing miRNA expression profiles we could demonstrate that neural-specific or enriched miRNAs show substance-specific expression patterns during neural differentiation of mESC when cells were being exposed to valproate or arsenite. Whole genome analysis as well as analysis of miRNA expression revealed that valproate may switch the lineage specification from neural to myogenic differentiation (upregulation of muscle-enriched miRNAs mir-206, mir-133a and mir-10a and downregulation of neuro-specific miRNAs mir-124a, mir-128 and mir-137). The downregulation of mir-128a and mir-124a in cells treated with NaVPA was stronger compared to the concurrent downregulation of the neuro-specific mir-9 or neuron marker βIII-tubulin. Furthermore, using our in vitro cell system we could confirm an aberrant expression of known VPA target genes and involve in neural tube closure. We can conclude that miRNA expression profiling is a suitable molecular endpoint to detect neurotoxicity. Further analyses of VPA-specific effects on miRNA expression may contribute to a better understanding of VPA-mediated toxicity mechanisms.

THE DEVELOPMENTAL NEUROTOXICITY OF LEAD IN 3D RAT PRIMARY NEURAL CELL CULTURES USING TRANSCRIPTOMICS AND METABOLOMICS APPROACHES.

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Tox-21c proposed a paradigm shift in the field of toxicology. Instead of relying on traditional animal experiments, the report proposes the application of the latest advances in science and technology to develop more relevant test strategies. The concept is that pathways of toxicity (PoT) can be identified using in vivo cell systems and omics’ approaches. The so-called “pathways of toxicity” are defined as cellular response pathways that, in sufficiently perturbed are expected to result in adverse health effects. An area of toxicology where Tox-21c could have a significant impact is developmental neurotoxicity (DNT). There is concern that exposures to environmental chemicals contribute to the increasing incidence of neurodevelopmental disorders in children. However, due to lack of DNT studies only very few substances have been identified as developmental neurotoxicants. This study aimed to develop an in vitro approach using metabolomics and transcriptomics for DNT assessment. A 3D rat primary neuronal organotypic model was exposed to lead chloride from day 7 up to 21. Quantitative measurement of genes expressed in different cell types (nestin, NF-200, S100β and MBP) and mass spectrometry based metabolomics measurements were performed. Treatment with lead chloride significantly altered the mRNA levels of all genes studied. Moreover, the mass spectrometry analysis showed differences in metabolite levels between control and treated cells in a concentration dependent manner. Further analysis of the altered metabolites should give mechanistic insight into the DNT of lead. This study demonstrates that gene expression and metabolomic analysis can be sensitive endpoints for DNT assessment. This work was sponsored by the Swedish Research Council and FDA.

AN AUTOMATED CELL-BASED HIGH-THROUGHPUT SCREEN FOR IDENTIFYING POTENTIAL DEVELOPMENTAL NEUROTOXINS.


There is an overwhelming backlog of environmental chemicals/contaminants that require testing for the potential to cause developmental neurotoxicity (DNT). Current in vivo methods are costly, time-consuming and require large numbers of animals to be sacrificed, presenting a major challenge for regulatory agencies to find alternative in vitro methods capable of predicting DNT risk to human populations. Broad scientific agreement exists on the value of prescreening substances for research prioritization and animal exposure levels. Neurodevelopmental involvement tight regulation of a multitude of cellular events such as proliferation, differentiation, neurite growth and apoptosis in the complex neuronal networks of the brain. Any perturbation of these events has the potential to cause adverse effects in humans. Therefore, cellular models need to measure multiple events in order to predict DNT with reasonable certainty. We have developed an in vitro assay for measuring disruption of the many of the key events in DNT using a subclonal line of PC-12 cells. High content imaging and automated data analysis of neuronal cell morphology, neurite outgrowth/initiation and cell viability were utilized to measure neurotoxic effects of a test set of environmental pollutants, including endocrine disruptors, heavy metals and pesticides in addition to representative pharmaceutical agents. The lowest observed effective dose (LOED), for both general toxicity and neurotoxicity, was determined for each compound. Some compounds such as retinoic acid exhibited neurotoxic potential with the general toxicity only occurring at the highest concentrations. Other compounds such as cadmium chloride primarily exhibited general toxicity with secondary effect of neurotoxicity occurring at higher concentrations. The results of these investigations indicate that by multiplexing measures of neurotoxicity, this system can predict DNT with reasonable sensitivity and specificity and has the potential to reduce the number of animals used and time needed for safety evaluations.

TOXICITY SCREENING WITH NEURAL PROGENITOR CELLS ON COMPLIANT HYDROGEL SURFACES.


Neurite outgrowth measurements, which quantify the extension of axons and dendrites, have been proposed for in vivo neurotoxicity screening. Neurite outgrowth is sensitive to known neurotoxins in neuronal monocultures grown on plastic surfaces. However, these cultures may not adequately represent in vivo environment, given the lack of astrocytes and the extreme stiffness of plastic compared to native tissue. Neural progenitor cells (NPCs) are an emerging model with which it is possible to access multiple aspects of neural development, including differentiation and neurite outgrowth. NPCs seeded onto plastic surfaces and exposed to 0.3–3 μM lithium chloride (LiCl) at pH 7.4. LiCl neurite outgrowth compared to controls. Exposures of 10 mM/L or greater were cytotoxic. NiTCPs are also reported to be sensitive to substrate modulus; neuronal differentiation is favored on compliant gels, whereas glial differentiation is favored on stiffer gels. Here we investigate whether substrate modulus impacts the toxicity profile of NPCs exposed to lithium chloride. PEG hydrogels were photopolymerized from solutions containing 5 wt% or 15 wt% polyethylene glycol diacrylate, 20 μg/mL, poly-L-ornithine, phosphate-buffered saline, and photoinitiator. Swelling studies indicate that 5 wt% hydrogels have a mesh size of 140 nm, corresponding to an estimated...
bulk modulus of 40 kPa, whereas 15 wt% hydrogels have a mesh size of 15 nm and an estimated bulk modulus of 1100 kPa. In contrast, tissue culture polystyrene has a modulus of ~10 Pa. Immunochemical staining shows that NPCs seeded onto hydrogel surfaces differentiate into neurons (beta-III-tubulin) and astrocytes (glial fibrillary acidic protein) after 7 days in culture. Preliminary analysis indicates that the neuron to astrocyte ratio increases with hydrogel compliance. Total neurite outgrowth is being assessed as a function of LiCl exposure.

PL 1706 LIMITATIONS OF PLATE READER-BASED HIGH-THROUGHPUT MEASUREMENTS OF DYNAMIC INTRACELLULAR SIGNALS.

Mainly due to their cost-effectiveness and speed, plate reader-based screening is the methodology of choice for high-throughput measurements of static signals, including cytotoxicity and apoptosis. However, high-throughput measurements can be challenging in case of organ- and cell type-specific endpoints that are dynamic and change dramatically within minutes (e.g., intracellular cAMP levels) or even seconds (e.g., intracellular calcium levels).

Regulation of the intracellular calcium concentration ([Ca2+]i) is critical for proper (neuronal) cell function. As a result, there is a high demand in hazard characterization and drug development for high-throughput measurements of [Ca2+]i, using multi-well microplate readers. This study therefore aimed at investigating the validity of microplate readers for these dynamic measurements.

We now show, using two different cell lines (PC12 and B35), two fluorescent calcium-sensitive dyes (Fluo-4 and Fura-2) and three different stimuli (high K+, ATP and acetylcholine) that the use of linear plate reader systems for measurements of [Ca2+]i, is subject to several limitations. Our data demonstrate that real-time kinetic measurements of [Ca2+]i can be confounded by erroneous sustained increases in fluorescence that depend on changes in volume rather than on changes in [Ca2+]i.

Moreover, compared to single cell fluorescence microscopy linear plate reader systems have limited sensitivity and lack single cell resolution. Probenecid is among the tools to prevent dye leakage and enhance the sensitivity of plate reader-based measurements of [Ca2+]i. However, our data demonstrate that probenecid effectively inhibits depolarization-evoked calcium influx thereby severely limiting its use for measurements of [Ca2+]i.

The use of current plate reader-based strategies for high throughput real-time kinetic measurements of [Ca2+]i thus appears associated with caveats and limitations that require further investigation.

PL 1708 OVERT TOXICITY AND BEHAVIORAL EFFECTS OF DEVELOPMENTAL EXPOSURE TO ORGANOPHOSPHATE FLAME RETARDANTS IN ZEBRAFISH.
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Concerns about the potential toxicity of organophosphate flame retardants (OPFRs), the primary replacements for the phased out brominated diphenyl ether (PentaBDE) mixture, have increased due to their persistence in the environment, high concentrations in residential dust, and structural similarity to neurotoxic organophosphate pesticides. We evaluated the developmental toxicity of four OPFRs (tris (1,3-dichloro-2-propyl) phosphate (TDCPP); tris (2-chloroethyl) phosphate (TCEP); tris (1-chloropropyl) phosphate (TCP); tris (2,3-dibromopropyl) phosphate (TDBPP)) and chlorpyrifos ethyl (CPF) using overt toxicity and locomotor activity assessments in zebrafish (Danio rerio). Embryos were reared in individual wells in 96-well plates, with exposures occurring from days 0-5 post-fertilization (pf). To assess overt toxicity (i.e., lethality, dysmorphology, hatching), chemicals were tested on the same plate (range=0.033-100 μM, ½ log increments; n=4/dose). On day 6 pf, a detailed visual malformation assessment and a high-content image analysis using a Cellomics® Array Scan® system with the Zebrafish V4 bioscipation was performed on each larva. For behavioral testing, chemicals were tested on separate plates (5 days post-fertilization) with exposures occurring from days 0-5 pf, 1/4 log increments; n=24-36/dose). On day 6 pf, larval swimming activity in both light and dark conditions was assessed with a video tracking system. Developmental exposure to ≤100 μM TCPP elicited no overt toxicity or behavioral effects. CPF, TDBPP, and TDCPP caused death or malformations (CPF: and TDCPP≤10 μM; TDBPP≤1 μM). Larval swimming activity was reduced to 75% of controls when exposed to 10 μM TDCPP. Behavioral testing in developmentally exposed larvae revealed decreased activity in the light phase for TDCPP- or TDBPP-treated embryos and decreased activity in the dark phase for CPF- or TCEP-treated embryos, indicating a potential for developmental neurotoxicity in zebrafish.

This abstract may not necessarily reflect official Agency policy.

PL 1709 LONG-TERM BEHAVIORAL DYSFUNCTION RESULTS FROM EMBRYONIC METHYLPHENIDATE EXPOSURE IN ZEBRAFISH.

With more adults being prescribed the stimulant medication methylphenidate (Ritalin®) to treat attention deficit hyperactivity disorder (ADHD) residual type, the risk of early developmental exposure arises when those taking this drug become pregnant. The zebrafish was used to study the persisting neurobehavioral effects of methylphenidate. Zebrafish have the advantages of cellular reporter systems, continuous visual access during development and molecular interventions such as morpholinos to help determine critical mechanisms underlying neurobehavioral teratogenicity. In earlier studies, we documented persisting neurobehavioral impairment in zebrafish after developmental exposure to the pesticide chlorpyrifos. This was associated with alterations in dopamine systems. Because methylphenidate is an indirect dopamine agonist, it might also cause persistent behavioral impairment after developmental exposure. Zebrafish embryos were exposed to the ADHD stimulant medication methylphenidate 0-5 days post fertilization (12.5-50 mg/l). Long-term behavioral effects were assessed in adult fish after embryonic exposure. Embryonic methylphenidate exposure at a dose of 50 mg/l in the water caused significant increases in dopamine, norepinephrine and serotonin on day 6, but not on day 30 after fertilization. More persistent effects were seen in behavioral function. In the result tank diving test of predatory avoidance developmental methylphenidate (50 mg/l) caused a significant reduction in the normal diving response. In the three-chamber spatial learning task early developmental methylphenidate this same dose caused a significant impairment in choice accuracy. These data show that early developmental exposure of zebrafish to methylphenidate causes long-term behavioral dysfunction. The identification of these functional deficits in zebrafish enables further studies with no overt toxicity observed with ≤100 μM TCEP. Behavioral testing in developmentally exposed larvae revealed decreased activity in the light phase for TDCPP- or TDBPP-treated embryos and decreased activity in the dark phase for CPF- or TCEP-treated embryos, indicating a potential for developmental neurotoxicity in zebrafish. This abstract may not necessarily reflect official Agency policy.
MicroRNAs with tissue-selective expression are promising biomarkers of drug-induced injury due to their rapid release post injury, stability in biofluids, and ability to be quantified using RT-qPCR. However, concerns regarding the best methods for measuring microRNAs in biofluids and choice of appropriate technical controls for data analysis need to be resolved. A collaborative study was initiated by the HESI Genomics committee to assess best practices for the reproducible quantification of injury-related microRNAs in serum, plasma, and urine. A single s.c. injection of 0.5 mg/kg isoproterenol that increased plasma levels of cardiac troponin I by >600-fold at 4 hr in a pilot study was used to induce cardiac injury in male Wistar rats. Blood was collected 4 hrs post injection, divided into equal portions for preparation of serum or plasma, pooled among 12 rats per group, and aliquots sent to 12 laboratories for analysis. Serum and plasma were analyzed for levels of cardiac- and skeletal muscle-selective microRNAs (miR-208, miR-499, miR-1). Data were normalized to levels of an endogenous reference (miR-16) and a spiked-in ath-miR-159a control. Comparison of data generated from multiple sites using a standard protocol and protocol variations will identify which preanalytical steps affect microRNA detection and quantification in biofluids in drug-induced injury models.

Potassium dichromate is a chemical compound widely used in industry and a common contaminant in the environment that induces renal S1-S2 proximal tubular lesions in rats. Studies were conducted in Sprague-Dawley rats to evaluate the utility of urinary microRNAs (miR) as novel biomarkers of nephrotoxicity in the context of a HESI collaborative program. Groups of 10 males were given a single subcutaneous injection of 5 and 15 mg/kg potassium dichromate in 0.9% NaCl and were sacrificed on Day 3. Urine was collected and kidney cortices were macrodissected for miR profiling by quantitative RT-PCR using TaqMan TLDA A and B rodent cards. Histologically, degeneration and necrosis of the proximal convoluted tubules were observed at 15 mg/kg potassium dichromate on Day 3. There were no changes in BUN but serum creatinine was slightly increased (1.2-fold) at 15 mg/kg. The urinary protein biomarkers of proximal tubular lesions N-acetyl-β-D-glucosaminidase and microalbumin were significantly increased at the high dose only on Day 3 (5- and 85-fold, respectively). Profiling experiments in kidney cortex showed that 15 miR were significantly up-regulated and 13 miRs were down-regulated at 15 mg/kg (False Discovery Rate corrected p-value ≤0.05). One miR was also found significantly down-regulated at 5 mg/kg in cortex. In urine, 109 miRs were found over-expressed at 15 mg/kg on Day 3, with marked increases (above 100-fold) versus controls for 5 of them, such as miR-339-3p and miR-134. Of note, 4 over-represented miRs in urine were found to be concomitantly under-expressed in the kidney cortex, suggesting a chemical-induced leakage of miRs from the injured kidney into the urine. These results indicate that urinary miRs could potentially be used as sensitive biomarkers of nephrotoxicity in rats.

MicroRNAs (miRNAs) are highly conserved, tissue-specific small regulatory RNA molecules that control many cellular processes, including response to xenobiotics. miRNAs can be secreted in the plasma, suggesting their potential as circulating biomarkers for disease and toxicity. This study was conducted to assess circulating miRNAs as biomarkers for liver injury in the Wistar rat. Acetaminophen (800 and 2000 mg/kg) was administered once to cause liver injury. The extent of hepatocellular damage was assessed microscopically and by measuring conventional (ALT and AST) and less established (GLDH and PON1) biomarkers at 3 and 24 h post-treatment. Expression of miRNAs in plasma was assessed using qPCR. Treatment-related findings in both dose groups at 24 h consisted of liver necrosis, increases of ALT, AST, and GLDH, and decrease of PON1. At 3 h, some low dose animals had liver necrosis without changes in circulating biomarkers. Concordant with reports in the mouse, miR-122, -192, -193, and -20a were elevated in the plasma at 3 and 24 h. In addition to the published results, circulating miR-10a-1, -365, -367, and -31 were also increased at 3 and 24 h.
The results demonstrate that circulating miRNAs are suitable biomarkers of liver damage, with urinary miRNA expression profiles of rats treated with hepatotoxicants. These preliminary data indicate that liver miRNAs can outperform ALT and AST, as well as GLDH and PON1, in terms of sensitivity for the detection of hepatotoxicity. The large concordance between these data and results published in mice supports that miRNAs have a strong potential as translational biomarkers. In addition, nucleic acids are easier to measure than proteins, especially across species. Additional qualification and data in other species, including man, should be generated.

**PL 1717 DEVELOPMENTAL PROGRAMMING: PRENATAL BISPHENOL A TREATMENT ALTERS FETAL OVARIAN GENE AND MRNA EXPRESSION.**

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Prenatal exposure to excess steroids, native or environmental, leads to reproductive disruptions. For instance, gestational testosterone (T) treatment disrupts ovarian function and alters ovarian gene expression in sheep. Prenatal BPA (BPA), an estrogen mimic, treatment also causes reproductive disruptions similar to the T females, albeit less severe. We hypothesized prenatal BPA treatment will disrupt ovarian gene expression as in T fetuses. Pregnant sheep were treated from days 30 to 90 of gestation with BPA (0 or 0.5 mg/kg BW, daily, s.c. in corn oil). Ovarian expression of several steroidogenic enzymes and steroid receptors was measured using quantitative RT-PCR at fetal days 65 (control [C]: n=5; BPA: n=5) and 90 (C: n=4; BPA: n=5). Data were analyzed using a 2-way ANOVA followed by Bonferroni post-hoc test. Expression of miRNAs was identified using Affymetrix GeneChip miRNA Array, summarized using the Robust MultiChip Average procedure and confirmed by reverse transcription. miRNA targets of differentially expressed miRNAs were identified by bioinformatic analysis (TargetScan 5.1). Maternal BPA treatment, similar to T treatment, increased Cyp19 and 5α-reductase expression at day 65. Further, BPA up-regulated 17 (>1.5-fold) and down-regulated 17 (>1.5-fold) miRNAs at day 90, many targets for steroidogenic or insulin metabolism genes. In summary, maternal BPA treatment expression of 17 fetal steroidogenic gene expression, specifically enzymes involved in conversion of androgens to estrogens or dihydrotestosterone, and 2 miRNAs involved in steroidogenic and insulin homeostasis, similar to disruptions seen in T fetuses. These results provide the first evidence that maternal BPA, at relevant exposure doses, alters the ovarian developmental trajectory in a large mammal species. Supported by NIH ES016541.

**PL 1715 EVALUATION OF URINARY MICRONRNAS AS NOVEL BIOMARKERS OF NEPHROTOXICITY.**


Urinary microRNA presents an alternative to protein biomarkers due to their stability in urine, easier and faster assay development in both rats and non-rodent species, and readiness for translation to human. Although recent collaborative efforts (PSTC, HESI) have led to the regulatory qualification of several biomarkers of drug-induced kidney injury, there are still unmet biomarker needs for glomerular structural injury and distal tubular injury. The objective of this study, part of a HESI collaborative program, was to characterize tissue-specific and/or pathology-related patterns of urinary microRNAs for drug-induced kidney injury in male Sprague-Dawley rats. Studies were conducted in rats treated with reference proximal tubular toxicants (cisplatin, gentamicin, potassium dichromate), reference glomerular toxicants (puromycin, adriamycin, antiluminal membrane antibody-mediated glomerulonephritis), and reference and/or proprietary distal tubular toxicants in different laboratories. Urine was collected and kidneys were macro- or micro-dissected for microRNA profiling and characterization of region-specific and/or patho-angiography microRNAs by quantitative RT-PCR. Initial results show measurable microRNAs in urine that were modulated following treatment with different nephrotoxins. Changes in the expression of kidney microRNAs were also observed after treatment. The multi-site and multi-toxicant approach employed by the HESI consortia, demonstrates that urinary microRNAs exhibit strong potential as sensitive, reproducible measures of renal toxicity.

**PL 1716 ACETAMINOPHEN AND CARBON TETRACHLORIDE-INDUCED CHANGES IN RAT URINARY MICRONRNAS.**

X. Yang, J. Greenhaw, Q. Shi, F. Qian, K. Davis, D. Mendrick, and W. F. Salminen.

MicroRNAs (miRNAs) are evolutionarily conserved small RNAs that affect a large range of physiological processes. miRNA expression profiles have been extensively investigated for distinguishing cancerous vs. non-cancerous tissue. A recent extension of this approach is using miRNA in cell-free body fluids to detect organ injury. This study tested the hypothesis that urinary miRNAs alterations are associated with liver injury induced by hepatotoxicants. Sprague-Dawley rats were administered one of two classical hepatotoxicants, acetaminophen (APAP) or carbon tetrachloride (CCL4), or no hepatotoxicant, pentanal (PCN), or one of two vehicle control articles (0.5% methylcellulose or corn oil). Urine samples were collected over a 24h period after a single oral dose of APAP (1250 μg/kg), CCL4 (2000 μg/kg) or PCN (2400 mg/kg). APAP and CCL4 induced liver injury based upon increased serum alanine and aspartate aminotransferase levels and histopathological findings, including liver necrosis. APAP and CCL4 both significantly increased the serum alanine and aspartate aminotransferase levels and histopathological findings, including liver necrosis. APAP and CCL4 both significantly increased the serum alanine and aspartate aminotransferase levels and histopathological findings, including liver necrosis. APAP and CCL4 both significantly increased the percent of cells in G1, and phospho-pRb. Additionally, breast carcinoma cells co-exposed to BPA and the anti-proliferative effects. In summary, exposure to BPA promotes breast carcinoma cells. In summary, exposure to BPA promotes breast carcinoma cells.
Guideline-compliant studies have failed to detect low dose BPA effects reported in many research studies. To address some possible reasons for this discrepancy, the present study included the following features: measurement of BPA levels in serum and study supplies, a low phytoestrogen diet, direct oral dosing of neonates, concurrent EE, groups, a naive control, and a broad range of endpoints related to reported toxicities. Test articles were administered by daily gavage in 0.3% carboxymethylcellulose to NCTR SD rats from GD 6 until parturition. Pups were directly dosed from PND 1. BPA doses were 2.5, 8, 25, 80, 260, 840, 2,700, 100,000, and 300,000 μg/kg body weight (bw)/day. EE, doses were 0.5 and 5.0 μg/kg bw/day. There were sporadic significant differences between the naïve and vehicle control group endpoints. Both EE, groups showed multiple dose-related effects in females, including effects on immature reproductive organ weights, delayed puberty, altered serum clinical chemistry, and altered estrous cycles and reproductive tract morphologies. In males, the high dose of EE, showed multiple adverse effects on reproductive organs and delayed puberty onset, while the only clear effect at the low dose was mammary gland hyperplasia. BPA affected gestational weight gain and the estrous cycle at 100,000 μg/kg/day EE increased weight gain depression) not seen with EE2 were also observed. In addition, EE2 and BPA-induced toxicities. Test articles were administered by daily gavage in 0.3% carboxymethylcellulose to NCTR SD rats from GD 6 until parturition. Pups were directly dosed from PND 1. BPA doses were 2.5, 8, 25, 80, 260, 840, 2,700, 100,000, and 300,000 μg/kg body weight (bw)/day. EE, doses were 0.5 and 5.0 μg/kg bw/day. There were sporadic significant differences between the naïve and vehicle control group endpoints. Both EE, groups showed multiple dose-related effects in females, including effects on immature reproductive organ weights, delayed puberty, altered serum clinical chemistry, and altered estrous cycles and reproductive tract morphologies. In males, the high dose of EE, showed multiple adverse effects on reproductive organs and delayed puberty onset, while the only clear effect at the low dose was mammary gland hyperplasia. BPA affected gestational weight gain and the estrous cycle at 100,000 μg/kg bw/day. Multiple reproductive organ and serum chemistry effects that were seen in EE2-treated animals were also observed in both sexes at 300,000 μg/kg BPA bw/day, although toxicities (e.g. body weight gain depression) not seen with EE, were also observed. In addition, EE, and high dose BPA showed mild renal effects in both sexes. Sporadic occurrences of pathological effects were observed in low dose BPA groups that did not show a consistent dose response in incidence or severity. Supported by FDA IAG # 224-07-0007/NIH IAG # YIES1027.
EXPOSURE TO THE CURRENT TOLERABLE DAILY INTAKE OF BISPHENOL A DURING ADULTHOOD DOES NOT ALTER OVULATION, BUT ALTERS THE FERTILIZING ABILITY OF OOCYTES IN FEMALE MICE.

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Oocytes are released from the ovary during ovulation to be fertilized by sperm cells. Bisphenol A (BPA), a plasticizer that leaches from plastics into food and water, has been suggested to impair oocytes, as well as to reduce ovulation and offspring. Concern about effects on ovarian function by exposure to the current tolerable daily intake (TDI, 50 μg/kg/d), however, has risen. Further, effects on ovarian function by BPA exposure in adulthood have not been investigated. This study evaluated whether exposure to the TDI of BPA during adulthood alters ovulation and the oocyte function. Female mice C57BL/6J (39 days old; n = 5–7) were exposed orally to BPA (50 μg/kg/d) or corn oil daily for 12–15 d. Effects of BPA on ovulation were assessed as changes in the number of oocytes released in response to exogenous gonadotropins, changes in estrous cyclicity, and changes in the numbers of preovulatory follicles and corpora lutea in ovarian histological sections. Effects of BPA on the fertilizing ability function were assessed as the fertilizing ability of oocytes in an in vitro fertilization assay and as changes in the diameter of the cumulus-oocyte complex (COC). BPA treated mice had reduced number of days spent on proestrus, but similar number of preovulatory follicles, oocytes and corpora lutea compared to control mice. BPA treated mice had decreased percentage of fertilized oocytes and reduced diameter of the COC compared to control mice. Similar effects were observed in mice treated with diethylstilbestrol, a positive control. In conclusion, exposure to the TDI of BPA during adulthood may not alter ovulation, but decreases the fertilizing ability of oocytes via alteration of the COC. Thus, exposure to BPA during adulthood may impair the oocyte and ovarian function. Support: Conacyt–Mexico.

BISPHENOL A INHIBITS FOLLICLE GROWTH OF CULTURED ANTRAL OVARIAN FOLLICLES IN MICE.

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Bisphenol A (BPA) is a plasticizer that is widely used in polycarbonate plastics. It has been reported to have some toxic effects on the female reproductive system. For example, BPA exposure has been inversely associated with the number and quality of oocytes retrieved among women undergoing in vitro fertilization. Moreover, previous studies reported that a 100 μg/ml dose of BPA inhibits steroidogenesis and antral follicle growth in mice. However, not much data are available regarding the potential toxic effects of BPA on mouse antral follicles at lower doses. This is important to study given the reported U-shaped dose response curve that is related to BPA exposure in other tissues. Hence, our current study was designed to evaluate the effects of low doses of BPA on mouse antral follicles. To perform our study, we mechanically isolated antral follicles from mouse ovaries (C57Bl6; ages: 50–54 days old) and cultured them in vehicle (dimethylsulfoxide; DMSO) or BPA (0.001-100 μg/ml) for 96 hours. Every 24 hours, follicle diameter was determined as a measure of follicular growth. BPA exposure did not affect follicle growth at 24 hours. However, at 48 hours, 100 μg/ml BPA (285.6±9.03 μm) significantly reduced follicle growth compared to DMSO (354.88±9.35 μm) and 0.1 μg/ml BPA (360.67±17.87 μm) treatment groups (p<0.05). At 72 and 96 hours, BPA 100 μg/ml continued to inhibit follicle growth compared to all treatment groups (at 96 hours: DMSO= 401.1±12.48μm; BPA 0.001 μg/ml = 357.74±16.87μm; BPA 0.01 μg/ml= 349.23±12.57μm; BPA 100 μg/ml = 285.73±8.81μm; p<0.05). None of the lower doses of BPA (0.001, 0.01, and 0.1 μg/ml) significantly inhibited follicle growth compared to DMSO at any time point. These data suggest that 100 μg/ml BPA, but not the tested lower doses of BPA, affects the growth of mouse antral follicles. Supported by: NIH RO1 ES019178 (JAF), a fellowship from Eli Lilly (TP), Billie Field Fellowships (ZRC and WW), the Environmental Toxicology Scholar Program (MSB), and the NIEHS Toxicology Training Program (BK).

APPLICATION OF MACHINE LEARNING IN THE DEVELOPMENT OF A RISK ASSESSMENT FRAMEWORK FOR EVALUATION OF ESTIMATES OF RELATIVE POTENCY FOR DIOXIN-LIKE COMPOUNDS.

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Potential health risks associated with exposure to mixtures of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and dioxin-like polychlorinated biphenyls (referred to as dioxin-like compounds or DLCs) are evaluated using toxic equivalency factors (TEFs). TEFs represent point estimates, even though they are based on relative estimates of potency (REPs) that often span several orders of magnitude. In order to move towards using REP distributions rather than single point estimates, experts have indicated the need for development of a consensus-based framework to weight REPs based on study quality and relevance for human health risk assessment. The objective of this study was to identify those study characteristics believed most important when evaluating REP quality and relevance, to develop a numerical approach for quantitatively weighting each REP and to apply such to the current REP database. Six main study characteristics (study type, study model, pharmacokinetics, REP derivation method, REP derivation quality, and endpoint) were identified as most important and form the backbone of the framework. Expert judgment was then used to categorize REPs based on these characteristics. A multinomial logistic classifier machine learning model was applied to develop an optimal mathematical model using the expert judgments for each REP. This model yielded numerical weights for each REP value, that when applied to the current REP database, resulted in weighted distributions of REPs for each congen. These distributions improve characterization of the variability and uncertainty inherent in the health risk estimates for this class of compounds, give risk managers the flexibility to tailor the desired level of protection to a specific situation, and facilitate establishment of a consistent level of protection for all congeners. (This abstract does not reflect the policies or views of NIEHS or NCI)

A GENOMICS-BASED BENCHMARK DOSE ANALYSES OF RELATIVE POTENCIES OF DIOXIN-LIKE COMPOUNDS IN PRIMARY RAT HEPATOCYTES.

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Toxic Equivalency Factors (TEFs) are central to characterizing risk associated with mixtures of dioxin-like compounds (DLCs), and genomic technologies have the potential of contributing to the estimation of meaningful TEF values. In this study, Sprague-Dawley primary hepatocytes were treated for 24 hours with 0.00001-100 nM 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), or 2,3,7,8-tetrachlorodibenzofuran (TCDF). Approximately 1600 genes were significantly altered by all 3 congeners by ANOVA (FDR ≤ 0.05). Hierarchical clustering revealed that these genes generally responded in a similar fashion to the 3 congeners. Automated benchmark dose (BMD) modeling was performed to derive BMD values for all individual gene changes as well as GeneGo Metacore canonical signaling pathways. TCDD-based relative potency values (REPs) based on the median BMD values for all individual gene changes were in close agreement with WHO TEF values for PeCDF (0.3) and TCDF (0.1), as were REPs based on specific aryl hydrocarbon receptor (AHR) battery genes. Functional enrichment analysis (FDR ≤ 0.05) of the 1600 genes identified 14 canonical signaling pathways, including the AHR signaling pathway, REPs based on the AHR signaling pathway were generally similar to WHO TEFs, while median REPs for the other 13 pathways were lower (PeCDF REP = 0.09, TCDF REP = 0.03). That the REPs derived from the AHR signaling pathway approximate the WHO TEFs is not unexpected given that the WHO REP database is comprised of many AHR-mediated biochemical endpoints (e.g. EROD) in rodents. REPs based on the other enriched pathways, potentially relevant to the mode of action and toxicity of DLCs, could provide meaningful measures of relative potency and thus might be considered for future addition to the WHO database.
In conclusion, the number of false predictions for solvents was not reduced when the number of false negatives (from 13 to 9 cases).\textit{metabolites increases the number of false positives (from 6 to 12 cases), and reduces toxicity (2-BE and TCE) and nephrotoxicity (HAL). The inclusion of predicted metabolites in the toxicity prediction models can be applied to direct the testing strategy for new solvents with those identified in vivo and, importantly, to assess potential use of QSAR methods to estimate RQs and TEs, the physicochemical, toxicological and biodegradability properties needed to predict RQs and TEs. To optimize the use of in silico predictions, an integrated approach for the use of multiple QSAR models, tools and approaches is needed.\textit{ }

\textbf{1727 USING QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS (QSAR) TO ESTABLISH TOXICITY/ENVIRONMENTAL SCORES (TES).} J. M. Ogden, P. Ruiz and J. Wheeler. Division of Toxicology and Environmental Medicine, CDC ATSDR, Atlanta, GA. Sponsor: M. Mumtaz.

The Agency for Toxic Substances and Disease Registry (ATSDR) uses Reportable Quantities (RQs) established by the Environmental Protection Agency (EPA) in order to prioritize chemicals subject to Toxicological Profile development. RQs are determined using two criteria. In the first criteria the intrinsic physicochemical (ignitability/reactivity) and toxicological properties (aquatic toxicity, acute mammalian toxicity, chronic toxicity, and potential carcinogenicity) of each chemical are evaluated. In the second criteria the susceptibility to biodegradation, hydrolysis, and photolysis (BHP) is evaluated. When an RQ is not available, the ATSDR uses the same criteria to develop a Toxicity/Environmental Score (TES). However, when sufficient original data are not available to assign a RQ/TE to chemical candidates Quantitative Structure-Activity Relationship (QSAR) methods can be used to computationally predict the physicochemical, toxicological and biodegradability properties needed to determine RQs and TEs. To evaluate the potential use of QSAR methods to estimate RQs and TEs, the physicochemical, toxicological and biodegradability properties of 232 chemicals were computationally-predicted, and QSAR RQs/TEs were estimated. QSAR predictions for rat oral LD50, headad minnow LC50, and BHP correlated strongly (74%, 43%, and 62%, respectively) with original data. QSAR could not predict a dose-response curve required to score chronic toxicity, however an alternate method combining developmental toxicity and chronic LOAELS is proposed. QSAR RQs/TEs were identical to original scores for 26% of chemicals (60% of scores were within 1 tier of original scores, and 14% of QSAR scores were higher than 1 tier). Thus, QSAR methods may be used as an alternative method to fill in data gaps needed for development of TEs. To optimize the use of in silico prediction for new substances. The aim of this research was to compare the in silico predicted effects for a number of solvents with those identified in vivo and, importantly, to assess whether the inclusion of predicted metabolites improves the predictivity of TEs. Metabolites were predicted using METEOR (Lhasa Ltd) and the OECD QSAR Toolbox. Alerts for toxicity of solvents and predicted phase I metabolites were generated using DEREK (Lhasa Ltd), the OECD QSAR Toolbox and TOPKAT® (Accelrys). In vivo toxicity data were obtained from North American and EU regulatory risk assessment reports.

Toxicity alerts for the solvents include developmental toxicity (ethanol [EtOH], isopropanol [IPA], carinogenicity (all except EtOH), hepatotoxicity (chlorofrom [CHL]), halothane [HAL], trichloroethylene [TCE]), nephrotoxicity (TCE) and skin toxicity (EtOH and CHL). These predictions indicate several false positives and few false negatives. For 2-butoxyethanol (2-BE), 26 unique phase 1 metabolites were predicted; 3 for EtOH: IPA 2; CHL 2; HAL 4; TCE 9. Additional alerts for toxicity emerged when predicted metabolites are included in the toxicity predictions: developmental toxicity, carcinogenicity and skin toxicity (all solvents), hepatotoxicity (2-BE and TCE) and nephrotoxicity (HAL). The inclusion of predicted metabolites increases the number of false positives (from 6 to 12 cases), and reduces the number of false negatives (from 13 to 9 cases). In conclusion, the number of false predictions for solvents was not reduced when including predicted metabolites. However, and importantly, the number of false negatives is reduced. Based on these findings it is recommended to include metabolism formation in toxicity predictions to prevent the postponement of potential toxicants in prioritization for experimental toxicity testing.
to test for sufficient similarity comparing benchmark dose (BMD) estimates between a reference mixture (with available experimental in vivo data) and observed mixtures (e.g., from exposure data) in data rich and data poor cases. The similarity region is defined by the radius around the BMD of the reference mixture (e.g., associated with a benchmark response of 20%), where the radius is the distance between the BMD and an a priori selected effective dose (e.g., ED50). A mixtures toxicity index may be used to compare the exposure region to a point of departure for mixtures considered sufficiently similar to the reference mixture. This strategy was applied to mixtures of pyrethroids identified from floor wipes from the National Environmental Health Survey of Child Care Centers and in vivo mixtures studies on neurobehavioral function. Accounting for the relative potency of the chemicals, the mixtures from 90% of the centers with at least detectable levels of pyrethroid(s) were determined to be sufficiently similar. The strategy uses a metric of distance to qualify as sufficient similarity—a systematic and reproducible approach without the assumption of additivity. Limitations include the need for public health stakeholders and regulators to agree on objective guidelines for defining the similarity region across a variety of mixtures and toxicity tests. (The authors gratefully acknowledge the support from #R01ES015276, #T32 ES007334 and #UL1RR031990.)

1732 EVALUATING TOOLS AND MODELS USED FOR QUANTITATIVE EXTRAPOLATION OF IN VITRO TO IN VIVO DATA FOR NEUROTOXICANTS.

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There are a number of risk management decisions, which range from prioritization for testing to quantitative risk assessments. The utility of in vitro studies in these decisions depends on how well the results of such data can be qualitatively and quantitatively extrapolated to in vivo responses. Extrapolation of in vitro data to in vivo responses includes both pharmacodynamic and pharmacokinetic considerations, and often assumes that media concentrations are equivalent to steady-state blood concentrations. For this assumption to be correct, the partitioning of the chemical between media and cells must be equivalent to the partitioning of the chemical between blood and tissue. The relationship between in vitro effects and in vivo responses was evaluated using deltamethrin and bifenthrin as test chemicals. In vitro data on chemical-induced decreases in cell firing in rat primary cortical cell cultures were compared to decreases in motor activity in vivo. Reverse dosimetry/toxicokinetic modeling using media concentrations estimated the administered in vivo dose within a factor of 2 for deltamethrin and factor of 2.3 for bifenthrin. However, when the data are compared on a cell or tissue concentration, reverse dosimetry/toxicokinetic modeling using cellular concentrations estimated the administered in vivo dose within a factor of 2 for deltamethrin and factor of 6-20 for bifenthrin. Accounting for cell:media partitioning reconciled in vitro/in vivo effect model is misspecified or that cellular concentrations are not accurately estimated by these procedures. Successful comparisons with deltamethrin but not bifenthrin indicate the need to examine these approaches for a wider set of pyrethroids. (This abstract does not reflect NIEHS or US EPA policy).

1733 INTEGRATED IN VITRO-IN SILICO APPROACH TO PREDICT DOSE-RESPONSE CURVES FOR IN VIVO DEVELOPMENTAL TOXICITY IN RAT AND HUMAN.
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The regulatory assessment of systemic toxicity is almost solely performed using animal models, providing in vivo dose-response curves. If in vitro toxicity data are to be used for human risk assessment, in vitro data will need to be translated to in vivo dose-response curves. These can be used to set a point of departure for deriving safe exposure limits. The present study shows our approach to extrapolate in vitro concentration-response curves to in vivo dose-response curves for developmental toxicity by combining in vitro toxicity data and in silico kinetic modeling. In vitro toxicity data obtained in the differentiation assay of the embryonic stem cell test (EST) were extrapolated to in vivo sensitivity data using physiologically based kinetic (PBK) modeling. We show that the integrated in vitro-in silico approach is able to predict developmental toxicity dose levels for glycol ethers and for retinoic acid in rats, reflected by the similarities of our predictions with the embryotoxic dose levels observed in rat studies. Combining the in vitro EST toxicity data with a human PBK model allows the prediction of dose-response curves for human developmental toxicity. This approach will therefore provide a means to reduce the need for animal testing in human risk assessment. *Louisse et al. Toxicological Sciences 118, 470-484 (2010)*

1734 THE ART OF NEGOTIATION: A FUNDAMENTAL SKILL FOR SCIENTISTS.
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Negotiation is an essential skill for scientists of every rank and job sector to navigate their career successfully, yet it is often not part of a scientist’s formal training. Fundamentally, negotiation culminates in the attainment of a mutually acceptable agreement between two or more parties—however there is an art to reaching such an agreement. Because negotiations typically occur behind closed doors, few will ever experience a negotiation until they represent one of the parties involved. In an ever-changing world it has become imperative to understand the nuances of negotiation, and this session offers attendees a unique opportunity to bring negotiations out in the open. This session will introduce scientists to the intricacies of negotiations in the workplace and to discuss idiosyncrasies in negotiation tactics across toxicology sectors. The session will be delivered in two segments: a formal lecture and a panel discussion delivered by speakers from academia, industry, and government. Our panel will deliver important information on the art of negotiation, addressing conflict styles and the basics of interest-based negotiation. The panel will then discuss their personal experiences in negotiation throughout their careers and address best practices in negotiation as it relates to their sector of toxicology. Topics covered will include preparation for negotiating, how to initiate negotiation, importance of body language, gender differences in negotiation, negotiating for salary and start-up in academia, negotiating for labor and representation at the bargaining table in government, and negotiation practices in the pharmaceutical industry. At the end of the session, participants will come away with a better understanding of how negotiations work and how to use them to their advantage.

1735 PLACING BISPHENOL A RISKS IN A HUMAN EXPOSURE CONTEXT: IS INTERNAL EXPOSURE TO BIOACTIVE BISPHENOL A IN HUMANS SIMILAR TO LEVELS IN AFFECTED RODENT TEST SPECIES?
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Human external and internal exposure to Bisphenol A (BPA) is widespread. Hydrolysis or leaching of unreacted monomer from polymeric products can release low levels of BPA leading to human exposure through the diet, handling of some paper products, and use of some medical devices utilized in neonatal/pediatric intensive care units. However, BPA undergoes substantial presystemic Phase II metabolism in the gut and liver following oral administration, producing inactive metabolites and limiting internal exposure to the active monomer, aglycone, or unconjugated BPA. Inconsistent reports of high (ng/ml) concentrations of aglycone BPA in human blood/tissue samples collected and/or analyzed in an uncontrolled manner and controversy regarding the pharmacokinetics of BPA in rodents and primates have fueled concerns that internal exposure to BPA may be high enough to cause endocrine disruption in humans. Recently published and emerging research funded by NIEHS/NTP, FDA, and EPA (STAR program) offer an exceptional scientific basis for assessing the significance of BPA exposure to human health. This session will introduce the BPA cause célèbre, present new data on internal exposures to unconjugated BPA in humans, the pharmacokinetics of aglycone and conjugated BPA in adult, neonatal, and fetal rodents and nonhuman primates, and present new rodent toxicity studies that concomitantly characterized internal BPA exposure and potentially adverse biological effects. These data will be synthesized and used to critically examine the hypothesis that human internal exposure to aglycone BPA is sufficiently high to produce a demonstrably adverse health outcome and identify key uncertainties and data needs. The conclusions will be discussed in a final session.
The purpose of this session is to provide information on the different certification options available to toxicologists and to highlight the benefits of certification. This session is intended for those who are considering certification and want to learn more about it. Practicing toxicologists come from different training backgrounds, are engaged in a diverse array of activities including research, risk assessment, product development, consulting, etc., and work in several different sectors. The SOT membership embodies this diversity and includes scientists from academia, government, nonprofits, and industry who practice toxicology in the United States and abroad. The diverse nature of the field makes certification particularly challenging and in truth many toxicologists are self-branded. However, several certification organizations exist worldwide, each with different foundations and requirements. Our panel of experts will explore what makes a toxicologist, understand the different certification options and their benefits, and identify opportunities for harmonization. Providing an overview of the requirements for each review body will be representatives from the American Board of Toxicology (ABT), the Academy of Toxicological Sciences (ATS), European Registered Toxicologists (ERT), and the Japanese Society of Toxicology (JST).

### 1737 GOOD LABORATORY PRACTICE (GLP) IN CHINA.

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China has experienced an increase in nonclinical studies conducted for submission to the US, Chinese, and other regulatory authorities. Both industry and regulators should be familiar with requirements and challenges in the emerging Chinese landscape. This session will review requirements and challenges of GLP regulations seen by both Chinese and US regulatory authorities, as well as provide industry perspectives on meeting and clarifying those challenges. Our panel of experts will highlight the current status of GLP laboratories certified by the Chinese State Food and Drug Administration (SFDA) and nonclinical GLP studies conducted in China for multinational regulatory submissions. Industry and regulatory leaders will participate in this session to share important perspectives to help achieve understanding and compliance with requirements. This particular topic will be useful to those interested in conducting nonclinical studies in China and those reviewing nonclinical studies conducted by Chinese contract research organizations (CROs).

### 1738 EPIGENETIC AND miRNA REGULATIONS IN CARCINOGENESIS: TOXICOLOGICAL IMPLICATIONS.

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Epigenetics is an increasingly evolving scientific field focused on mechanisms involving heritable gene expression profiles and phenotypic changes fundamental for normal development without alterations in nucleotide sequence. DNA methylation and acetylation patterns, histone modifications, germ-line reprogramming and noncoding RNAs mediate epigenetic regulations. Increasing evidence suggests that disruption and alteration of normal epigenetic regulation mechanisms are fundamental in cancer development and progression. Recent evidence shows that exposure to air toxics and environmental pollutants triggers changes in microRNA (miRNA) expression profiles. These cellular changes reveal novel mechanisms through which toxicants may induce adverse health effects, including the development of leukemia and liver carcinogenesis. In addition, arsenic has been implicated in the development of human skin, lung, bladder, liver, and prostate cancers. Histone modifications have been suggested as epigenetic mechanisms related to arsenic-induced carcinogenesis. Identification and development of new therapeutic strategies with specific and selective targets in the epigenetic machinery are of utmost importance. Recent research shows that detecting alterations in miRNA expression has been associated with early stages of tumor development. These findings suggest that miRNAs may play an important role in cancer risk assessment. Alternatively, histone deacetylase inhibitors are effective in the treatment of hemato- logical malignancies such as leukemia in children. Epigenetic alterations that result in carcinogenesis stress the importance of research efforts to characterize associated molecular mechanisms and highlight current public health issues.

### 1739 EPINEFFECTS OF FORMALDEHYDE EXPOSURE.

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Formaldehyde is a common indoor and outdoor air pollutant known to cause cancer in the upper respiratory tract, and possibly leukemia. Despite its adverse health effects, the mechanisms underlying formaldehyde-induced disease remain largely unknown. Research investigations have uncovered microRNAs (miRNAs) as key post-transcriptional regulators of gene expression that may influence cellular disease state. While studies have compared miRNA expression patterns between diseased and healthy tissue, this is the first study to examine perturbations in global miRNA levels resulting from formaldehyde exposure. We set out to investigate whether cellular miRNA expression profiles are modified by formaldehyde exposure. We hypothesized that formaldehyde exposure disrupts miRNA expression levels within human lung cells, representing a novel epigenetic mechanism through which formaldehyde may induce disease. Human lung epithelial cells were grown at air-liquid interface and exposed to gaseous formaldehyde at 1 ppm. Small RNAs were collected and analyzed for miRNA expression using microarray analysis. Formaldehyde exposure was found to significantly decrease the expression levels of 89 miRNAs. In particular, miR-181a showed the largest decrease in expression upon exposure. Interestingly, this miRNA is recognized for its involvement in leukemia development, and it is known to target inflammation-related miRNAs. Molecular network analysis of predicted miRNA transcript targets revealed that formaldehyde exposure potentially alters signaling pathways associated with cancer and inflammation via epigenetic mechanisms. In conclusion, formaldehyde alters miRNA patterns which regulate gene expression, potentially leading to disease. Future work will investigate epigenetic modifications resulting from formaldehyde exposure in vivo throughout several target tissues.

### 1740 ARSENIC-INDUCED ALTERATIONS IN GLOBAL POSTTRANSLATIONAL HISTONE MODIFICATIONS AMONG ADULTS IN BANGLADESH.

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Arsenic is classified as a group 1 human carcinogen and is implicated in the development of multiple types of cancers. It has been suggested that As-induced carcinogenesis may involve As-induced epigenetic dysregulation. We have detected significant differences in the global levels of several histone tail acetylation and methylation marks, including histone 3, lysine 9 acetylation (H3K9ac), histone 3 lysine 9 di-methylation (H3K9m2), histone 3 lysine 4 tri-methylation (H3K4me3) and histone 3 lysine 27 tri-methylation (H3K27me3), in human peripheral mononuclear cells (PMBCs) collected from individuals exposed to varying levels of As in drinking water in Bangladesh. H3K4me3 and H3K27me3 changes appear to be gender specific. We measured histone modifications from the same individuals collected at three time points: weeks 0, 12, and 24 and saw that methylation marks are fairly stable, while the acetylation marks appear to be more dynamic. Changes in the global levels of histone marks were primarily measured using a novel ELISA method. Our results suggest that chronic As exposure via drinking water in adults alters global levels of post-translational histone marks by increasing the global levels of H3K9 di-methylation and H3K27 tri-methylation (females only), which are marks of transcriptional repression. Decreasing the global levels of H3K9 acetylation and H3K4 tri-methylation (males only) marks is usually associated with repressed chromatin structure and gene silencing. These respective alterations in the post-translational histone modifications may imply that chronic As exposure may be associated with global transcriptional repression.
and J. Chandra.

accumulation of subsequent epigenetic abnormalities during progression of the car-

mental epigenetic events that promote liver carcinogenesis by causing a profound

both genotoxic and nongenotoxic hepatocarcinogenesis may be one of the funda-

ulation of several microRNAs, e.g. miR-22 and miR-29 family, that target miRNA

key one-carbon metabolism genes, including Mat1a, Ahcy, Mthfd1 and Cbs.

livers of mice fed a methyl-deficient diet we found the down-regulation of several

diet) and genotoxic (2-acetylaminofluorene; 2-AAF) carcinogens. Notably, in the

tenance of the cellular epigenome. We conducted experiments to examine the un-

derstanding mechanisms of cancer progression and prevention. Accumulating evi-

those epigenetic events that drive cell transformation is crucially important for un-

ance abnormalities. However, it is highly unlikely that all of these epigenetic

sion abnormalities. During early embryonic develop-

ment and remain inactive in most cells and tissues. Although the complex tran-

scriptional control mechanisms of L1 are not well understood, L1 reactivation has

been described in several human cancers following exposure of mouse or human

cells to BaP. We investigated the epigenetic mechanisms involved in L1 silencing in

mouse and human cells as well as the epigenetic modifications that contribute to L1

reactivation following BaP exposure. We found that pRB and E2F interact with

human and mouse L1 elements, and contribute to both maintenance of histone

heterochromatin silencing marks H3 K9me3 and H4 K20me3, and to the recruit-

ment of histone deacetylases (HDAC) HDAC1 and HDAC2 coexpressors to L1

5'UTR. Challenge of HeLa cells with BaP reduces the recruitment of DNA methyl-

transferase-1 (DNMT1) but not pRB to the L1 promoter, and increases the levels of

H3K4me3 and H3K9Ac, epigenetic marks for transcriptionally-active chromatin.

Also, long-term exposure to BaP resulted in decreased cytosine methylation at sev-

eral loci within the L1 5'UTR. We conclude that genetic reactivation of L1 by BaP

involves an ordered cascade of epigenetic events that begin with nucleosomal his-

tone modifications and is completed with alterations in DNMT1 recruitment to

the L1 promoter and reduced DNA methylation of CpG islands.

1742 EPIGENETIC AND miRNA DYSREGULATION IN LIVER

NONGENOTOXIC AND GENOTOXIC TUMORIGENESIS.

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Currently, it is well-established that carcinogenesis, in addition to various genetic

changes, is associated with the accumulation of multiple epigenetic and miRNA ex-

pression abnormalities. However, it is highly unlikely that all of these epigenetic

changes play a causative role in tumorigenesis. In this respect, the identification of

those epigenetic events that drive cell transformation is crucially important for un-

derstanding mechanisms of cancer progression and prevention. Accumulating evi-

dence suggests that impaired functioning of one-carbon metabolism may influence

cancer incidence and progression because of its integral role in the regulation of sev-

eral main interdependent cellular metabolic processes critical for the proper main-

tenance of the cellular genome. We conducted experiments to examine the un-

derlying mechanisms of one-carbon metabolism dysregulation during the early

stages of rodent hepatocarcinogenesis induced by nongenotoxic (methyl-deficient diet)

and genotoxic (2-acetylaminofluorene: 2-AAF) carcinogens. Notably, in the

livers of mice fed a methyl-deficient diet we found the down-regulation of several

key one-carbon metabolism genes, including Marla, Ahec, Mthfd1 and Cbs. Impor-

tantly, these changes were accompanied by marked functional alterations the level of in one-carbon metabolites. Similar changes were detected in rat liver during

2-AAF-induced hepatocarcinogenesis. Mechanistically, inhibition of expression of these genes was associated with epigenetic alterations in the promoters and up-regu-

lation of several microRNAs, e.g. miR-22 and miR-29 family, that target miRNA

of these genes. The results of the present study suggest that epigenetically- or

miRNA-mediated down-regulation of the one-carbon metabolism genes during both

nongenotenic and nongenotoxic hepatocarcinogenesis may be one of the funda-

mental epigenetic events that promote liver carcinogenesis by causing a profound

accumulation of subsequent epigenetic abnormalities during progression of the car-

cinogenic process.

1743 OXIDATIVE STRESS BASED STRATEGIES FOR

ENHANCING THE EFFICACY OF HISTONE

DEACETYLASE INHIBITORS (HDACi) FOR THE

TREATMENT OF LEUKEMIA.

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Despite advances in the treatment of pediatric leukemia, twenty percent of children
die due to relapse or refractory disease. Furthermore, survivors have an increased

risk of death compared to age matched cohorts due to late effects of their therapy.

Identification and development of new therapeutic strategies with specific and se-

lective anti-leukemia activity are of utmost importance for these patients. Histone

decaetaylase inhibitors (HDACi), have been shown to be effective in the treatment of

hematological malignancies. The major accepted mechanism of action for these
drugs is through hyperacetylation of histones. Interestingly, several studies have in-
dicated that HDACi cause oxidative stress, which contributes to the cytotoxicity of

these drugs. Combining HDACi with other agents that cause further oxidative

stress might enhance the efficacy of HDACi for the treatment of leukemia. The ty-

rosphostin inhibitor adaphostin is one such agent. Adaphostin was originally identi-

cified as a tyrosine kinase inhibitor, but subsequent studies defined oxidative stress as

its primary mechanism of action. Furthermore, adaphostin demonstrates selectivity

for leukemia cells as compared to normal lymphocytes. We addressed the utility of

combining HDACi and adaphostin in a leukemia model system. Our preliminary

data shows strong synergy between two structurally different HDACi (entinostat and

vorinostat) and adaphostin resulting in apoptosis. Results showed a threefold in

crease in DNA fragmentation when cells were treated with adaphostin plus enti-

nostat and a sixfold increase with adaphostin plus vorinostat, compared to DNA

fragmentation with HDACi alone. Furthermore, these combinations resulted in a
twofold increase in superoxide levels, suggesting that oxidative stress plays an im-

portant role in the induction of apoptosis. Our focus is to understand the mecha-

nism involved in the synergy between these agents, which will allow the identifica-

tion of better strategies to treat leukemia.

1744 NONCLINICAL SAFETY ASSESSMENT OF DUAL-

TARGETING BIOTHERAPEUTICS.

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Administration, Silver Spring, MD.

Engineering of protein-based biotherapeutics has advanced significantly in recent

years to delivering novel molecules having exceptional target specificity. Initial anti-

body-based therapies were designed to target a single epitope. More recently, how-

ever, multitargeting antibodies, including nanobodies, are being designed to bind

and modulate multiple cellular targets having coordinated biological pathways.

These novel and highly specific constructs present new challenges for assessing

safety in the nonclinical setting, including identification of pharmacologically rele-

vant species, creative study designs that support clinical development in the pres-

ence of species dependent pharmacokinetic behavior, and antidrug antibody assay

implementation strategies for multi-antidotype immunological responses that may

interfere with pharmacological/toxicological data interpretation. This impor-

tant topic will generate interest in this issue and bring about the discussion and

challenges with regard to assessing the safety of these cutting-edge biotherapeutic

modalities. Our panel of experts will begin by providing a brief overview of protein

engineering technologies used in the design of dual-targeting biotherapeutics with

the aim of providing a basic understanding of the technologies and particularly the

toxicity-relevant aspects of construct design, such as the influence of amino acid

sequence homology and glycosylation patterns on species-relevant pharmacologic

action. We will follow the introduction with a series of three case studies that illus-

trate the unique issues faced when developing clinical trial-enabling strategies,

study design, and data interpretation. Finally, a brief commentary from the regula-

tory perspective on the need for nonclinical safety and regulatory scientists to part-

ner when faced with these new challenges will close out the session. At the conclu-

sion of the session a summary of the challenges and provocative commentary on the

future directions of nonclinical safety assessment of dual-targeting biotherapeutics

will be presented.

1745 DUAL-TARGET CONSTRUCT ENGINEERING FOR

TARGET SPECIFICITY AND EFFICACY.

J. Cochran. Stanford University, Palo Alto, CA. Sponsor: M. Bogdanffy

Dual-specific and multi-specific proteins that recognize two or more clinical targets

have generated great interest in the pharmaceutical and biotechnology arenas. This

burgeoning interest has afforded exciting new drug candidates with the potential
for enhanced efficacy, reduced side effects, and lower costs of development and production compared to monospecific therapies. Bispecific proteins have been shown to potentiate the activity of more than one clinical target on the same cell, or bind receptors that bring different types of cells together in the body with interesting and important biological outcomes. Several main strategies have been used to create multi-specific proteins, including chemical conjugation of protein domains, recombinant expression of promiscuous proteins with binding sites that recognize multiple targets, and engineering proteins that contain multiple binding epitopes. Rational and combinatorial methods that have been used to generate such proteins will be discussed. Antibody-based fusion proteins have dominated this development space, but multi-specific therapeutics generated from natural or engineered proteins are emerging as attractive alternatives, often with advantages including increased stability and expression yields. While multi-specific proteins have already been created with activities that are greater than the sum of their parts, challenges remain such as identifying which clinical targets or functionalities will be best to combine, and the inability to alter the dosage of the binding therapeutic. The potential for these proteins, in interest in multi-specific proteins will continue to grow at a rapid pace, especially as these biologics continue to demonstrate success in pre-clinical and clinical trials.

**1746 NONCLINICAL ASSESSMENT OF BISPECIFIC T CELL-ENGGAGING ANTIBODIES.**

B. Rattel. Micromet AG, Munich, Germany. Sponsor: M. Bogdanoff.

Certain bispecific antibodies can transiently link tumor cells with resting polyclonal T cells for induction of a surface target antigen-dependent redirected lysis of tumor cells. One example is blinatumomab, which belongs to a class of bispecific biologics called BiTE® antibodies (for bispecific T cell engager). Blinatumomab consists of two covalently linked single-chain antibody variable domains (scFv) directed against CD3 and CD19, respectively. Durable objective responses to single-agent therapy with blinatumomab have been demonstrated in patients with refractory non-Hodgkin’s lymphoma (NHL) and B-precursor acute lymphocytic leukemia (ALL), and clearance of bone marrow from tumor cells below the limit of detection has been observed in patients with minimal residual ALL. In vivo, blinatumomab and other BiTE antibodies activate T cells in a highly conditional manner that is dependent on the presence of target cells. Blinatumomab belongs to a first generation of BiTE antibodies that cross-react only with respective antigens from chimpanzees. To facilitate in vivo safety testing of this first generation of BiTE antibodies, surrogate mice that are cross-reactive with murine antigens were generated and will be discussed. Furthermore an example will be presented where such surrogate BiTE approaches did not work, and the clinical starting dose had to be determined solely based on MABEL data and PK modeling. A new generation of BiTE antibodies is now available that is fully human in sequence and cross-reacts with a wide variety of non-human primates including cynomolgus macaques. The pharmacological characterization of BiTE antibodies includes in-depth analysis of their effects on tumor as well as on T cells. Various models are available for in vivo efficacy testing. For instance, xenotransplanted mice are reconstituted with human effector T cells after establishment of solid tumors. Strategies for nonclinical assessment of BiTE antibodies with different cross-reactivity profiles and with specificity for various tumor-associated antigens will be presented.

**1747 COMPUTATIONAL SAFETY AND REGULATORY STRATEGIES USED IN DEVELOPING A NOVEL BISPECIFIC MOLECULE IN ONCOLOGY.**

K. Olivier, Merrimack Pharmaceuticals, Inc., Cambridge, MA.

Bispecific molecules can greatly increase the ability to inhibit tumor growth through avidity. The potentiation created when utilizing two receptors in the same growth factor receptor pathway has been shown to better inhibit tumor growth compared to single target monoclonal therapy alone. Network biology suggested that a bispecific molecule that docks to an overexpressed target on tumor cells, allowing greater accumulation of therapeutic molecules at the site of the tumor, which subsequently binds and inhibits a key molecule in tumor growth could be efficacious. MM-111 was designed as a bispecific antibody consisting of a HER2 docking arm and a HER3 effector arm linked via a proprietary modified human serum albumin sequence. HER2 overexpression is known to occur in certain patients with breast, lung and stomach cancer. Both HER2 and HER3, also known as ErbB2 and ErbB3 respectively, are members of the ErbB family of receptors, whose activation is commonly linked with cancer. Safety studies supporting the MM-111 IND included repeat-dose toxicity studies in rodent and nonrodent models, and a tissue cross-reactivity study in representative species. Maximum feasible doses were tested in both species, based on the anticipated clinical dose formulation. The only MM-111 related finding in some rodents administered any dose level of MM-111, up to 500 mg/kg (NOAEL), was a minimal to mild 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 periarterial findings were largely reversible after a 4-week treatment free recovery period. Network Biology and the safety and regulatory considerations for the development of MM-111, a bispecific antibody targeting HER2 & HER3 for oncology indications, will be described.

**1748 NONCLINICAL CHARACTERIZATION OF A HER3 AND EGFR DUAL-ACTION ANTIBODY IN CYNOMOLGUS MONKEYS.**


MEHD7945A is an engineered monoclonal antibody (mAb) in which each of the two antigen binding fragments is capable of binding to human and cynomolgus monkey HER3 and EGFR with high affinity. MEHD7945A binds to rodent EGFR but not rodent HER3; thus, the cynomolgus monkey was selected as the appropriate species for safety assessment. MEHD7945A blocks ligand binding to both receptors and demonstrates equivalent or superior efficacy to other anti-EGFR or anti-HER3 mAbs in mouse xenograft models. Dysregulation of EGFR and HER3 signaling has been implicated in tumorigenesis and tumor progression. EGFR is a clinically validated target, although inhibition is associated with dermatologic toxicity in non-clinical and clinical studies. In contrast, relatively little is known about safety liabilities associated with HER3 inhibition. The nonclinical development of MEHD7945A included a pilot toxicity study in female cynomolgus monkeys to compare the relative dermatologic toxicity of MEHD7945A to cetuximab, a clinically approved anti-EGFR mAb, and to screen for unique toxicities that may be associated with HER3 and EGFR combination. Animals were administered cetuximab (25 mg/kg) or MEHD7945A (12.5 or 25 mg/kg) IV weekly for 5 weeks. Consistent with prior reports, dermatologic toxicity was observed between the 3rd and 4th doses in all animals given cetuximab, whereas only 1 of 3 animals treated with MEHD7945A at 25 mg/kg had evidence of dermatologic toxicity that was of lesser severity and had later onset. No animals given MEHD7945A at 12.5 mg/kg showed gross evidence of dermatologic toxicity. TK profiles confirmed comparable exposure between animals dosed with cetuximab and MEHD7945A at 25 mg/kg; thus the reduced relative toxicity was not due to differential drug exposure. In a 12-week GLP repeat dose toxicity study no dermatological toxicity was observed after weekly IV administration of MEHD7945A at doses up to 30 mg/kg. These data suggest that MEHD7945A is well tolerated and may offer an improved clinical safety profile relative to approved therapies.

**1749 REGULATORY PERSPECTIVE ON DUAL-TARGETING BIOTHERAPEUTICS: APPROACHES, TRANSLATION OF NONCLINICAL FINDINGS, AND CHALLENGES.**

A. Pilaro, CDER, US FDA, Silver Spring, MD.

This presentation will provide a discussion of the regulatory issues regarding the nonclinical development of bispecific or dual-targeting biopharmaceuticals, in a question and answer format. Questions and challenges regarding nonclinical safety testing and interpretation of the findings, with the goal of defining safety for first-in-human studies of these products will be presented. The US regulatory perspective, based on FDA CDER’s interpretation of the current revised ICH S6(R1) Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals guidance. Interactive questions and discussion from the participants will be encouraged.

**1750 CHALLENGES AND FUTURE DIRECTIONS OF MULTITARGETING BIOTHERAPEUTIC APPROACHES.**

W. Ku, Boehringer Ingelheim, Ridgefield, CT.

A brief summary perspective on the challenges and future opportunities for the nonclinical safety assessment of multtargeting biotherapeutics will be presented. The need for close partnering between nonclinical safety and drug discovery at the exploratory stage of preclinical development followed by rapid advancement of candidates to the clinic, will most likely be important to achieve more efficient and successful transition of these molecules to clinical development. Also, clinical differentiation over novel single target or other ‘standard of care’ therapies will be critical. This highlights the important role of translational medicine and the availability of biomarkers for earlier proof of mechanism or clinical concept, as well as the need for novel nonclinical safety strategies that rapidly advance candidates to the clinic. Concepts that will be highlighted include 1) the importance of applying available target knowledge and its limitations in predicting optimized target combinations.
The prevalence of atopic diseases has increased significantly during the last decades in developed countries. The composition of the intestinal flora plays a potential role in the development of the infant’s immune system. Evidence in infants indicates that the composition of the microflora might relate to the induction and severity of allergies. Therefore, modulation of the microbiota has been investigated intensively as one of the novel anti-allergy strategies. Human milk is full of components that can influence the immune system. Prebiotics are non-digestible oligosaccharides (OS) present in human milk in large amounts that promote the growth and activity of commensal bacteria, mainly Bifidobacteria. Based on the analysis of human milk OS, a specific prebiotic OS mixture of 90% short chain galacto-oligosaccharides and 10% long chain galacto-oligosaccharides has been developed. The immune modulatory effects of this specific OS mixture and its combination with the probiotic strain Bifidobacterium breve M-16 (synbiotics) in several in vitro, animal and clinical trial studies will be discussed. Findings demonstrate that oral administration of specific OS or a synthetic mixture appears to modulate the immune system. Future research aims at the identification of the underlying mechanisms and at further examination of the relationship between early supplementation and effects later in life.
The 2010 Gulf of Mexico oil crisis was the worst environmental pollution disaster in US history. By the time the well was capped, more than 200 million gallons of crude oil poured into the Gulf over an 87-day period. To combat the crisis, a marine toxicology strategy was deployed to decrease the toxic potential of the crude oil inshore species by increasing the toxic potential to offshore species. Thus, over two million gallons of chemical dispersants were applied to the oil, which prevented oil accumulation at the ocean surface and, instead, moved it into the water column and onto the ocean floor. This approach decreased the amount of surface oil reaching inshore waters and beaches. However, it is unclear if it ultimately decreased toxicity to inshore species because the acute and chronic toxicity of dispersants, dispersed oil, and oil-related metals in the water column are unknown. Also unknown are the toxic outcomes of this approach for offshore species. During this session our panel of experts will present and discuss some of the first studies to evaluate the impact of this toxicological strategy considering the toxicity of crude oil, dispersants, dispersed oil, and oil-related metals on benthic and pelagic species using a combination of field and laboratory studies. Species presented will span from microbes and invertebrates, to fish and whales with some consideration of human health effects. Outcomes discussed will range from simple survival studies to more subtle effects on reproduction and DNA integrity.

The Deepwater Horizon Disaster (also referred to as the BP oil spill, the Gulf of Mexico oil spill, the BP oil disaster, or the Macondo blowout) occurred on April 20th 2010. Methane gas from the Macondo wellhead (that was being closed off) leaked up onto the drill rig and exploded, killing 11 people and starting the largest oil spill in the history of the US. By the time the well was capped, more than 200 million gallons of oil, and 20 million gallons of chemical dispersants were applied to the oil, which prevented oil accumulation at the ocean surface and, instead, moved it into the water column and onto the ocean floor. This approach decreased the amount of surface oil reaching inshore waters and beaches. However, it is unclear if it ultimately decreased toxicity to inshore species because the acute and chronic toxicity of dispersants, dispersed oil, and oil-related metals in the water column are unknown. Also unknown are the toxic outcomes of this approach for offshore species. During this session our panel of experts will present and discuss some of the first studies to evaluate the impact of this toxicological strategy considering the toxicity of crude oil, dispersants, dispersed oil, and oil-related metals on benthic and pelagic species using a combination of field and laboratory studies. Species presented will span from microbes and invertebrates, to fish and whales with some consideration of human health effects. Outcomes discussed will range from simple survival studies to more subtle effects on reproduction and DNA integrity.

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Free radicals are known to cause a multitude of effects in living systems, which span the spectrum of blunt injury to signal transduction pathways. Although it is important to understand the role of oxidative stress in toxicological pathways, the assay identification of the specific free radical initiators and mediators can provide insight into the source(s) of these species and their modulation, and their unique downstream targets that lead to activation of toxicity pathways. There are many diverse methods that are used to detect free radicals such as ROS (qualifies as oxidative stress) but the diversity in the quality of data obtained can vary considerably. This session will focus on characterizing the free radical intermediates themselves and describe the consequences of their generation in terms of toxicological effects. These methods will be used to demonstrate the importance in making an association with a particular oxidant with specific toxic outcomes. We will discuss various approaches to determine free radical species and highlight their advantages, disadvantages, and where improvements can be made. There are many issues to consider including electron paramagnetic resonance (EPR) spectroscopy which is considered the gold standard for free radical detection and identification but entails technical challenges and specialized expertise. Analytical methods such as HPLC and MS represent a more general approach but likely require previous findings with EPR to rationalize their use. Spectrophotometric and fluorimetric assays are quite popular, but either lack specificity or exaggerate findings due to the detection of artifacts. More recently, the detection of free radicals on macromolecules (protein, DNA) by immunoassays has presented an unprecedented opportunity to sensitively observe free radicals in *vitro* and *in vivo*. The discussions will focus on what and where each approach succeeds and fails and how each technique can be used to identify specific free radicals in pathways to toxicity.
Free radical metabolites of drugs generally receive less attention than more stable electrophilic metabolites. The pathways to free radical metabolite formation are well known but determining the consequences of their reactions with cellular targets that leads to toxicity requires considerable effort. I will present the formation of two distinct free radical metabolites (N-centered radicals and carbon-centered phenyl radicals) from aromatic amine drugs and determine their presence through their reactions with various cellular molecules. I will illustrate these concepts by emphasizing how studies with electron paramagnetic resonance (EPR) spectroscopy, oxygen analysis, HPLC, and immunoassays can be used to carry out these studies. I will demonstrate how these reactions are potentially involved in the pathways to haematological toxicity associated with the aromatic amine drug class.

Chelation therapy is a life saving treatment for childhood lead poisoning. However, chelation therapy is not without its risks and deaths from the treatment have been described in the literature, most recently 3 deaths attributed to the use of Na EDTA to treat lead poisoning, arteriosclerosis and autism. Chelating agents are available on the internet and have only recently come under scrutiny by the US Food and Drug Administration (FDA). This presentation will provide information on the use of Succimer to reduce blood lead levels among children whose levels were extremely high, provide an update on the WHO chelation recommendations and information from FDA regarding off-label use of chelation medications.

Chelation therapy is a very important therapy to lessen the occurrence of health consequences from the exposure to high levels of metals including selected radionuclides. However, the efficacy of treatment long after exposure has not been consistently demonstrated. Chelation in cases where exposures are limited can compromise health. Furthermore, chelation has been used in attempts to treat diseases for which evidence shows that even slightly elevated Pb levels are associated with impaired cognitive functioning and behavior problems in children, there may be growing pressure for clinicians to prescribe chelation therapy at only moderately elevated blood Pb levels. Chelation therapy is also used in off-label applications without clear indication of heavy metal poisoning, such as in the treatment of children with autism spectrum disorder (ASD) and other disorders. Our recent studies in both rodent and primate models of childhood Pb poisoning found that succimer was efficacious in ameliorating some areas of Pb-induced cognitive and affective dysfunction, and that these benefits were not directly associated with reductions in blood or brain Pb levels. Alarmingly, our studies have also shown that succimer treatment of animals not previously exposed to Pb produced significant, lasting neurobehavioral deficits. These findings substantiate the potential efficacy of succimer in Pb-poisoned children, and they raise concerns over the safety of the drug when administered in the absence of Pb poisoning, as is generally the case with ASD children.
may accelerate the excretion of heavy metals, the therapeautic value of this treatment in terms of decreased morbidity and mortality is largely untested. Recent research suggests that the use of these drugs in such settings may be associated with deleterious effects. Careful attention to risk-benefit issues is necessary, particularly in clinical situations where the contribution of heavy metals to the patient’s illness is in question.

1776 COOPERATIVE EPIEDEMIOLOGY AND TOXICOLOGY RESEARCH: HEI’S NATIONAL PARTICLE COMPONENT TOXICITY (NPACT) INITIATIVE.

M. J. Campen1 and G. Sunshine2, 1University of New Mexico, Albuquerque, NM and 2Health Effects Institute, Boston, MA.

In 2006, the Health Effects Institute (HEI) funded two major studies to address the comparative toxicity of components of particulate matter (PM) at multiple places across the United States where PM components and the sources of PM would differ. The goal of the program was to integrate toxicological and epidemiological approaches to address this issue. Both teams, one led by Sverre Vedal at the University of Washington, the other by Mort Lippmann at New York University (NYU), investigated the effects of PM components on cardiovascular endpoints in the same strain of mouse, ApoE knockout, but took contrasting approaches. In research conducted at Lovelace Respiratory Research Institute, Dr. Vedal’s team exposed the mice to well-characterized, lab-generated pollutant atmospheres that included vehicular—diesel + gasoline engine emissions—resuspended road dust, and secondary nitrates and sulfate particles. Dr. Lippmann’s team exposed the mice to particles concentrated from ambient air at four sites across the US. The NYU team also collected PM samples of different size ranges—ultrafine, fine, and coarse—at these sites to evaluate cardiovascular effects in another strain of mice. The epidemiological analyses in both studies used well-established cohorts with participants throughout the US and with some overlap between the locations studied by both groups. Dr. Vedal’s team focused on evaluating associations between long-term exposure to PM components with cardiovascular endpoints in participants in the Multi-Ethnic Study of Atherosclerosis (MESA) and Women’s Health Initiative (WHI) studies. Dr. Lippmann’s team evaluated associations in multiple cities between PM components and daily mortality and hospital admissions endpoints, as well as between exposures to components and annual mortality in the American Cancer Society (ACS) cohort. These integrated studies provide important lessons on how to design and execute population and laboratory-based research in a cooperative manner.

1777 THE UNIVERSITY OF WASHINGTON (UW)-LOVELACE RESPIRATORY RESEARCH INSTITUTE (LRRRI) NPACT INITIATIVE ON THE CARDIOVASCULAR HEALTH EFFECTS OF PM2.5 COMPONENTS.


It is not known which components of fine particulate matter (PM2.5) are most detrimental to human health. The UW-LRRRI NPACT aims to identify toxic PM2.5 component cardiovascular effects using both toxicologic and observational approaches. The observational study component of NPACT focuses on estimating individual-level long-term concentrations of PM2.5 components and makes use of two cohorts: (1) the Multi-Ethnic Study of Atherosclerosis (MESA) and Women’s Health Initiative (WHI) studies. Dr. Lippmann’s team evaluated associations in multiple cities between PM components and daily mortality and hospital admissions endpoints, as well as between exposures to components and annual mortality in the American Cancer Society (ACS) cohort. These integrated studies provide important lessons on how to design and execute population and laboratory-based research in a cooperative manner.

1778 CARDIOVASCULAR TOXICITY OF SIMULATED COMPLEX AIR POLLUTION ATMOSPHERES.

J. D. McDonald1, M. Campen2 and A. K. Lund1, 1Toxicology Division, Lovelace Respiratory Research Institute, Albuquerque, NM and 2Pharmaceutical Sciences, University of New Mexico, Albuquerque, NM.

Epidemiological associations between air pollutants and cardiovascular mortality have been reported in numerous studies. Contrasts in air pollution composition by regional associations and exposure to specific types of sources of environmental air pollutants may account for discrepancies among the epidemiological findings. To examine the effects of these multiple sources and combinations on progression of atherosclerosis, male ApoE−/− mice, on a high fat diet, were exposed to different mixtures of PM components, and to a control group (PM). Exposure to PM and a less-extreme F-MVE, but not PM alone, resulted in enhanced vascular constriction, as determined by myography, and also vascular TBARS levels. Additionally, exposure to PM2.5 resulted in a significant elevation in expression of factors associated with progression of atherosclerosis, including endothelin (ET)-1 and matrix metalloproteinase (MMP)-2 and -9 expression and activity, which was further increased by combining secondary PM (S,N). Exposure to MVE and to a lesser extent F-MVE and MVE combined with secondary PM, also resulted in increased plasma oxidized low density lipoprotein (oxLDL) levels and expression of its endothelial cell receptor, LOX-1. Increased expression of oxLDL and LOX-1 expression, with MVE exposure, was also correlated with increased macrophage/monocyte (MOMA-2) infiltration in the arterial wall and atherosclerotic plaque regions in ApoE−/− mice. These findings identify key mechanistic pathways which may account for epidemiological findings of increased cardiovascular morbidity and mortality resulting from exposure to air pollution and provide insight as to which components of ambient air pollution are most toxic.

1779 OVERVIEW OF THE NEW YORK UNIVERSITY NPACT INITIATIVE ON THE HEALTH EFFECTS OF PM COMPONENTS.

M. Lippmann. Environmental Medicine, New York University, Tuxedo Park, NY. Sponsor: M. Campen.

Our objectives were to: 1) to identify PM2.5 components and sources most closely associated with acute and chronic health effects in humans, and in a mouse model of atherosclerosis in US airsheds with a variety of PM2.5 compositions; and 2) examine the effects of toxic PM2.5 component cardiovascular effects on progression of atherosclerosis, male ApoE−/− mice, on a high fat diet, exposure to different mixtures of PM components, and to a control group (PM). Exposure to PM and a less-extreme F-MVE, but not PM alone, resulted in enhanced vascular constriction, as determined by myography, and also vascular TBARS levels. Additionally, exposure to PM2.5 resulted in a significant elevation in expression of factors associated with progression of atherosclerosis, including endothelin (ET)-1 and matrix metalloproteinase (MMP)-2 and -9 expression and activity, which was further increased by combining secondary PM (S,N). Exposure to MVE and to a lesser extent F-MVE and MVE combined with secondary PM, also resulted in increased plasma oxidized low density lipoprotein (oxLDL) levels and expression of its endothelial cell receptor, LOX-1. Increased expression of oxLDL and LOX-1 expression, with MVE exposure, was also correlated with increased macrophage/monocyte (MOMA-2) infiltration in the arterial wall and atherosclerotic plaque regions in ApoE−/− mice. These findings identify key mechanistic pathways which may account for epidemiological findings of increased cardiovascular morbidity and mortality resulting from exposure to air pollution and provide insight as to which components of ambient air pollution are most toxic.

1780 ALTERATIONS OF CARDIAC FUNCTION AND PLAQUE PROGRESSION IN APOE−/− MICE BY SUBCHRONIC INHALATION EXPOSURE OF CONCENTRATED AMBIENT PM2.5: THE ROLES OF PM COMPONENTS AND SOURCE CATEGORIES.

L. Chen. Environmental Medicine, New York University, Tuxedo Park, NY.

Ambient PM2.5 (particulate matter with aerodynamic diameters < 2.5 μm) is associated with alterations in the autonomic nervous system and cardiac function, but there are significant response variations. We studied the effects of concentrated PM2.5 (CAP) and PM2.5 components in Seattle, WA (Sa), and compared the results with those obtained in simultaneous studies at the Mount Sinai School of Medicine (MS) in Manhattan, NY, and at Sterling Forest (SF) in Tuxedo, NY.
A strong association between ambient PM and adverse health effects has been consistently reported. Because PM toxicity has shown differences depending on particle size, season, and location, it has become clear that mass concentration alone is not the best indicator of PM-induced health effects. We hypothesized that differences in the PM composition account for these reported varied effects. A high volume cascade impactor was used to collect 306 size-fractioned PM samples from five U.S. cities. In vitro analysis was conducted in a human pulmonary microvascular endothelial cell line (HPMEC-ST1.6R) and a human bronchial epithelial cell line (BEAS-2B). Results show that size and season have significant effects on reactive oxygen species (ROS) formation when data are separated by city. For example, winter coarse samples from Los Angeles, CA elicited a greater production of ROS than the corresponding summer samples, while the opposite was true for the fine and ultrafine samples. Following 6 and 24 h treatments with PM, mRNA abundance levels for markers of ROS and cellular inflammation also showed differences in expression depending on size, city, and duration of exposure. The most notable expression changes were in murine model using oropharyngeal aspiration of PM, PMN levels measured in BAL did not correlate with in vitro ROS production suggesting that in vitro ROS production may not be the best in vitro indicator of PM toxicity.

In vivo PM composition was identified by ICP-MS and correlations were made between specific constituents and biological endpoints. Our results support the hypothesis that the elemental composition of PM drives PM-induced health effects. Future studies aim to link particular emission sources to measured PM effects via source apportionment analysis.
Since genes do not operate individually but rather through concerted interactions, analyzing and visualizing networks of genes would provide important mechanistic information, especially when including additional functional parameters, such as exposure and effect biomarker measurements. Conventional methods as hierarchical clustering and correlation analyses are frequently used to address these complex interactions, but are limited as they do not provide causal relationships. Therefore, our aim was to investigate the added value of Bayesian network analysis and connectivity mapping, both following the conventional methods, concerning whole genome transcriptomic analysis in the context of environmental carcinogenesis. We investigated transcriptomic responses to cigarette smoking in humans associated with plasma cotinine levels as biomarker of exposure and aromatic DNA-adducts as biomarker of effect. Hierarchical clustering analysis revealed clusters with biological relevance and the correlation analysis showed significant correlations with previously identified genes, (e.g., CYP1B1 significantly correlated with DNA-adducts).

Most of the identified genes were connected to cotinine levels. Both Bayesian network analysis and connectivity mapping added valuable biologically relevant information on the dependence relationships between genes and the phenotypic parameters. Moreover, novel gene-gene interactions have been observed for multiple genes, which were directly and indirectly connected to cotinine levels. These results emphasize the added value of Bayesian network analysis and connectivity mapping in the phenotypic anchoring of transcriptomic responses.

We have shown earlier that Quantitative Structure-Activity Relationships (QSAR) models utilizing chemical descriptors of compounds improved accuracy for predicting complex toxicity endpoints when combined with biological descriptors derived from in vitro assays. However, in our recent study (Low et al., 2011. Chem Res Toxicol., 24:1251-62) of 127 drugs from the Japanese Toxicogenomics Project (Open TG-GATE), hybrid models did not show higher accuracy than those using toxicogenomics descriptors only. Innovative hybrid approaches, other than simply pooling chemical and biological descriptors together, may be required to optimize the concomitant use of chemical and short term biological assay data for improved toxicity prediction. To this end, we have developed multi-space (MS) kNN modeling approach. For each compound, two sets of k nearest neighbors are independently identified in the chemical and toxicogenomic descriptor spaces using the Tanimoto similarity metric. Predicted hepatotoxicity is calculated by the weighted average of all 2k neighbors’ hepatotoxicities. For the TG-GATE dataset, MS-kNN attained the best external Correct Classification Rate (CCR_MS = 79 +/- 2%, CCR_Toxicogenomics = 74 +/- 2%, CCR_Hybrid = 71 +/- 2%, CCR_Chemical = 59 +/- 2%; p = 0.04) Similar analysis of a second, Iconix data set using 24h gene expression as biological descriptors to model liver carcinogenicity showed the same trend (CCR_Toxicogenomics > CCR_Hybrid > CCR_Chemical). Both MS-kNN and toxicogenomics models afforded the best CCR (83%). We posit that MS-kNN represents a novel hybrid approach employing chemical descriptors and short term biological assays to achieve the improved toxicity prediction. Furthermore, MS-kNN models also afford improved interpretation of both biological and chemical features responsible for toxicity as well as extended applicability domains for predicting toxicity of diverse chemicals.
1792  A MODE-OF-ACTION-BASED QSAR APPROACH TO IMPROVE UNDERSTANDING OF DEVELOPMENTAL TOXICITY.

C. Yang1, A. P. Worth2, K. B. Arvidson3 and A. M. Richard4. 1Altamira, LLC, Columbus, OH, 2EC JRC, Ispra, Italy, 3OFAS, US FDA CFSAN, College Park, MD and 4NCCCT, US EPA ORD, Research Triangle Park, NC.

QSAR models of developmental toxicity (devtox) have met with limited regulatory acceptance due to the use of ill-defined endpoints, lack of biological interpretability, and poor model performance. More generally, the lack of biological inference of many QSAR models is often due to a disconnect between the training sets and modeling activities. To this end, we initiated a mode-of-action (MoA) QSAR approach in which biological context and interpretation guide the construction of training sets and selection of descriptors. We previously implemented the MoA concepts of DNA electrophilic reactivity and interactions involved in mutagenicity, clastogenicity, and rodent tumorigenicity into the US FDA CSFAN Chemical Evaluation and Risk Estimation System (CERES). We here extend this approach to a new set of MoA QSAR models and chemotypes for developmental defects in pre-natal devtox studies. A consolidated devtox database was created from several high quality datasets, including ToxCast, US FDA drug-related additives, and ILSI DevTox. Based on these data, mechanistically-based QSAR training sets for particular phenotypic effects (e.g., cleft palate) were created by grouping chemicals using high-level biological events linked to putative toxicity pathways. An example is presented of MoA categories built from ToxCast and literature-based evidence of high-level biological events linked to putative toxicity pathways. An example is presented of MoA categories built from ToxCast and literature-based evidence of high-level biological events linked to putative toxicity pathways. An example is presented of MoA categories built from ToxCast and literature-based evidence of high-level biological events linked to putative toxicity pathways. An example is presented of MoA categories built from ToxCast and literature-based evidence of high-level biological events linked to putative toxicity pathways.

1793  IN SILICO PREDICTIVE MODELS FOR INHIBITION OF CYTOCHROME P450 3A4 AND 2D6.

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In silico models can speed the early screening process of assessing potential drug interactions and has become an integral part of drug development. Metabolic-based drug-drug interactions can cause significant clinical issues in human drug exposure, leading to adverse effects and toxicity. In a US FDA-approved research collaboration, we built in silico predictive quantitative structure-activity relationship models using BioEpisteme (Prous Institute predictive software) for the inhibition of CYP3A4 and CYP2D6. CYP3A4/2D6 inhibitors were given a binary value to indicate their activities in the training sets. Molecular descriptors of the training set drugs were generated using the Molecular Operating Environment (Chemical Computing Group). Cross validation and external validation studies were used to assess predictive performance of these custom models. Performance was also appraised using regulatory classification of inhibitors per US FDA Guidance. Cross-validation depicted statistically significant results: CYP3A4 87.8%, CYP2D6 74.7%, concordance, with 94.7% and 88.3% sensitivity, respectively. External validation of the models using test sets containing known inhibitors for each enzyme (by US FDA Guidance) confirmed predictive performance. The use of our models can be a potential and cost-effective preliminary approach to predict if an investigational drug is an inhibitor of metabolizing enzymes which can then be confirmed or explored further through in vitro and in vivo studies, and thus serve as a guide to CYP liability.

1794  AHR-MEDIATED GENE EXPRESSION ACROSS MULTIPLE DEVELOPING MOUSE TISSUES.

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) exerts a broad range of toxicity mediated by the aryl hydrocarbon receptor (AhR). However, the role of AhR in development and the mechanism of TCDD-induced developmental toxicity remain largely unknown. We hypothesized that transcriptional changes through activation of the AhR mediates TCDD-induced developmental toxicity. To evaluate this hypothesis, global gene expression profiles were examined in three tissues (heart, lung, and liver) of the Ahr-/-(KO) and wild type (WT) mice embryos exposed in utero to 5 μg/kg TCDD or vehicle (Veh) on GD11.5 and terminated on GD13.5. Principal component analysis indicates that tissue is the largest source of variation with many more genes differentially expressed between tissues than between different genotypes/treatment groups within the same tissue. ANOVA analysis with p < 0.002 and fold change > 1.2 as cut-offs were used to determine the genes with significantly changed expression across various groups. Consistent with previous reports, changes in gene expression are much fewer after TCDD treatment in Ahr-/ than WT mice for the lung and liver, suggesting that TCDD-induced gene expression changes are primarily AhR-dependent in these tissues. Gene expression changes are greater in KO_Veh vs WT_Veh than in WT_TCDD vs WT_Veh for the heart and liver, whereas in lung, more genes are changed by TCDD treatment than by loss of AhR. Of numerous genes with significantly changed expression in KO_Veh vs WT_Veh across the three tissues, only acetylated dioxxygenase 1 and E2F transcription factor 6 are common. In addition, Cyp1a1 is the only common gene significantly induced by TCDD in all three tissues of the WT mice. Cyp1a1 is highly induced in the liver and lung with -70 and -30 fold increases, respectively, but is much less induced in the heart (+4-fold). In summary, this study indicates that effects of AhR ablation and/or TCDD treatment at the transcriptional level are tissue-specific. (This abstract does not represent NIHES or ATSDR policy).

1795  ACTIVITY PROFILES OF 676 TOXICANT PHASE II COMPOUNDS IN 231 BIOCHEMICAL HIGH-THROUGHPUT SCREENING ASSAYS.


Understanding potential health risks posed by environmental chemicals is a significant challenge elevated by large numbers of diverse chemicals with generally uncharacterized exposures, mechanisms and toxicities. The present study is a performance evaluation and critical analysis of 231 high-throughput cell-free assay results for 676 chemicals (including a number of failed pharmaceuticals, alternative plasticizers and food additives) in Phase II of EPA’s ToxCast™ project, and comparison to previous results for 309 ToxCast Phase I compounds. Biochemical high-throughput screening profiled G-protein-coupled and nuclear receptors, kinases, phosphatases, CYPs, histone deacetylases, ion channels and transporters. A primary screen tested all Phase II chemicals at 25μM concentration (or 10μM for CYP assays) and a secondary screen re-tested over 14,000 chemical-assay pairs in 8-point concentration series from 0.023 to 50μM (or 0.009–20μM for CYPs). Mapping relationships on half-maximal activity concentration (AC50) revealed 5484 active chemical-assay pairs for 510 unique chemicals and 216 unique assays. On average a chemical affected 2.5 assays and an assay was affected by 7.5 chemicals at AC50≤μM, versus 3.4 and 3.7, respectively for Phase I, but the percent affected remained constant (1%). Among the most promiscuous chemicals were tributyltin methacrylate, crystal violet, and tributyltin chloride; the most promiscuous assays were CYP2C19, CYP2C9, and the dopamine transporter. Known (e.g. caffeine perturbation of adenosine receptors and carbosulfan inhibiting acetylcholinesterase activity) and unknown (e.g. cyclopamine binding ion channels and methotrexate binding to somatostatin receptors) chemical activities were observed. A combination of these in vitro results along with in vivo toxicity data are being used to generate hypotheses about potential molecular initiating events associated with adverse outcomes for this diverse chemical set. This abstract does not necessarily reflect US EPA policy.
with liquid HD and surface decontamination there is an appreciable, solvent-ex- tractable depot of free HD representing multiple vesicating doses. This investiga- tion characterised the toxicological relevance of this reservoir by extraction using three different techniques. Methods. In vitro: “Franz-type” diffusion cells containing dermated human and pig skin at 32°C, with stirred ethanol-water (50:50 v/v) receptor. 14C-HD was used to trace full dose distributions in the system fol- lowing application of each of the extraction techniques. In vivo lesions raised on large white pigs by contact with filter paper soaked in liquid HD and treated immedi- ately and at 1 and 3 hours, primary measure of effect – histopathology at 7 days. Extraction techniques were tape stripping, and extraction with either tetraglyme (30% v/v in water) or kerosene respectively. Results. A rapidly equili- brating, finite reservoir of 14C-HD was observed in human and pig skin. In vitro within 2 min of application of liquid 14C-HD, persisting for up to 6 h and solvent- extractable following surface decontamination by dry swabbing. Gas chromatogra- phy – mass spectrometry has shown that the majority of the extractable fraction, at least at the earlier time points (≤3 h), was free 14C-HD equivalent to multiple vesicating doses. The majority of the reservoir could be extracted with tetraglyme (30% v/v in water) or kerosene or by tape stripping the stratum corneum, all meth- ods were similarly efficient out to ~6 h following contamination. In vitro the lesions were not changed by extraction unless performed within 5 minutes of contact. Conclusion. Removal of the reservoir is unlikely to benefit the patient unless carried out immediately, but may affect contamination control. © Crown Copyright 2011.

PL 1799 PUPILARY LIGHT RESPONSE IN A GUINEA PIG MODEL EXPOSED TO ORGANOPHOSPHATE AGENTS.

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Here we report a study to more precisely define and quantify the relationship between organophosphate agent exposure, including pesticides and nerve agents, with cholinesterase inhibition and the ocular biomarkers that are induced. A thorough investigation of these relationships was conducted to subsequently refine pupillary algorithms for the automated detection of organophosphate exposures with greater specificity regarding level of exposure, the extent of cholinesterase inhibition, and the temporal presentation and persistence of the ocular biomarkers. A guinea pig model was exposed to varying concentrations of parathion (pesticide), soman and VX (nerve agents) to methodically detail both the temporal and quantitative occur- rence of pupillary deficits (anticholinesterase biomarkers) to determine the most sensitive, accurate diagnostic algorithms in these animal models. Dose-response curves and temporal-response curves for both pupillary deficits and generalized symptoms were developed for each agent used. Based upon previous studies dose ranges were conducted from the LD50 to 3000-fold below the LD50 to incorpo- rate lethal and sub-lethal exposures without decreasing the potential sensitivity of the ocular biomarkers. In addition, cholinesterase assays were performed at various time points post-exposure. Trends were identified for 14C-HD, VX and soman in the guinea pig model will allow improved correlation to human data that already exist and map a direct relationship between exposure, enzyme inhibition, and ocular deficits that will be vital for future development of both diagnostic and treatment protocols.

PL 1800 EFFICACY OF BIFUNCTIONAL AND NONSTEROIDAL ANTIINFLAMMATORY COMPOUNDS AGAINST VAPOR-INDUCED SULFUR MUSTARD INJURY IN A HAIRLESS MOUSE VESICANT MODEL (HMVM).

Y. Chang1, J. D. Wang1, R. A. Hahn1, M. K. Gordon1, M. C. Babin2, S. C. Young1, N. D. Heindel1, L. D. Laskin1 and D. R. Gercke2. 1Pharmacology and Toxicology, Ernest Mario School of Pharmacy, Rutgers University, Piscataway, NJ, 2Batelle Biological Research Center, Columbus, OH, 3Chemistry, Lehigh University, Bethlehem, PA and 4Environmental and Occupational Medicine, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, Piscataway, NJ. Effective medical countermeasures against chemical vesicant exposure such as sulfur mustard [bis(2-chloroethyl) sulfide, SM] have yet to be established. Possible countermeasures candidates include bifunctional compounds that have dual inhibition of cholinergic activity and inflammation, two pathways that appear to exacerbate tissue damage after vesicant exposure. Using a SKH-1 mouse vapor cup model, we have tested the efficacy of a novel bifunctional compound designed to act as a non- steroidal antiinflammatory drug (NSAID) and an acetylcholinesterase inhibitor. The drug was formulated in ethyl oleate vehicle and applied to mouse backs 24 hours after sulfur mustard vapor exposure. Additional applications were made 48 and 72 hours after exposure. Punch biopsies were then collected and the tissue sam- ple divided for histology, genomic, and protein analyses. Draize score and H & E microscopic analyses indicated that following dosing of human or pig skin

PL 1796 IN SILICO IDENTIFICATION OF BISPHENOL A MOLECULAR TARGETS.

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Bisphenol A (2,2-bis-(p-hydroxyphenyl)-2-propane, BPA) is a known endocrine disrupting chemical used in the fabrication of plastics, resins and flame retardants. Its biological properties are mainly explained based on its capacity to bind the estro- gen receptor (ER). However, its role on other molecular mechanisms involving protein targets different from this nuclear receptor is still under debate. In order to identify plausible binding proteins for BPA, a docking study with Autodock Vina was performed using 269 proteins representative of different pathological condi- tions that were previously selected by executing a data mining search in PubMed. Coordinates of target proteins were downloaded from Protein Data Bank (PDB) and prepared in SYBYL 8.1 program package. BPA structure was drawn and optimized by DFT at the B3LYP/6-31G level in Gaussian 0.3, the grid box center was determined utilizing MGLTools, and a blind docking strategy was performed to recognize protein-ligand complexes. Calculated binding affinities were then e- mployed for ranking proteins. Repetitions of 100 runs and conformational analyses with LigandScout 2.0 were carried out on complexes presenting best ligand affini- ties. Validation protocols on blind tested protein complexes showed that modeled BPA structure always selected the specific BPA binding site, after a whole protein blind docking procedure with Autodock Vina. The proteins that exhibited greatest in silico affinities for BPA were the estrogen-related receptor gamma (ERRγ, -9.9 Kcal/mol), and the C/EBP-like kinase isoforms 4, 1 and 2 (C/EBPα/β/δ and C/EBPκ), with affinity values -9.5 and -9.0 Kcal/mol respectively. These last proteins are involved in the control of RNA splicing. Moreover, molecular targets related to diabetes, breast cancer, and circadian rhythm showed moderate affinities with values around ~8.0 Kcal/mol. These results suggest that BPA may be acting on targets different from ER, eventually influencing several sig- naling pathways involved in disease.

PL 1797 BIOCHEMICAL AND CELLULAR CHANGES ASSOCIATED WITH METHYL ISOCYANATE-INDUCED PULMONARY FIBROSIS IN C57BL/6 MICE.

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Methyl isocyanate (MIC) is classified as a Level 2 Toxic Industrial Chemical and is considered an “agent of opportunity” that could cause mass casualties if released during terrorist or storage tanks were breached. Long-term effects in survivors of the MIC release in Bhopal, India in 1984 include pulmonary fibrosis. The purpose of this study was to determine potential mechanisms of MIC-induced fibrosis. Groups of 12 female C57BL/6 mice were administered 0, 2.6, 3.9, and 5.3 mg MIC/kg body weight by intracheal instillation. Six mice/group were euthanized 24 h and 7 days post-dosing. The lungs were harvested, fixed, and stained with hematoxylin and eosin. Neutrophils counts in lavage fluid. Lung tissue was examined for evidence of oxida- tive stress (thiobarbiturate reactive substances; TBARS), apoptosis (TUNEL assay), collage deposition (hydroxyproline), and histopathology. MIC exposure caused no mortality or sustained clinical signs of toxicity. Neutrophil numbers in lavage fluid were increased at 24 h and 7 days post-dosing. Dose-dependent in- creases in concentrations of cytokines IL-6, MCP-1, Eotaxin, and TGFβ1 in lavage fluid occurred at 24 h, with levels in high-dose group significantly increased com- pared to controls. TBARS/mg protein was significantly increased (30–50%) at 7 h in all dose groups. MIC-induced statistically significant increases in apoptotic cells/100 lung cells counted, with 12–16 fold and 6–11 fold increases seen at 24 h and 7 days respectively. Histological changes included peribronchial, perivascular and alveolar septal wall mononuclear cell infiltrates. Focal areas of minimal-to-mild fi- broblast/myofibroblast proliferation were observed in 12% and 23% of mid- and high-dose lungs examined. MIC increased lung hydroxyproline content by ap- proximately 25% in all dose groups at 7 days. These changes occurred in the absence of mortality and parallel those seen in the rodent models of bloomycin-induced pul- monary fibrosis. This research was supported by intramural funds.

PL 1798 THE TOXICOLOGICAL RELEVANCE OF THE CUTANEOUS RESERVOIR OF SULPHUR MUSTARD.

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Experimental investigations of sulfur mustard (HD) burns during the First World War demonstrated a reservoir of un-reacted HD in human skin. More recent evi- dence from an in vitro system indicated that following dosing of human or pig skin
analysis of tissue samples showed improvement for the bifunctional compound. Samples exposed to SM alone appeared to have an acute inflammatory response with markers of inflammation such as Cox-2. II.1B, CXC12, MMP-9 increased as determined by RT-PCR. Samples treated with the bifunctional compound showed a reduction in Cox-2 and MMP-9 protein by Western blot analysis. Immunofluorescence analysis demonstrated that the basement membrane zone between the epidermis and dermis was improved after treatment with the bifunctional compound as shown by specific antibodies for laminin-332, Ki67, Keratin10, Cox-2, and MMP-9. Taken together, this data supports the potential of using bifunctional compounds as medical countermeasures against vesicant-induced skin damage. Supported by ES005022, ES004738, EY00956, and NIAMS U54AR050703.

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1801 ANTI-INFLAMMATORY PROPERTIES EXHIBITED BY ARYL HYDROCARBON RECEPTOR (AHR) LIGANDS, INDOLE-3-CARBINOL, AND 3,3'-DIINDOLYL METHANE DURING STAPHYLOCOCCAL ENTEROTOXIN B-INDUCED IMMUNE SYSTEM ACTIVATION.

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Staphylococcal enterotoxin B (SEB) is an exotoxin produced by the Staphylococcus aureus bacterium. This toxin is classified as a “superantigen” because of its ability to directly bind T cell receptors (TCR) with MHC II class receptors of antigen presenting cells (APCs), bypassing the normal antigen processing and presentation mechanism and activating a large proportion of T cells. Commonly associated with classic food poisoning, SEB has also been shown to induce toxic shock syndrome (TSS), and more recently gained attention as a potential biological warfare agent since it is easily aerosolized. In the current study, we tested the potential use of indole-3-carbinol (I3C) and one of its byproducts, 3,3'-diindolylmethan (DIM), two AHR ligands, on SEB-induced activation of T cells and cytokine production. To this end, we evaluated the ability of I3C and DIM to reduce T cell activation mediated by SEB in CD3+Vbeta+ T cells both in vitro and in vivo. Administration of I3C and DIM into C57BL/6 mice challenged with SEB was able to significantly reduce the number of SEB-specific CD3+Vbeta+ cells. There was also a decrease in CD69 expression, a marker associated with activation of T lymphocytes, and expression of pro-inflammatory cytokines, such as TNF-alpha. Using TUNEL assay and caspase inhibitor studies, we were able to show that not only did I3C and DIM induce apoptosis in SEB-activated T cells, but also that this observed apoptotic cell death was mediated primarily through extrinsic pathway. We confirmed that I3C and DIM were inducing apoptotic responses in SEB-activated T cells by using an Ahr-specific antagonist. Taken together, our results demonstrate that I3C and DIM, which act as AHR ligands, are potentially promising candidates to ameliorate the toxic effects induced by SEB exposure.

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1802 EFFECT OF SOLVENTS ON THE STABILITY OF CHLOROETHYL ETHYL SULFIDE (CEES).

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Chloroethyl-ethyl sulfide (CEES), a commonly used surrogate for sulfur mustard (HD), is similarly hydrophobic. Although the half-life of aqueous HD is only 4-8 min, its slow rate of solubility in aqueous solution substantially extends its overall half-life. Treatment of cells in culture with either HD or CEES is difficult; polar organic solvents are required to dissolve either compound in aqueous solution. While these solvents may increase cellular exposure to the vesicants, they may also shorten the vesicant’s half-life by increasing degradation through reactions with the solvent or water. Although ethanol is typically used to dissolve vesicants, even low doses of ethanol (0.1%) can have off-target effects. Therefore, in the current study, we investigated the use of DMSO as a solvent for CEES. CEES (1.7 M) was found to be stable in DMSO at room temperature for at least 24 hours as measured by 1H-NMR, whereas heating in DMSO at 70°C for 30 min or longer caused measurable degradation. The presence of 10% water did not affect the rate of CEES degradation at room temperature over 24 hr. Under constant agitation, the half-life of CEES pre-dissolved in DMSO, followed by dilution in aqueous solution (3.5 min) was less than that of CEES pre-dissolved in ethanol (5.5 min) or CEES added directly to phosphate buffered saline (11.5 min). These studies indicate that while CEES is effective as a stock solution in DMSO for short time periods at room temperature, further dilution in aqueous media enhances its reactivity. DMSO is therefore suitable as an alternative to ethanol for CEES.

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1803 THERAPEUTIC EFFICACY OF SILIBININ IN ATTENUATING SULFUR MUSTARD-INDUCED SKIN INJURIES.


Sulfur mustard (HD), a chemical warfare agent, inflicts delayed blistering and incapacitating injuries on skin. To identify effective therapeutic interventions against HD-induced skin injuries, efficacy studies were carried out employing HD analog 2-chloroethyl ethyl sulfide (CEES)-induced injury biomarkers established from our earlier studies in skin cells and SKH-1 hairless mice. The results of these studies demonstrate strong therapeutic potential of silibinin (SB), a natural flavonone with proven antioxidant, anti-inflammatory and anti-cancer properties, in attenuating CEES-induced skin injuries and oxidative stress. In skin cells, SB (10 μM) treatment 0.5 h after CEES (0.5 mM) exposure caused a significant reversal in CEES-induced decrease in cell viability, apoptotic and necrotic cell death, DNA damage, and increased oxidative stress. SB (1 mg) topical treatment onto SKH-1 hairless mouse skin 30 min after CEES (2 mg) exposure, was most effective in reversal of CEES-induced increases in skin bi-fold (62%) and epidermal thickness (85%), apoptotic cell death (70%), myeloperoxidase activity (complete reversal), increases in INOS, COX-2 and MMP-9 protein levels (> 90%), and activation of transcription factors NF-κB and AP-1 (complete reversal). Similar SB treatment was also effective in attenuating CEES-induced oxidative stress measured by 4-hydroxy-2-nonenal and 5,5-dimethyl-2-(8-octanoic acid)-1-pyrolline N-oxide protein adduction formation, and 8-oxo-deoxyguanosine levels. Our previous studies have demonstrated the involvement of oxidative stress in CEES-induced toxic responses in skin cells and mouse skin tissue. Reversal of CEES-induced oxidative stress by SB evidenced in this study indicates the antioxidant therapeutic efficacy of SB in attenuating CEES-induced skin injuries. Together, these findings support further optimization of SB rescue treatment in the HD skin toxicity model and its clinical development as an effective treatment for skin injuries by vesicants.

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1804 IDENTIFICATION OF EXPOSURE BIOMARKERS TO BIOLOGICAL TOXINS SEB, MICROCYSTIN LR, AND T2.

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The objective of this research was to define elimination kinetics/disposition of biomarkers of exposure to Staphylococcal Enterotoxin B (SEB), Microcystin LR and T2. This is conducted through conduct of tissue distribution and pharmacokinetic studies in rodents and non-human primates (NHP), modeling of disposition and elimination characteristics, selection of optimal excretion matrix to utilize for biomarker targeting, and identification of biomarkers that can be used to link to toxin exposure. Custom 14C labeled toxin was developed that allowed disposition and excreta analysis. Both radioactivity and ELISA analysis of parent compound were conducted in Sprague-Dawley rats after oral administration and NHP (Cynomolgus macaque) after oral or intratracheal administration. Sprague-Dawley rats were observed up to 72 hr post administration. Tissues and blood were collected at 0.25, 1, 4, 8, 12, 24, 48, 72 hr. Urine and feces were collected for 72 hr in rodents and NHP SEB showed rapid absorption from the GI tract, and metabolism of the parent compound within 1-2 hours after dose administration. The metabolism was confirmed by the absence of parent compound coupled to the presence of radioactivity remaining in tissues, plasma and excreta. Peptide sequencing found several unique peptide sequences that could be used to detect SEB exposure. Microcystin LR showed high concentrations of parent compound and a number of metabolites in excreta and peripheral organs. It was was detected in both urine and feces 24-72 hr after dose administration. Interestingly, the radioactivity did show excretion at earlier time points. T2 is rapidly absorbed and excreted in both urine and feces for 48 h after exposure. Both parent T2 and a number of promising metabolite biomarkers were observed. Work supported by the Defense Threat Reduction Agency.

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1805 MECHANISMS OF SULFUR MUSTARD ANALOGS, 2-CHLOROETHYL ETHYL SULFIDE AND NITROGEN MUSTARD-INDUCED DNA DAMAGE IN SKIN CELLS.

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Sulfur mustard (SM), an alkylating chemical warfare agent, causes vesication and severe damage to the skin. The major reported cause of SM-induced cytotoxicity is DNA damage, which could result either via alklylation- or oxidative stress-related
Mechanisms. Accordingly, to understand the role of these cytotoxic effects, we em-
ployed 2-chloroethyl ethyl sulfide (CEES), a monofunctional analog of SM. CEES exposure to mouse skin epidermal JB6 cells and dermal fibroblasts caused DNA-
damage (phospho H2AX and p53 and comet tail extent moment), oxidative stress and oxidative DNA damage (8-OHdG levels). In further studies distinguishing be-
tween oxidative and direct DNA-damaging effects of CEES, pretreatment with glut-
tathione (GSH) or anticipating trolox caused a 50% reduction in CEES-induced oxidative stress and oxidative DNA damage. However, only GSH decreased CEES-induced total DNA damage, probably through formation of GSH-CEES conjugate detected by LC-MS analysis, indicating that oxidative stress may play a minor role in CEES-induced DNA damage. Unlike SM [forms both DNA interstrand cross links (ICL) and adducts], CEES can only form DNA adducts. Therefore, we further expanded our studies with nitrogen mustard (NM), a bifunctional analog of SM. Notably, SM studies in the laboratory setting are limited, as they require special facilities. DNA damage was assessed in JB6 cells via comet assay, which confirmed that NM acts as a strong DNA ICL forming agent. Trypan blue exclusion assay indicated that 0.75μM NM-induced arrest in JB6 cell growth at 24h, suggesting a possible cell-cycle arrest, thereby allowing the cells to process DNA ICLs. An increase in comet length and phospho H2AX and p53 at 16 and 24h following NM exposure also indicated DNA damage and the initiation of repair machineries. Studies are underway to understand the molecular mechanisms involved in repair of NM-induced DNA damage that can ultimately help us develop therapeutic strategies against NM- and SM-induced skin toxicity.

1806 PULMONARY FIBROTIC RESPONSE FROM INHALED MULTIWALLED CARBON NANOTUBE EXPOSURE IN MICE.


Inhalation exposure studies of mice were conducted with co-milled with multiwalled carbon nan-
tubes (MWCNTs) to assess the fibrotic potential of this manufactured carbon nanomaterial. To address the hypothesis that MWCNTs cause persistent morpho-
logic changes, male C57BL/6 mice were exposed in a whole-body inhalation system to a 5 mg/m3 MWCNT aerosol for 5 hours/day for 12 days (4 times/week for 3 weeks). At the end of inhalation exposures, lungs were preserved by vascular perfusion of fixative while inflated with air at 1, 14, and 84 days post inhalation exposure. A separate, clean-air control group was also studied. Sections were prepared to analyze the distribution of lung burden following inhalation exposure. Morphometric measurements of Sirius Red staining were used to assess the connective tissue response. At day 1 post-exposure 86±42 and 14±6% of the percent lung burden (mean±SE, N=5) were in the alveolar and airway regions, respectively. Distribution within the alveolar region of was 57±6, 7±5 and 20±4% percent in alveo-
lar macrophages, alveolar airspaces and alveolar tissue, respectively. The mean linear intercept, a measure of the degree of alveolar expansion, was not significantly dif-
ferent between groups being 29±5.0, 29.6±6.0, 29.1±0.5 and 29±4.0 microns for clean-air controls, 1, 14 and 84 days MWCNT groups, respectively. The con-
nective tissue in the alveolar region of MWCNT-exposed mice demonstrated a pro-
gressive increase in thickness over time (0.17±0.02, 0.22±0.02 and 0.25±0.03 for 1, 14 and 84 days) and was significantly different from clean-air controls (0.16±0.02) at 84 days. Despite the relatively low fraction of the lung burden being noted wherein fiber-laden alveolar macrophages had accumulated. This finding was characterized by infiltrates of inflam-
matory cells and some thickening of interstitial walls and minimal to slight hy-
pertrophy/hyperplasia of Type II epithelial cells for the 2.5 and 25 mg/m3 groups, respectively. Three months following exposure this inflammation was still present, but less severe. A non-specific inflammatory response was also noted in the nasal passages of exposed animals. Extrapulmonary fibers, occurring as single or very few fibers were sporadically noted in other organs; however no adverse pathologic re-
actions were noted in the tissues containing these fibers. Therefore, the NOAEL for VGCF™-H nanofibers is considered to be 0.54 mg/m3 (4.9 fibers/cc) for male and female rats.

1808 LINKING NANOMATERIAL PHYSICAL PARAMETERS TO TOXICITY: A SYSTEMATIC ASSESSMENT OF GOLD NANOMATERIALS.

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Gold nanomaterials (Au NMs) have been studied for their incorporation in biolog-
cal applications due to their unique surface Plasmon resonance. Previous reports identified size and surface charge as critical in mediating the biological response, while other studies describe the importance of shape. However, within the nan-
otoxicology literature, comparisons of NMs are typically made across a variety of cell models using different biological assays. This study reports the evaluation of Au NMs through the HaCaT keratinocyte cell line and the same assays in order to pro-
vide a comprehensive assessment of Au NM physical parameters. Previously, surface charge was shown to mediate the mechanism of toxicity in Au spheres, and Au spheres were less toxic than rods2. In addition, altering the rod aspect ratio (AR) and surface chemistry impacted toxicity3,2. To further explore shape, this study evaluated nanocubes (NC; 50 nm), nanospheres (NS; 20, 50 nm), nanofillets (NF; AR=2), and nanorods (NR; AR=3) with a tannic acid surface using HaCaT cells. Typically, Au NRs coated with PEG display minimal cellular uptake. However, TEM imaging identified increased uptake in Au NP and NR when tannic acid was on the surface. Furthermore, cell viability was examined (MTS assay) at concentra-
tions of 25, 50 and 100 μg/ml, and cell morphology was assessed using FDA/PI stained data. The viability demonstrated a shape and concentration dependent response, with the ranking of toxicity: 20 nm NS < 60 nm NS < 50 nm NC < NP (AR=2)- NR (AR=3). Furthermore, changes in gene expression (25 μg/ml) demonstrated a shape dependent increase in stress response genes. This data in combination with previous data, illustrated that several physical parameters mediated Au NM toxicity in ker-
atinoocytes, emphasizing the link between NM characterization and toxicity.

1809 THE ROLE OF IL-1β SIGNALING IN NICKEL ASSOCIATED MULTIWALLED CARBON NANOTUBE-INDUCED ACUTE PULMONARY EOSINOPHILIA.

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Exposure to certain engineered nanomaterials (ENM) has been associated with pathological changes in animal models raising concern that human health effects will emerge with increasing use of ENM, such as multiwalled carbon nanotubes (MWCNT). We had previously shown that a correlation exists between the amount of nickel associated with MWCNT and assembly of the NLRP3 inflam-
mation (NLRP3) resulting in conversation of pro-IL-1β to the active form. Furthermore, we have shown that NLRP3 activation in vitro correlates strongly with lung inflammation and pathology. In this study, we investigated the role of IL-
1 receptor signaling in the induction of acute pulmonary eosinophilia. C57Bl/6 and IL-1 receptor null mice (IL-1R−/−) mice instilled with MWCNTs FA04 (2.54%Ni) and FA21 (5.54% Nickel) underwent whole lung lavage at 24 hours, 4, 7 and 14 days post-exposure. Differential cell counts, Eosinophil Peroxidase Assay (EPO), flow cytometry and histological evaluation of tissue sections were per-
formed. Analysis of the results revealed that 24 hours of exposure to MWCNT FA21 was effective in inducing pulmonary inflammation as indicated by eosinophil influx into the airways of wild type mice. The cell differential count and EPO assay confirmed the presence of eosinophils in the airway of MWCNT exposed WT mice. The initial acute inflammatory response was diminished in mice deficient for the IL-1 receptor. However, in later time points, this inflammatory response was heightened in the lungs of IL-1R−/− mice, compared to the wild type mice. These data support an important role for IL-1β signaling in the regulation of the inflam-
matory response and a potential mechanism for the clearance of Ni-MWCNT from the lung. This work was supported by NIH grants NRSA F32 ES019816 RC2-ES018742 and P20-RR017670.
1810 MAST CELLS AND THE IL-33/ST2 AXIS ARE ESSENTIAL DETERMINANTS OF CARBON NANOTUBE TOXICITY.

P. Kato1, X. Wang1, R. Urkanza2, S. C. Hilderbrand3, C. J. Wingard1 and J. M. Brown1, 1Pharmacology & Toxicology, East Carolina University, Greenville, NC and 2Department of Physiology, East Carolina University, Greenville, NC.

The use and production of nanomaterials such as multi-walled carbon nanotubes (MWCNTs) has significantly increased in recent years due to their versatility in numerous applications thereby raising concern about the potentially hazardous impacts on human health. Mast cells are known to play an important role in several pathological conditions, including allergy, asthma and cardiovascular disease. The aim of this study was to examine mast cell mediated mechanisms of respiratory and cardiovascular toxicity following exposure to MWCNTs. We assessed inflammatory and fibrotic responses in the lungs as well as cardiac ischemia-reperfusion (IR) injury responses in C57BL/6, K\textsuperscript{IR}-/- (mast cell deficient), K\textsuperscript{IR}-/- reconstituted with bone marrow derived mast cells, and IL-33 receptor (ST2 \textsuperscript{+}) deficient mice following oropharyngeal aspiration of MWCNTs. C57BL/6 mice instilled with MWCNTs exhibited significant pulmonary inflammation as evidenced by increased numbers of neutrophils and macrophages that were associated with elevated IL-33. Furthermore, impaired pulmonary function (compliance and elastance) was observed along with increased granuloma formation, collagen content and deposition in C57BL/6 mice 30 days following instillation of MWCNTs. In addition to adverse pulmonary effects, myocardial infarction was exacerbated in C57BL/6 mice 1 day following exposure to MWCNTs. These adverse pulmonary and cardiovascular responses elicited by MWCNTs in C57BL/6 mice were all significantly attenuated in K\textsuperscript{IR}-/- mast cell deficient mice as well as ST2 \textsuperscript{+} mice, and conversely, were restored in K\textsuperscript{IR}-/- mice reconstituted with mast cells. These findings demonstrate an unrecognized, but critical role for the IL-33/ST2 axis and mast cell activation in mediating MWCNT toxicity. This work supported by NIH RO1 ES019311 (JMB) and RO1 ES016246 (JW).

1811 DIFFERENT EFFECT OF MULTIWALLED CARBON NANOTUBES IN LUNG TUMORIGENESIS.

J. Kim1,2, A. Minai-Tehrani1, B. Kang1, S. Hong1, J. Shin1, S. Park1 and M. Chun1,2, 1Laboratory of Toxicology College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea and 2Department of Nano Fusion Technology, Graduate School of Convergence Science and Technology, Seoul National University, Seoul, Republic of Korea.

Carbon nanotubes (CNTs) have gained great interest due to its unique and superb properties such as ultra-high Young's modulus and tensile strength, which makes them promising in serving as a reinforcement of composite materials with desired mechanical properties, however, the high aspect ratio of carbon nanotubes (CNT), a feature they share with asbestos, is likely the key factor for reported toxicity of certain CNT. In this study, we compared pulmonary toxicity and carcinogenicity of two different types of MWCNTs; pristine multi-walled carbon nanotube (PMWNT-CNT), and acid treated multiwalled carbon nanotube (TMWNT-CNT). We administered PMWNTs, TMWNTs and sterilized saline as vehicle control into murine lung by intratracheal instillation and the animals were sacrificed at the time point of 1 month, 6 months, 1 year following injection (n=5/group). Genetic instability and tumor occurrence were examined by Comet assay and histopathological analysis respectively. In mice treated with 0.1mg PMWNT-CNT, adenocarcinoma was observed in 2 of 5 mice while TMWNT-CNT treated mice did not show. Previously, we reported that MWCNTs could cause genetically unstable status even though the status was transient. Here, we report that PMWNTs which were not cleared from the lung could develop tumors in the lung of mice. Western blot analysis showed that Cathepsin D was significantly increased in mice instilled with 0.1mg/mouse of PMWNT-CNT compared to mice treated with TMWNT-CNT. Anti-apoptotic protein, Bcl-2 also showed the same pattern of change. Our results demonstrated that cathepsin D induced excessive apoptosis leading to regeneration response in the lung that may result in uncontrolled replication and genetically unstable status.

1812 EVALUATION OF LUNG RESPONSE IN GOLDEN SYRIAN HAMSTERS TO INHALED SINGLEWALLED CARBON NANOTUBES.

K. V. Biljani1, H. R. Sukhija1 and J. M. Cerreta1,1Pharmaceutical Sciences, St. John's University, Queens, NY. Sponsor: L. Trombetta.

Singlewalled carbon nanotubes (SWCNTs) are graphene sheets rolled into cylinders that have diameters of few nanometers (nm) and lengths that may extend to micrometers. Carbon nanotubes have several applications, i.e. as superconductor material and in manufacture of biosensors. Human exposure may occur in the production and use of SWCNTs and such exposure has the potential of causing injury. To evaluate the potential harmful effects of inhalation exposure to SWCNT, Golden Syrian Hamsters were divided into: a control group that was exposed to an aerosol of autoclaved distilled water and a treated group that was exposed to aerosolized SWCNT at 2mg/m\textsuperscript{3} for 4, 8 or 14 days. Hamsters were euthanized one day following completion of exposures, and the lungs were removed and processed for scanning (SEM) and transmission (TEM). Macrophages in bronchoalveolar lavage (BAL) cytosin preparations were assessed for Tumor Necrosis Factor-\textalpha Receptor 1 (TNFR1) and Endothelin Type A receptor (ETA). Results from SEM demonstrated thickening of alveolar septa and deposition of aggregates of SWCNTs along the alveolar walls. TEM of 8 and 14 day SWCNTs treated hamster lungs demonstrated a distorted ultrastructure with deposition of aggregates of bundles of SWCNTs in the alveolar spaces and lung cell surfaces that were ruffled and irregular. Percent TNFR1 positive cells per field were significantly increased in the hamsters exposed to SWCNT for 4, 8 or 14 days as compared to their respective control groups (p<0.0001). Immunofluorescence analysis of ETA receptor demonstrated that in comparison to the control groups, animals treated with SWCNTs for 4, 8 or 14 days had increased ETA receptor levels. Data from this study indicate that inhaled SWCNT can reach alveolar spaces and enter cells. Further, that such particles can cause injury and altered levels of TNFR1 and ETA.

1813 90-DAY INHALATION TOXICITY STUDY WITH CARBON NANOFIBERS IN RATS: FIBER-RELATED EFFECTS ON THE RESPIRATORY TRACT DO NOT IMPACT THE CARDOVASCULAR SYSTEM.

D. B. Washeir1, Y. Muro2, K. L. Reed3, S. R. Frame1 and M. P. DeLorme1, 1DuPont Haskell Lab., Newark, DE and 2Showa Denko K.K., Tokyo, Japan.

The aim of this study was to investigate the potential cardiovascular (CV) effects following a subchronic exposure to VGCF™-H carbon nanofibers in male and female SD rats. Groups of rats were exposed nose-only, 6 h/d, 5 d/wk to 0.54, (4.9 b/cc) 2.5 (56 b/cc), or 25 (252 b/cc) mg/m\textsuperscript{3} over a 90-day period. Groups of animals from the high and control group were allowed a 90 day recovery period to determine the reversibility of any effects observed at the end of the exposure period. In addition to histopathological evaluation of the respiratory tract, bronchoalveolar lavage (BAL) was also used to assess pulmonary inflammation. CV-specific endpoints evaluated were prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen (FBGN), platelets (PLT), C-Reactive protein (CRP) and cardiac specific serum enzymes (CPK, LDH, AST) as well as cell proliferation (CP) of cardiac tissue using BrdU staining. Exposure to 2.5 and 25 mg/m\textsuperscript{3} resulted in a minimal and slight inflammation of the terminal bronchiol and alveolar duct areas, respectively, as well as significant lung weight increases in rats exposed at the high concentration. Evaluation of BAL fluid biomarkers (PMNs, LDH, ALKP, microprotein) were significantly elevated (vs. air controls) only controls exposed to 25 mg/m\textsuperscript{3}. These findings indicated sustained but low-grade inflammation. With respect to cardiovascular effects, serum enzymes, coagulation parameters (PT, aPTT, FBGN and PLT) as well as CRP were unaffected by VGCF™-H exposure at any exposure concentration and no increases in CP were measured in the cardiac tissue. Therefore, the VGCF™-H induced pulmonary inflammatory response was not associated with adverse changes in systemic, cardiovascular endpoints in this study.

1814 INTERDISCIPLINARY IN VITRO APPROACH TO EVALUATE THE TOXIC POTENTIAL OF ENGINEERED NANOMATERIALS: GOLD NANOPARTICLES AND CARBON NANOTUBES AS CASE STUDIES.

Y. Zhang1, W. Salmi2, Q. Shi1, X. Yang1, Y. Jones1, A. Keasling1, A. Paredes1, S. F. Ali3, T. Chen4, S. Linder3, T. Mudalige5, Y. Xu6, A. S. Biros7 and P. Howard8, 1Office of Scientific Coordination, and Nanotechnology Core Facility, NCTRUS FDA, Jefferson, AR; 2Division of Systems Biology, NCTRUS FDA, Jefferson, AR; 3Division of Toxicology, NCTRUS FDA, Jefferson, AR; 4Division of Genetic and Molecular Toxicology, NCTRUS FDA, Jefferson, AR; 5Arkansas Regional Laboratory, US FDA, Jefferson, AR; 6and Nanotechnology Center, University of Arkansas at Little Rock, Little Rock, AR.

Gold nanoparticles (Au NPs) and single walled carbon nanotubes (SWCNTs) exhibit unique chemical and physical properties that are attractive for many biomedical applications. We examined their uptake and toxicity in vitro in primary rat hepatocytes, mouse leukemic macrophages (RAW 264.7), and rat adrenal pheochromocytoma cells (PC12). Cytotoxicity was assessed by examining cell membrane integrity and mitochondrial activity (LDH, XTT and MTT assay) and oxidative stress (DCF assay; reduced glutathione levels; PCR array for ROS responsive gene expression). Uptake and subcellular localization were monitored using SOT 2012 Annual Meeting 389
and M. L.}

1Department of Environmental and Occupational Health, Michigan State University, East Lansing, MI and 2Department of Integrative Toxicology, Michigan State University, East Lansing, MI.

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E. B. Evans1, R. Priston1, J. Willoughby2, H. N. Wagner2 and J. M. McKim

360 The effects of prochloraz, atrazine, ph-
330 -estradiol (~4-fold). rhCG and FSK also elicited temporal
330 treatment of BLTK1 cells with recombinant human chorionic gonadotropin
320 Cyp11a1, Cyp17a1, Hsd3b1, and Hsd17b3 required for steroidogenesis.
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300 promoter/simian virus 40 T-antigen fusion gene, express Star, Cypl1a1, Cypl7a1, Hsd3b1, and Hsd17b3 required for steroidogenesis. Treatment of BLTK1 cells with recombinant human chorionic gonadotropin (rhCG) and forskolin (FSK) induced cAMP (~100-fold), progesterone (~8-fold), T levels (~4-fold), and 17β-estradiol (~4-fold), rhCG and FSK also elicited temporal and dose-dependent steroidogenic gene expression, including induction of Star (rhCG ~10-fold, FSK ~8-fold and Hsd17b3 (rhCG ~10-fold, FSK ~20-fold), and down-regulation of Insl3 (~2-fold for rhCG), while there were no time or dose effects on Cypl1a1 and Hsd3b1 expression. The effects of prochloraz, atrazine, phthalates (di- and mono- esters), triclosan and glyphosate suggest that alterations to steroidogenesis can occur by different pathways. For example, prochloraz alone had no effects, but when co-treated with rhCG, T induction was inhibited by 50% compared to rhCG alone. Collectively, these data suggest that BLTK1 cells are not only an excellent model for evaluating reproductive and developmental toxicants but can also be used to elucidate adverse outcome pathways affecting Leydig cell steroidogenesis.

1815 BLTK1 MURINE LEYDIG CELLS AS A NOVEL MODEL FOR EVALUATING THE STEROIDIGENIC EFFECTS OF REPRODUCTIVE AND DEVELOPMENTAL TOXICANTS.

R. Jaremba1, A. L. Forgacs1, Q. Ding1, I. T. Huhtaniemi2, N. A. Rahman1 and T. R. Zacharewski1, 2Department of Biochemistry and Molecular Biology, Center for Integrative Toxicology, Michigan State University, East Lansing, MI and Department of Physiology, University of Turku, Turku, Finland.

Gonadal steroidogenesis, a target for endocrine disruption, is essential for proper reproductive and developmental development. As the primary site of testosterone (T) synthesis, Leydig cells are an important model to evaluate the effects of drugs, chemicals, natural products and contaminants as well as their metabolites and mixtures on steroido-

360 get LT-PCR and western analysis demonstrate BLTK1 cells, a Leydig cell line isolated from a testicular tumor that developed in a transgenic mouse expressing the mouse intron 5 promoter/simian virus 40 T-antigen fusion gene, express Star. Cyp11a1, Cyp17a1, Hsd3b1, and Hsd17b3 required for steroidogenesis.

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in both humans and wildlife. Regulatory mandates requiring endocrine disruption risk assessment for consumer products present major obstacles to current testing procedures that are expensive, time consuming, and require a large number of animals. The obstacles can be addressed by an automated procedure to assess a chemical’s endocrine activity in vitro as a pre-screen prior to investigating potential organ- ism and environmental impact. An automated in vitro assay that categorized and prioritized endocrine disruptors on their ability to alter endocrine receptor activity would also reduce animal testing. We thus developed cell-based assay workflows to efficiently test chemicals for their ability to activate GPR-tagged estrogen receptor alpha (ERα) or androgen receptor (AR). These receptors form nuclear foci in response to stimulation that can be easily quantified by automated fluorescence imaging. In addition to nuclear foci, other properties of individual cells are simultaneously measured resulting in direct assessment of compound toxicity, comparison to positive controls, and overall mechanism of action. Compared to currently validated in vitro endocrine disruption assays, this cell-based functional assay resulted in high specificity and sensitivity (>80%) on a panel of compounds that included pesticides, plasticizers, and pharmaceuticals. The ERα assay detected bisphenol A at ~66 ppb, which is more sensitive than the current allowable intake limits from several regulatory agencies. These assays were developed following EPA and ICCVAM guidelines for endocrine disruption assays and provide functional in vitro determination of receptor activity resulting in a more thorough assessment of the potential for in vitro endocrine disruption.

**1820 SCREENING FOR ENDOCRINE DISRUPTORS IN PRODUCT DEVELOPMENT: TIERED TESTING USING IN VITRO AND IN VIVO ASSAYS.**


The development and application of in vitro screening tools significantly enhanced our ability to detect indicators of relevant toxicological potential. The early detection of toxicity during substance development is a key element in the decision making process to promote or discontinue development of new chemicals/active ingredients and thereby optimizes the efficient use of resources and reduces animal testing. Here, we present our approach to reduce and refine animal testing to detect substances with an endocrine mode of action. Most endocrine disruptors interact with hormone receptors or steroid biosynthesis/metabolism, thereby modifying the physiological function of endogenous hormones. Therefore, in this study receptor mediated endocrine effects were assessed using the yeast based receptor mediated transcriptional activation YES/YAS assays and effects on steroid hormone biosynthesis were assessed using the human cell line H295R screening assay based on OPPTS 890.1550 and OECD TG 456. The effects observed in vitro were confirmed in an in vivo repeated dose study in which plasma samples were obtained and analyzed for their metabolome profile. This analysis allowed us to confirm or disconfirm amongst many other modes of action, the potential in vitro endocrine effect of a compound. Therefore, specific, but additional in vitro studies such as the Hershberger assay (OECD TG 441) or uterotrophic assay (OECD TG 440) are rarely needed. This approach combined with the use of modern toxicological techniques has significantly increased the efficient allocation of resources and reduced animal use and thereby presents an important contribution to animal welfare in product development. We present here data on 14 reference substances for which the in vitro YES/YAS and steroidogenesis assays and the in vivo metabolome analysis were performed to assess their putative endocrine mode of action.

**1821 COMPUTATIONAL MODELING OF HYPOTHALAMIC-PITUITARY-GONADAL AXIS TO PREDICT ADAPTIVE RESPONSES IN FEMALE FATHEAD MINNOWS EXPOSED TO AN AROMATASE INHIBITOR.**

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Exposure to endocrine disrupting chemicals can affect reproduction and development in both humans and wildlife. We are developing a mechanistic computational model of the hypothalamic-pituitary-gonadal (HPG) axis in female fathead minnows to predict dose-response and time-course (DRT) behaviors for endocrine effects of the aromatase inhibitor, fadrozole (FAD). The model includes two feedback regulatory loops within the HPG axis that mediate adaptive responses to endocrine stress. The regulatory loop controlling the secretion of luteiniz- ing hormone (LH) and follicle-stimulating hormone (FSH) from the brain, and the other regulates LH and FSH receptor recycling in the ovary. Data on plasma E2 and ovarian CYP19A mRNA from two experiments with a post-exposure recovery phase were used to develop and evaluate the model. In the experiments, fathead minnows were exposed to FAD at 0, 3, or 30 μg/L for 8 days followed by a 20-day recovery phase (experiment 1) or to FAD at 0, 0.5, or 30 μg/L for 8 days followed by a 20-day recovery phase (experiment 2). Adaptive changes in plasma E2 levels occurred during exposure and overshoot occurred post-exposure. Model parameters were estimated using E2 concentrations for 0, 0.5, and 3 μg/L FAD doses. The model predicted dynamic E2 concentrations for 0, 0.5, and 3 μg/L FAD close to time-course measurements. This abstract does not necessarily reflect US Environmental Protection Agency policy.

**1822 ICCVAM RECOMMENDATIONS AND LIMITATIONS OF THE BG1LUC ER TA TEST METHOD FOR IDENTIFYING ESTROGEN RECEPTOR AGONISTS AND ANTAGONISTS.**

D. Hattan1, K. Carlson1, A. Jacobs3, J. Bray3, W. Casey4 and W. Stokes4. 1CFSAN, US FDA, College Park, MD, 2CPSC, Bethesda, MD, 3CDER, US FDA, Silver Spring, MD and 4NTP/NICEATM, NIEHS, Research Triangle Park, NC.

ICCVAM recently completed an evaluation of the BG1Luc estrogen receptor (ER) transcriptional activation (TA) test method. An international interlaboratory validation study was conducted to determine the usefulness and limitations of the BG1 Luc ER TA test method as a screening tool to identify substances with in vitro ER agonist and antagonist activity. Three laboratories (one each from the US, Europe, and Japan) tested coded reference chemicals up to three times each. Results were similar across the three participating laboratories. For the agonist protocol, only one of the 35 reference substances that produced a definitive result was discordant (false negative) with existing reference data from other in vitro ER TA assays. For the antagonist protocol, all 25 reference substances that produced a definitive result were concordant with existing reference data from other in vitro ER TA assays. The BG1Luc ER TA test method results were also compared to results from the only in vitro ER TA test method currently included in national and international regulatory testing guidelines (i.e., US EPA OPPTS 890.1300/OECD Test Guideline [TG] 455), resulting in identical accuracy statistics when each method tested the same agonist reference chemicals. ICCVAM concluded that the accuracy of this assay is at least equivalent to US EPA OPPTS 890.1300/OECD Test Guideline 455 test methods. Thus, the BG1Luc ER TA method may be applicable to the US EPA Endocrine Disruptor Screening Program. ICCVAM considered the peer review panel report, public comments, and the comments of SACATM in preparing the ICCVAM final test method recommendations. ICCVAM recommends that the BG1Luc Estrogen Receptor ER TA test method be used as a screening assay to identify substances with in vitro agonist and antagonist activity.

**1823 ICCVAM PERFORMANCE STANDARDS FOR THE BG1LUC ER TA TEST METHOD.**

W. Casey1, D. Hattan1, K. Carlson1, A. Jacobs3, J. Bray3, J. Hamm3, P. Ceger3, D. Allen4 and W. Stokes4. 1CFSAN, US FDA, College Park, MD, 2CPSC, Bethesda, MD, 3CDER, US FDA, Silver Spring, MD and 4NTP/NICEATM, NIEHS, Research Triangle Park, NC.

Performance standards can be used to evaluate the accuracy and reliability of proposed test methods that are functionally and mechanistically similar to an accepted test method. ICCVAM recently recommended performance standards for the BG1Luc estrogen receptor (ER) transcriptional activation (TA) test method. The performance standards were based on results from an international interlaboratory validation study, and include essential test method components, reference substances, and standards for accuracy and reliability. Essential components include: a cell line that endogenously expresses human ERs and is stably transfected with a reporter gene, use of a soluble mimic with cell culture media, a defined concentration limit for agonist (1 mM) or antagonist (10 μM) testing, evaluation of cytotoxicity, a reference estrogen, anti-estrogen, and positive and solvent controls. The reference substances should be accurately quantitated, both positive and negative. When the reference substances are evaluated in a newly proposed method, the accuracy and reliability should approximate those of the validated ER TA method: accuracy of 100% (34/34), sensitivity of 100% (27/27), specificity of 100% (77), false positive rate of 0% (0/77), and false negative rate of 0% (0/77) for agonists; and accuracy of 100% (10/10), sensitivity of 100% (3/3), specificity of 100% (7/7), false positive rate of 0% (0/7), and false negative rate of 0% (0/3) for antagonists. Although it is not realistic to expect test methods to perform identically, the basis for any discordant results should be discussed along with the impact.
on the proposed use. These ICCVAM performance standards are expected to facilitate the efficient evaluation of new test methods proposed for evaluation of ER agonist and/or antagonist activity. ILS staff supported by NIEHS Contract N01-ES-35504.

1824 BIOENERGETICS CHARACTERIZATION OF HUMAN-INDUCED PLURIPOTENT STEM (IPS) CELL-DERIVED CARDIOMYOCYTES GROWN IN DIFFERENT CARBON SOURCES.

P. Rana, S. Engle, B. Anson and Y. Will. Compound Safety Prediction, Pfizer Global Research & Development, Groton, CT and Cellular Dynamics International, Madison, WI.

iCells™ (Cellular Dynamics International, Madison, WI) are highly purified human cardiomyocytes derived from induced pluripotent stem (iPS) cells through differentiation and purification protocols. Adult heart prefers fatty acids for energy utilization, however very little is known about the bioenergetics of iCells and their preferred substrate for energy utilization. Cells grown in high glucose are mostly glycolytic because they tend to use glycolysis for energy production despite of having fully functioning mitochondria. Here, we investigated the bioenergetics of iCells grown in different substrate media (high-glucose, low-glucose, galactose, and fatty acids (oleic acid and palmitate)) and their preferred substrate for energy utilization using the XF96 flux analyzer. We investigated effect of mitochondrial modulators (Rotenone, Antimycin, and Oligomycin) on iCells growing in the different substrate media. We observed that iCells grown in galactose and fatty acids media were more sensitive to mitochondrial modulators compared to cells grown in high/glucose media. iCells grown in galactose and fatty acids media displayed higher mitochondrial reserve capacity and maximum respiratory capacity compared to cells cultured in high/glucose media. Furthermore, complete inhibition of oxidative phosphorylation (OXPHOS) reduced ATP levels in cells growing in galactose and fatty acids suggesting that these cells have limited ability to increase glycolysis to preserve energy levels under these conditions. In contrast, inhibition of OXPHOS did not reduce ATP levels in cells grown in high/glucose suggesting an increase in glycolytic activity. In summary, cultured iCell cardiomyocytes can use a diverse set of substrates, including high and low-glucose, galactose and fatty acids. However, only iCells grown in galactose and fatty acids display an aerobic (heart-like) phenotype.

1825 THE ROLE OF METABOLISM IN DICLOFENAC-INDUCED INTESTINAL TOXICITY IN RAT AND HUMAN IN VITRO.


Sponsor: A. Vickers

The use of Diclofenac (DCF), a non-steroidal anti-inflammatory drug is associated with severe gastrointestinal side-effects. The mechanisms of drug-induced intestinal toxicity are largely unknown due to the lack of in vitro models. In vivo rat studies suggested that reactive metabolites of DCF especially diclofenac acyl-glucuronide (DAE) produced by liver played an important role in intestinal toxicity. Whether DCF is directly toxic to the intestine is not known. In this study, human as well as rat precision-cut intestinal slices (PCIS) are used as an in vitro model to investigate the mechanism of DCF-induced intestinal toxicity. PCIS from rats and 4 human individuals were incubated with different concentrations of DCF, metabolite formation as well as toxicity were tested. ATP content and morphology were used as markers for toxicity. Dose-dependent toxicity of DCF was shown for both species: 200 µM DCF caused a significant decrease in ATP and morphological damage in rats. Human intestinal slices were more resistant to DCF and significant damage was induced at concentrations ≥400 µM. Minor amounts of hydroxylated DCF as well as DAG were detected in rat and human PCIS; but with a large variation among human individuals. Drug-protein adducts were detected by immunohistochemistry staining using anti-DCF antibody. To investigate the role of DAE in the mechanism of toxicity, the PCIS were incubated with DCF in the presence of a non-toxic concentration of the glucuronidation inhibitor boron
eol (0.5 mM). Borneol effectively decreased the formation of DAG but did not further reduce the toxicity compared with DCF alone.

In conclusion, using PCIS as an in vitro model we show that DCF is directly toxic to the human and rat intestine. Human intestine appears less sensitive to DCF-induced toxicity. Similar metabolite profiles are found in rat and human intestinal slices, meanwhile drug-protein adducts are detected. However the role of the reactive metabolite DAG in DCF-induced intestinal toxicity could not be confirmed.

1826 ASSESSMENT OF AN IN VITRO HUMAN INTESTINAL EPITHELIAL CELL MODEL FOR EVALUATION OF PROTEIN CYTOTOXICITY.

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Historically, the toxicity of individual proteins has been evaluated in mice or other laboratory animals by oral exposure and requires multiple grams of protein. However, it is generally appreciated that proteins that are toxic to humans and other animals following oral exposure exhibit toxicity toward intestinal cells or systemic toxicity following absorption from the gastrointestinal (GI) system. In this study, we evaluated in vitro indicators of cytotoxicity and cellular health using the human intestinal epithelial cell line T84 grown on permeable Transwell®TM filters. This well characterized in vitro culturing method results in the formation of a polarized intestinal epithelial monolayer that exhibits a functional barrier and serves as a model of the human GI system. Intestinal epithelial monolayers were exposed on the apical surface to a diversity of well-known innocuous proteins and multiple toxic proteins produced by bacterial pathogens, allergens from milk and peanuts, and toxic plant lectins. Our objective was to determine whether this in vitro method has the potential to complement or substitute for acute oral toxicity studies in animals. This study describes the physiological impact of proteins on cell function by monitoring cytotoxicity indicators (LDH release and MTT reduction), integrity/permeability changes (3H-inulin flux, HRP flux and trans-epithelial electrical resistance) as well as activation of inflammatory readouts (IL-8, IL-6). Our results demonstrate that multiple toxic proteins exert reproducible effects on various physiological metrics of the intestinal in vitro system. We conclude that further evaluation of the intestinal barrier in vitro system is warranted as it may prove useful as an additional tool to analyze proteins with unknown toxicity profiles, particularly in the evaluation of low abundance target proteins.

1827 DNA ADDUCTS FORMED IN FETAL TURKEY LIVER BY AROMATIC AMINE-CONTAINING LOCAL ANESTHETICS.

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Lidocaine (lido), Prilocaine (prilo) and Novocaine (novo) are widely used as local anesthetics either individually or in combination. Metabolically, they are cleaved to release single ring aromatic amines, 2,6-xylidine, p-toluidine and aniline, respectively. We have previously shown that lido > prilo form DNA adducts in the nasal mucosa and liver of treated F344 rats via their aromatic amine metabolites, although at lower levels than equal molar quantities of their respective amines (Duan, J.-D., et al. 2008) Drug Metab. Dis. 36, 1470-1475). The aim of our study was to assess the ability of these compounds to form DNA adducts in fetal turkey livers as an alternative genotoxicity testing assay for these and other similar compounds. Fertilized turkey eggs (average weight 85 g) were incubated at 37°C and 60% humidity. The development of the eggs was monitored with a strong flashlight and/or a digital egg monitor (Avitronics, England) and those which did not develop were removed. A single injection of doses ranging from 200-2000 µmoles per egg of lido, prilo and 50-400 µmoles per egg of novo into the air sac of the turkey eggs in 200 µl water was made at day 23 of development (5 days before hatching). Water served as the vehicle control. After 24 hours, the eggs were opened and the livers were removed. Two repeat injections of lido and prilo were made on day 22 and 23, and three repeat injections were done on days 21, 22 and 23. Survival was proportional to dose with toxicity being lido > prilo > novo. DNA adducts were detected in livers of treated eggs: prilo > lido > novo or their corresponding aromatic amines. These results are similar to those found in rats for lido and prilo except that the levels of lido DNA adducts were greater than those from prilo.

1828 DETERMINATION OF SKIN AND AIRWAY GENOTOXICITY POTENTIAL USING THE NORMAL HUMAN 3-DIMENSIONAL (NUH-3D) EPIAIRWAY, EPIODERM, AND EPIFTM IN VITRO HUMAN MODELS AND THE COMET ASSAY.


Determination of genotoxicity potential is an important factor for safety assessment of chemicals that may be present in drugs, consumer products, occupational chemicals or environmental pollutants. Due to recently enacted legislation including
REACH and a ban on animal testing of cosmetics by the 7th Amendment to the Cosmetics Directive, which results in higher animal testing costs and increases the need for predictive non-animal tests. For the assessment of genotoxicity, currently, in vitro methods are urgently needed. Commonly used in vitro genotoxicity assays produce a high false positive rate, limiting their utility for predicting human genotoxicity. In addition, genotoxic assays based on immortalized cell lines typically suffer from significant drawbacks including deficient function of p53, NFκB and other important genotoxicity related cellular pathways. Furthermore, for assessment of organ-specific genotoxicity, NHu-3D organotypic in vitro human tissue models with in vivo-like barrier function and metabolic capability will have improved biological relevance and predictive ability. The current poster describes application of the comet assay for genotoxicity screening with NHu-3D airway (EpiAirway) and skin (EpiDerm-EFT) tissues. These organotypic models reproduce the 3D structure, barrier function and xenobiotic metabolizing capabilities of in vivo epithelial tissues. In vitro tissues were digested with trypsin to produce cell suspensions for Comet Assay experiments. Comets were visually scored in duplicate tissues and % tail DNA was determined. Untreated control samples produced low background comet scores that were independent of the donor cells. Treatment of cells with direct genotoxins such as methyl methane sulfonate and 4-nitroquinoline, or indirect genotoxins that require metabolism such as benzo(a)pyrene or cyclophosphamide, produced statistically significant, dose-dependent increases in % tail DNA. Thus, the Comet Assay appears to be a promising approach to in vitro genotoxicity testing in airway and dermal tissues.

**1829 IN SILICO METHODS OF GENOTOXICITY PREDICTION: CAN IT BE USED RELIABLY FOR PREDICTION OF IN VITRO/IN VIVO GENOTOXICITY?**

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The main purpose of carrying out this study was to test the reliability of already available models for prediction of genotoxicity and determining if the genotoxicity in animal models can be reliably predicted. In this study, we first used a pharmaceutical and nonpharmaceutical dataset to test the sensitivity, specificity and concordance of available models in TOPKAT, DEREK, Lazar, and various QSAR models such as Binary QSAR model, Bayesian Model, k-NN (k-nearest neighbor algorithm) method, which are developed using MOE, Discovery Studio 2.5.0, and ToxMatch v1.06, respectively. As most of the available models do not predict the genotoxicity of compounds in animal models, and after checking concordance of these models for Ames mutagenicity, we developed a QSAR for prediction of genotoxicity in an animal model. After carrying out internal validation by (leave-one-out method) and external validation (using training-set and test-set) of these models, we screened a set of 14 new chemical entities. We also screened their metabolites for genotoxicity and finally selected 4 compounds based on the predictions, for further testing by Micro-nucleus and Comet Assay in mice. Results demonstrated that these models can be successfully used for screening of in vivo genotoxicity, provided that most of the factors (e.g., metabolism, mechanism of toxicity, etc.) are taken into consideration. Highest accuracy of prediction shown by any QSAR model in this study was 75.32%.

**1830 USE OF NORMAL HUMAN 3-DIMENSIONAL (NHU-3D) TISSUE MODELS (EPIDERM, EPIAIRWAY) FOR NANOTOXICOLOGY APPLICATIONS**

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Nanomaterials are increasingly utilized in numerous commercial applications where dermal contact, inhalation, or oral ingestion is likely. However, their toxicological properties are largely unknown. Potential adverse effects of nanoparticle exposure include alterations in gene expression, cytotoxicity, and genotoxicity. Nanomaterials can enter the body by interacting with, and eventually crossing, epithelial barriers including the skin, airway, and intestinal epithelium. Once inside the body, additional interactions with internal organs such as the heart, liver, brain, kidney, and others, are possible. Therefore, there is an urgent need for animal alternative tissue models that can be utilized for toxicological evaluation of nanoparticle materials. This poster summarizes results from experiments using in vitro NHu-3D skin (EpiDerm, EpiDerm-EFT) and airway (EpiAirway) models and well characterized nanomaterials. Using confocal microscopy, we observed penetration of fluorescently-labeled polystyrene nano-beads into NHu-3D tissue models, EpiAirway and EpiDerm tissues. In addition, comet assay genotoxicity experiments showed dose-dependent increases in %Tail DNA after treatment of EpiDerm tissues with: a) single wall carbon nanotubes (1-4 nm), Fullerenes C60 (avg particle size = 120 nm), ultra fine titanium oxide (5-10 nm), and crystalline silica (avg particle size = 450 nm). These studies and others already in the literature demonstrate that in vitro NHu-3D models are useful tools for the study of nanoparticle interactions and potential toxicologic effects on epithelial tissues.

**1831 THE USE OF DERMAL AND LUNG IN VITRO MODELS TO EVALUATE POTENTIAL OCCUPATIONAL EFFEC TSTHS OF MILITARY FUELS.**

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Given the extensive use of fuels within the U.S. Armed Forces, fuel exposure remains an area of concern with regard to the occupational health risks to military personnel. The potential health risks, coupled with many new fuels in the form of previously untested alternative and bio-based fuels, increases the need for reliable methods to systematically assess potential risk among fuels. While animal models are well validated and historically used to evaluate the effects of fuel exposure, there is a demand for in vitro methods capable of rapidly and economically screening these effects in order to accommodate the number of fuels. Toward this goal, two dermal and one lung in vitro models were used to evaluate a series of military alternative and bio-based jet fuels in comparison to the conventional fuel, Jet Propulsion 8 (JP-8). As a means to assess dermal effects, a 3-D human skin model was used and found to be resilient to the fuels tested when exposed to 50 μl/9 mm tissue for up to 40 h. Alternatively, a human epidermal keratinocyte cell line exposed for only 10 min resulted in an immediate loss (30%) in viability following JP-8 exposure, with similar losses for other fuels. Viability continued to decrease over the next 24 h to 50% for JP-8 exposed cells, while control cells maintained similar viability levels of viability allowing comparisons between the fuels. As a separate screen designed to assess the effect of fuel exposure on the lung, human lung epithelial cells were exposed to fuels, directly or as vapor, in the presence of macrophages. Vapor exposure resulted in changes in viability that could be differentiated between fuels, while direct exposure did not. Numerous cytokines were also evaluated with only minor differences observed. Taken together, these in vitro models show the potential to provide a rapid and cost-effective means of screening the toxicological effects of alternative fuels in comparison to conventional fuels (sponsored by Air Force Surgeon General).

**1832 PREDICTING PHOTOSENSITIZATION USING AN IN VITRO METHOD.**

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The safety of OTC drugs, cosmetics and personal care products is an important part of product development. Standard tests for chemical sensitization have depended on animals. Amendment 7 of the European Cosmetics Directive requires the development of in vitro methods that replace animal usage. Although some in vitro approaches are under evaluation, SenCeeTox® is the only method designed to identify sensitization potential and provide a potency category. This assay relies on a concentration response of genes controlled by the antioxidant response element (ARE), cytotoxicity, direct reactivity, solubility, and dermal permeability to predict the sensitization potential. The aim of this study was to determine if an in vitro alternative method (SenCeeTox®) could be used to identify photoallergens. Four known photo activated chemicals (oxybenzone, avobenzene, octisalate, and padimate-O) relevant to the OTC drug/cosmetic industry were selected. Glycerol, p-benzoquinone, and naproxen were used as negative controls, while ciprofloxacin and TSA were used as positive controls for photoactivation. Test compounds were prepared in DMSO and then diluted into PBS. The samples were divided into two groups, one exposed to 6J/cm2 UVA light, and one that remained in the dark. Following the light exposure, an aliquot was removed and evaluated for direct reactivity using glutathione (GSH) depletion. A second aliquot was mixed with culture medium and applied to a human keratinocyte (HaCaT) cell in 96-well plates. Following a 24 h exposure, cells were assessed for cytotoxicity (MTT) and ARE controlled gene expression using RT-PCR. Analysis of cell viability, gene expression, and reactivity data indicate that SenCeeTox® may be useful for identifying chemicals that are photoallergens.
1833 ADOPTING TESTING CONDITIONS OF THE VALIDATED CFU-GM ASSAY FOR HEMATOPOIETIC STEM CELLS IN A MICROWELL FORMAT.

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The validated clonogenic assay for the colony forming unit-granulocyte/macrophage (CFU-GM) progenitor can be used to determine exposure associated with severe neutropenia and to quantify differences in susceptibility of different species to bone marrow toxicants. Despite its acceptance by ECVAM as an alternative to animal testing, the CFU-GM assay is unsuitable for use in drug discovery or screening toxicology settings due to low throughput and specialized techniques. In culture conditions adopted from the CFU-GM assay, CD34+ stem cells showed proliferation and differentiation into CD11b+, CD13+, CD117+ and myeloperoxidase+ daughter cells over 7-14 days of liquid culture. By day 14, cell numbers were sufficiently high to quantify IC90 values for myelosuppressive chemotherapeutic agents (vinca alkaloids, taxanes and a topoisomerase I inhibitor), which were indistinguishable from IC90 values derived from CFU-GM assays. Secreted IL6 and IL8 increased by ~60- and ~3000-fold, respectively, over the 14-day period, consistent with myeloid differentiation in response to rGM-CSF.

Accelerated CD34+ cell proliferation and differentiation from additional cytokines decreased the accuracy of the IC90 values, similar to previous results from adding day period, consistent with myeloid differentiation in response to rGM-CSF.

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1834 EVALUATING IN VITRO MODELS FOR THE PREDICTION OF CNS EXPOSURE TO CHEMICAL ENTITIES.

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In chemical discovery, an important early phase is the investigation of central nervous system (CNS) exposure and hence potential neurotoxicity of new chemical entities. In vitro models can be used to predict the exposure of compounds in the CNS. The characteristics of the model will influence the degree of predictivity. An optimum model should be representative of the in vivo blood brain barrier (BBB) and possess key physiological characteristics such as tight junction formation and functional activity of key transporter proteins such as P-glycoprotein (P-gp). Culturing brain endothelial cells has proved challenging and as a consequent often surrogate models such as Madin Darby canine kidney (MDCK) cells transfected with human P-gp are utilized as they offer a high throughput solution. The limitations of the MDCK model include the specificity of the overexpressed protein to measure the interaction of chemical entities with human P-gp. This study explores the utility of MDCK cells with an advanced primary porcine in vitro BBB model to aid the prediction of CNS exposure. The distribution of compounds into the CNS is influenced by plasma and tissue protein binding as well as passive and active transport processes across the BBB. An integrated approach involving the measure of free plasma and tissue concentration by equilibrium dialysis and permeability data generated from the in vitro BBB model was utilised to identify improved in vivo predictions. In vitro Kp (Kp, pred) was determined for a set of eleven test compounds with a range of physicochemical properties. Kp, pred obtained from the porcine BBB model showed 55% of compounds within 3-fold of observed in vivo data compared to 25% generated from MDCK cells. In particular, MDCK cells underpredicted the distribution of P-gp substrates, namely Amupreravir, Risperidone and Saquinavir whereas the porcine BBB model showed improved predictions of observed Kp for these compounds indicating that this model may provide a better prediction of CNS exposure possibly owing to the more relevant physiologically of the system.

1835 PARACRINE SIGNALING IN A NOVEL MODEL FOR HEPATOPOCYTE-PREADIPOCYTE COCULTURES.

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Obesity is a serious public health problem and a major risk factor for cardiovascular disease, certain types of cancer, and type 2 diabetes. Hence there is a need for physiologically relevant cellular models that can mimic adipogenesis. Currently, most cellular models utilize single cell type cultures of 3T3-L1 preadipocytes that are induced to undergo adipogenesis. However the effect of other cell types on preadipocyte growth and differentiation has not yet been elucidated. Since secreted factors from the liver are distributed throughout the body, as are products of drug metabolism, the effects of such paracrine signals and/or drug metabolites on adipocyte function is relevant. In this study we have used a proprietary technology developed in our laboratory, termed Discrète Multi Organe Co-culture (IdMOCTM) for the co-culture of 3T3-L1 preadipocytes and hepatocytes. Using Rosiglitazone to induce adipogenesis along with quantitative PCR for the amplification of AP2, an early marker for adipogenic differentiation, we show that adipogenesis can be induced reproducibly within 48 hours. We have optimized this assay using various concentrations of MatrigelTM for cell adhesion and media for concomitant culture of the two cell types. In this co-culture mode, we demonstrate the presence of hepatic factors that positively influence preadipocyte proliferation and adipogenic differentiation. To determine the specificity of Rosiglitazone action, which occurs presumably via its metabolites, N-desmethyl rosiglitazone and p-hydroxy rosiglitazone, we investigated the effect of the major metabolite entero-GYPC28, namely, theophylline was used. A decrease in adipogenesis in the presence of trimethoprim indicates the role of metabolism in driving drug-induced differentiation. Such a model is physiologically relevant in testing drugs that affect adipogenesis as it provides the paracrine signals and xenobiotic metabolizing capacity of hepatocytes in simultaneous culture with preadipocytes.

1836 DEVELOPMENT OF AN IN VITRO ACUTE TOXICITY PANEL TO ASSESS THE TOXICITY OF EXTRACTS FROM SINGLE-USE BIOPHARMACEUTICAL PROCESSING PRODUCTS.

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Assessing the toxicity of extractables from biopharmaceutical processing products is required for the safety assessment of these products. A standard protocol for this type of evaluation does not currently exist. As a result, an in vivo method described in the USP <88> guidelines for Biological Reactivity Tests is often used as a default for evaluating potential toxicity. This protocol requires the intravenous administration of large volumes (50 ml/kg) of extracts to mice. The resulting effects on hemo-dynamics are a common cause of failure for materials undergoing USP testing. In addition, the current test requires significant animal use for the production of the material and new product screening. In order to improve testing speed and reliability and reduce requirements for animal testing, this study presents and evaluates an in vitro screening method that could more accurately identify a risk for in vivo toxicity. Rat (H4IE) hepatoma cells were used to assess the toxicity of various dilutions of extracts generated per USP criteria (0.9% saline, 1 hour at 121 degrees C) from different biopharmaceutical purification product chemistries and raw material solutions. Cytotoxicity was determined by measuring several critical markers of cell health, which included membrane integrity (alpha-GST) and cell mass (propidium iodide) following 24 hour exposures. The in vitro TC50 values were derived and compared to results from in vivo exposures of similar dilutions. In all cases, the TC50 of the extracts predicted the dilution needed to successfully pass the in vivo acute toxicity test, displaying excellent accuracy and sensitivity. It is concluded that the in vitro screening of extracts and raw material solutions is a useful tool to assess potential toxicity issues associated with biopharmaceutical processing products, and the inclusion of these assays can provide important toxicity information, while minimizing animal testing, particularly during the product development phase.
access bioinformatics tools. The slices viability was significantly improved at 60 and 80% of oxygen. However, these parameters were significantly lower compared to the uncultured PCLS. Testosterone metabolism was comparable between the tested conditions. The transcriptome analysis revealed that oxygen did not affect gene expression, but the in vivo/ex vivo comparison identified 2524 differentially expressed genes. Within the altered gene functional clusters we found genes related to phase I and II drug metabolism, inflammation, peroxisomal and mitochondrial activity, and bile acid metabolism. Based on the biochemical and morphological characteristics PCLS cultured at higher oxygen concentrations had improved quality. Surprisingly, oxygen did not affect gene expression suggesting that neither low (20%) nor high (80%) oxygen levels cause hypoxia or hyperoxia, respectively. The alternations in gene expression in PCLS compared to the in vivo situation indicate that several processes are altered however, active testosterone metabolism, indicates sufficient preservation of liver functions.

1838 HUMAN LIVER MICROTISSUES AS A NOVEL SYSTEM TO PREDICT INFLAMMATION-MEDIATED TOXICITY.

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Adverse idiosyncratic drug reactions are a prime reason for drug attrition. Although these reactions occur very rarely, they represent a major risk for pharmaceutical companies. The hepatotoxic potential of the withdrawn drug Trovafloxacin, for example, was not recognized in preclinical studies. Only recently, animal studies showed that inflammation is the main trigger for Trovafloxacin-induced idiosyncratic toxicity. However, conventional in vitro methods were not able to mimic this toxicity. In order to establish a system capable of assessing such complex hepatotoxicity, we generated human liver microtissues. The liver microtissues are composed of primary human hepatocytes and non-parenchymal cells. They are generated by gravity enforced self-assembly in hanging drops. These scaffold-free liver microtissues are produced in a standard 96-well format, which is automation compatible. In contrast to standard 2D-sandwich culture, liver microtissues were stable and viable over several weeks in culture. The secretion of albumin and urea was consistently higher than in 2D-culture. In addition, functionality of the major cytochromes was maintained over the culture time. Morphological characterization revealed incorporation of Kupffer macrophages in the liver microtissues. Stimulation of macrophages with lipopolysaccharide resulted in secretion of pro-inflammatory cytokines, such as interleukin-6. Co-treatment of liver microtissues with LPS and the known hepatotoxic drug Trovafloxacin resulted in LPS-dependent hepatotoxicity. However, the safe drug Levofloxacin did not induce toxicity. Our newly described model system is therefore capable of predicting rare idiosyncratic adverse drug reactions.

1839 HUMAN AND MOUSE PRECISION-CUT LIVER SLICES AS TRANSLATIONAL MODEL FOR IDIOSYNCRATIC HEPATOTOXICITY OF CLOZAPINE.

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Idiosyncratic drug reactions (IDRs) are adverse drug reactions that are rare, sporadic, unpredicted by clinical trials, unrelated to drugs pharmacology and occur without relation to time or dose. IDRs may arise from drug interaction with inflammation that renders the liver more sensitive to injury resulting in increased toxicity. With the aim to develop a translational model to unravel the mechanism behind IDRs and to find biomarkers that can detect them, we used mouse and human precision-cut liver slices (PCLS) to study the influence of inflammatory reactions on the toxicity of drugs. PCLS technology is receiving increased attention as a potential ex vivo toxicological model because PCLS retain the normal tissue architecture of an intact liver with all its cell types in their natural environment. PCLS from mouse and human were incubated with clozapine (CZ) or its toxic analog olanzapine (OZ), in the presence or absence of lipopolysaccharide (LPS), an inflammation inducer. Toxicity (ATP), cytokine production (CBA), transcriptomics and kinomics [microarrays] data were assessed. CZ was more toxic in mouse than human and OZ was not toxic in both. LPS aggravated the toxicity of CZ and had no influence on OZ toxicity in both mouse and human PCLS. Moreover, LPS induced an inflammatory response and increased among others TNFα, IFN-γ. IL-6 and IL-1β and their production was altered by CZ and OZ in PCLS. Both CZ and OZ decreased LPS-induced IFN-γ production but only CZ increased LPS-induced IL-1β production in both mouse and human PCLS. CZ upregulated many more genes and activated many more pathways than OZ in the presence of LPS in human PCLS. Transcriptomics data showed that complement pathways appeared to be implicated exclusively in the CZ-LPS group and not in other treatment groups in human PCLS. Based on these results, PCLS appear to be a promising ex vivo model to study mechanisms behind IDRs.

1840 MICROARRAY ANALYSIS OF GENE EXPRESSION CHANGES DUE TO CHILLING INJURY IN PRECISION-CUT LIVER SLICES (PCLS).

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Successful cryopreservation of PCLS would allow the building of a tissue bank and reduce the use of laboratory animals. During vitrification, tissue may be damaged by toxicity of the cryoprotective agent (CPA), chilling injury (injury due to temperature reduction per se) and injury from ice crystal formation. The mechanism of chilling injury is unknown. We aim to identify critical genes and signaling pathways responding to chilling injury by microarray analysis to enhance insight into its mechanism and to facilitate development of vitrification methods for PCLS. Rat PCLS impregnated with CPA were cooled to -15 °C and held for 10 min to induce chilling injury. Changes in mRNA expression profile in the slices after cooling were compared to those exposed to the CPAs only using Affymetrix arrays. Differential Scanning Calorimetry (DSC) indicated that ice crystal formation was prevented in slices loaded with CPA during cooling. CPA toxicity appeared negligible (ATP content >95% of control slices). By cooling at -15°C, the ATP content was decreased by 20-30% compared to that of slices exposed to CPA, indicating moderate chilling injury. Microarray analysis showed that chilling-induced gene expression changes were clearly distinguishable from those induced by the CPAs. 1800 genes were changed (FDR<0.05) with 30 genes up- and 6 genes down-regulated 1.5 fold. IL-1 and MAPK pathways were among the most interesting regulated pathways.

In conclusion: we were able to separate chilling injury from other damaging events that occur during PCLS vitrification, and identified changes in gene expression and signaling pathways due to chilling injury by microarray analysis. This is the first effort to investigate the mechanism of chilling injury in integrated tissue by microarray analysis under conditions in which other sources of injury are absent.

1841 DOSIMETRY METHODS FOR TOBACCO SMOKE AEROSOL IN VITRO EXPOSURES.

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Recent technological developments in the testing of tobacco products have seen the widespread introduction of equipment that is capable of exposing cultured cells or media to the whole smoke aerosol, adding an additional capability to the well-established regimes for testing tobacco smoke total particulate matter in vitro for genotoxicity and cytotoxicity. Two dosimetry methods have been developed, for the gas and particulate phases of smoke aerosol respectively. The methods measure atmospheric carbon monoxide and smoke particulate concentrations, within diluted smoke used to expose air-liquid-interface cultures. Carbon monoxide was measured spectrophotometrically. Particulate concentration was measured by light scattering. Whole smoke aerosol was mutagenic in an Ames test, inducing 3 and 5-fold increases in revertants, with strains TA100 and YG1042, respectively. Cells on an agar surface, in the presence of S-9, were exposed to several dilutions of smoke aerosol, from 3 to 4 μg/mL references cigarettes smoked under ISO conditions. Averaged over the exposure period 24 hours) the carbon monoxide dose range was 0.5-4 μg/mL air. The particulate dose range was also 0.5-4 μg/mL air. The carbon dioxide and particulate dose ranges were similar, because their smoke yields are similar. The results showed that the cells were exposed to the particulate and gas phases of tobacco smoke, and that the exposures can be controlled incrementally to generate dose responses in an Ames test. The relative response of TA100 and YG1042 is consistent with YG1042’s increased sensitivity to aromatic amines and nitroarenes. These dosimetry methods can be applied to most current tobacco smoke exposure techniques. They are also used by other sectors, contributing to common dosimetry methods. Further work will measure the resolving power of in vitro smoke aerosol dose responses, compared to that of conventional total particulate matter.
1842 CYTOTOXIC AND INFLAMMATORY EFFECTS OF TOBACCO-FATTY ACIDS: COMPARATIVE RESPONSE FROM MONO VERSUS COCULTURED HUMAN LUNG CELLS IN VITRO.

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The presence of macrophage mediated inflammation in the lung is considered to be a hallmark for smoking conditions in smokers. Therefore, coculturing the lung epithelial cells with macrophage cells will model more closely the steady state in vivo situations in animals and humans and will provide a better in vitro model to investigate the smoke mediated effects. We compared the cytotoxic and inflammatory responses of several free fatty acids (FFAs) in human lung epithelial cells, BEAS-2B in the absence or presence of human macrophage-like cells. U-937 in order to determine the role of macrophage cells. FFAs are present at about 1% concentration in a standard tobacco blend with a transfer rate of up to 30% in smoke. The dose-dependent (2.5–20 μM) and time-dependent (1, 2, 3, 4, 7 day) cytotoxicity and inflammatory potentials were determined by the neutral red dye uptake in cells and interleukin-8 (IL-8) measurement in cell supernatant, respectively. The cytotoxicity was mainly observed at high doses, 10 and 20 μM for the majority of FFAs used. Overall, higher cytotoxicity was observed when BEAS-2B cells were cocultured with U937 as compared to BEAS-2B alone. The cytotoxicity rankings of both mono- and cocultures were same and as follows; Linoleic acid > Oleic acid > Palmitic acid > Stearic acid. A dose- and time-dependent IL-8 release was observed for all FFAs in the absence or presence of U937 cocultured with BEAS-2B cells. A higher inflammatory response from cocultured cells was observed relative to BEAS-2B cells alone. The inflammatory rankings of both mono- and cocultures were same and as follows; Stearic acid > Linoleic acid > Oleic acid = Palmitic acid. Overall, this time-dependent study demonstrated that the coculturing the inflammatory cells with lung epithelial cells is relevant model for acute and sub-chronic smoke exposure and also important since it demonstrated not only enhanced cytotoxicity but also played an important role in higher inflammatory response.

1843 IMPACT OF TEST CONDITIONS ON IN VITRO CYTOTOXICITY MEASUREMENTS OF MAINSTREAM CIGARETTE SMOKE USING A WHOLE SMOKE EXPOSURE SYSTEM.

X. Li, P. Shang, C. Nie, F. Xie, H. Liu and J. Xie, Key Laboratory of Tobacco Chemistry, Zhengzhou Tobacco Research Institute of CNCTC, Zhengzhou, China. Sponsor: R. Meng.

The purpose of this study was to evaluate the impact of several factors on smoke cytotoxicity measurements in an in vitro whole smoke exposure system. Effects of incubation time after smoke exposure, cell types used, and smoke regimens on the cytotoxicity of cigarette smoke were investigated. Mainstream cigarette smoke was generated from the 3RAF reference cigarettes using a VC10 smoking robot under the ISO regimen (35/60/2 without blocking of filter ventilation) or the HCl regimen (55/30/2 with complete blocking of filter ventilation). Cells were exposed to fresh whole smoke (WS) in the VITROCELL® system, and cytotoxicity was evaluated using the neutral red uptake (NRU) assay. Results showed 24 hrs after smoke exposure appeared to be an optimal time-point to assess smoke cytotoxicity. CHO cells were more sensitive to smoke-induced cytotoxic effects than A549 cells. Smoke regimen evaluation was conducted in both cigarette smoke condensate (CSC) and WS. For CSC testing, the cytotoxicity decreased going from the ISO regimen to HCl regimen on a per unit of total particulate matter (TPM) basis. For WS exposure testing, cytotoxicity under the ISO regimen was less than that under the HCl regimen when smoke doses were expressed as % of cigarette smoke; notably, when smoke doses were converted to TPM (μg), cytotoxicity under the HCl regimen was less than that under the ISO regimen. A clear dose-response relationship between cell viability and smoke doses was observed under all test conditions. These data indicated that this in vitro smoke exposure system can be a useful tool to study the toxicological effects of WS, and the test conditions can have an important impact on the results of cytotoxicity evaluation of cigarette smoke.

1844 HUMAN 3D AIRWAY MODELS TO PREDICT IN VIVO AVAILABILITY UPON INHALATION.

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Human 3D airway models are promising models for safety and efficacy evaluation of compounds targeting the airways. The two most important reasons are (1) the models are fully differentiated and functional (incl. metabolic activity, mucus production and cilia beating) and (2) they are cultured in an air-liquid interface allowing exposure to particles, vapour and particulate aerosols via the air (relevant exposure). Besides healthy tissue, several pathologies (asthma, COPD) are available. It is anticipated that these models may predict a more realistic bioavailability of inhalated compounds instead of using a 100% uptake as default. To demonstrate this, we assessed absorption of several compounds by the MucilAir™ human 3D model (EpithelioLab). 3D inserts were resource intensive and animal cost- and time-consuming test substances via droplets on the tissue surface. Absorption was determined using liquid scintillation counting of the receptor fluid (basal side), cellular fraction and (apical) washing fluid. The model was able to differentiate between compounds utilizing various transport mechanisms, including passive uptake transcellularly (e.g. caffeine) or paracellularly (e.g. mannitol). Impaired respiratory tissue is expected to have an altered uptake of substances, which in part may explain the strong response of asthma to inhalation of mannitol. Therefore, uptake of mannitol and other substances was also assessed with inserts obtained from asthmatic patients. Increased uptake of mannitol was found compared to healthy donors, but the response depended heavily on the donor. This suggests a large interindividual variation in diseased tissues, in contrast to that of control tissues. In future, we will further assess the applicability of these models for exposure via the air (dynamic test atmosphere) and compare the results with in vivo inhalation data. Ultimately, these models may be useful in the safety evaluation of compounds for which the airways are the primary route of exposure.

1845 TESTING OF OECD REFERENCE NANOMATERIALS (NM-SERIES) IN RAT PRECISION-CUT LUNG SLICES.

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The OECD has defined reference nanomaterials (NM) to be tested in different endpoints concerning human health and environmental safety (1) in order to evaluate if the toxicity of nanomaterials can be linked to their physico-chemical properties. For nanomaterials, inhalation presents the major exposure route of concern and can be assessed using acute inhalation toxicity studies in rodents. However, these in vivo experiments are resource intensive and animal consuming. The OECD working party on nanomaterials has named several alternative methods as being of particular interest for testing of nanomaterials; among them are the precision-cut lung slices model (PCLS) to estimate respiratory toxicity. We have tested all 16 NM in PCLS measuring cytotoxicity as well as apoptotic, oxidative stress and inflammatory response of the tissues. For in vitro exposure of PCLS the test material was dispersed in medium. Since it is the nature of these materials to change their surface characteristics and agglomeration state in different environments, a standardized dispersion method (nanoCare) using bovine serum albumin as a stabilizing agent, was used. Particle size-distributions of the nanomaterial dispersions were characterized via analytical ultracentrifugation and found the nanoparticles well dispersed. Silver and zinc oxide but none of the other NM showed cytotoxicity to the lung tissue in the tested concentrations. However, differences in cytokine profiles among the NM were observed and showed several correlations to the results obtained in vivo inhalation or instillation studies.

1846 RESPIRATORY TOXICITY IN VITRO: IN HOUSE VALIDATION OF THE EPIAIRWAY MODEL.

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One of the main routes for substance uptake is by inhalation. In vivo experiments for inhalation toxicity are time and animal consuming. Thus several in vitro methods aim to replace or reduce and refine the in vivo experiments. Human 3D-tissue models are commercially available reconstructed from different donors (normal, smokers, chronic obstructive pulmonary diseases) which show a normal bronchiolar type II epithelial tissue that reveals a pseudostratified epithelial structure, numerous microvilli and cilia on the apical surface of the cultures. The presence of tight junctions and mucus secretion has also been confirmed comparable to the in vivo situation. These 3D-models are cultured on a porous membrane as air-liquid interface. Test substances can be applied apically, either as solution or with an aerosol-inducer.

In our in house validation to test the strengths, handling and reproducibility of such 3D-model systems as well as determining the correlation between in vivo inhalation data, we have assessed here the EpiAirwayTM model from MatTek, USA. A set of 20 substances were selected with known in vivo toxicity data and mode of action. The substances were tested in the EpiAirway model an in parallel, in 3T3 cell line to assess putative unspecific cytotoxic effects of the test substances.
A comparison of toxicity data from the 3D-model (and 3T3 cells) and the in vivo data revealed that the model is only predictive of respiratory toxicity in vivo for a subset of substances with specific modes of action. The EpiAirway™ model has proven to be robust, showing high reproducibility between pre- and main-tests as well as in the concurrent controls but it will need a strict definition of its applicability domain or further development of the test protocol to achieve a wider applicability.

The state-of-the-art method for testing inhalable substances in vitro is the "air-liquid-interface" culture technique. During this procedure, cell lines, primary cells from the human lung or complex ex vivo models like precision cut lung slices are presented to the test atmosphere on microporous membranes. Sensitivity and relevance of the basic technique have been demonstrated in a round-robin prevalida-
tion study in Germany. However, there were still major limiting factors until now leading to a loss of control of the individual exposure and a lack of insight into the immediate cellular effect of the test substances. These limitations were addressed by a newly developed cell culture and exposure system (P.R.T. by Fraunhofer ITEM) allowing cell culture, exposure to test substances and in situ live cell fluorescence analysis at the same time. Immortalized human lung cells (A549) and primary human airway epithelial cells were exposed to clean air, ozone or smoke aerosol. The cellular mitochondrial membrane potential, lipid oxidation, and intracellular oxidative species were monitored during exposures. Also, the particle deposition in the system was investigated. The results show that (1) immediate effects of inhala-
tion substances were clearly detectable in situ during the exposure at low, sub-toxic doses; (2) single long-term exposures (6 hours) could be performed without loss of viability in the controls; (3) effects of environmentally relevant ozone concentra-
tions (100 ppb) on the cells could be detected online and (4) particles were de-
positioned effectively from aerosols. Revised methods for testing inhalable substances in vitro are needed in the future to overcome limitations of the actual testing proce-
dures and meet demands of varied in vitro testing approaches like repeated exposure protocols. By introduction of a much higher practicability, expanded technical pos-
sibilities, an enhanced exposure environment for the cells and online monitoring of the cellular status, the improved cell culture and exposure procedure makes an im-
portant contribution in this direction.

The Toxicity Testing in the 21st Century report has stimulated a change in hazard testing strategies from animal-based studies to in vitro cell-based assays. To trans-
form conventional submerged cell cultures to an air-liquid interface (ALI) sys-

A comparison of toxicity data from the 3D-model (and 3T3 cells) and the in vivo data revealed that the model is only predictive of respiratory toxicity in vivo for a subset of substances with specific modes of action. The EpiAirway™ model has proven to be robust, showing high reproducibility between pre- and main-tests as well as in the concurrent controls but it will need a strict definition of its applica-
tility domain or further development of the test protocol to achieve a wider appli-
cability. However, CYP1A1/1B1 activity could be induced. Study designs using

CYP1A1/1B1 activity was assessed with the P450-Glo™ assay after induction with
tion from conventional submerged cell cultures to an air-liquid interface (ALI) sys-

The cell line BEAS-2B, derived from normal human bronchial epithelium, has
been considered as a potentially suitable lung cell system for in vitro testing of to-
bacco products. However, its metabolic capability has not yet been fully charac-
terized. Here, the metabolic competency of BEAS-2B was compared with a metaboli-
cally inactive cell system A549 and two metabolically active cell systems HepG2 and
HepaRG. Activity profiles were generated for four cytochrome P450 enzymes.
CYP1A1/1B1 activity was assessed with the P450-Glo™ assay after induction with

Conversely, the enzyme activity of CYP1A2, CYP2A6/2A13 and CYP2E1 in
BEAS-2B was marginal, not exceeding 2-fold than that seen in A549. In contrast,
CYP1A1/1B1 and CYP2E1 activity were 913- and 23-fold increased in HepG2, re-
spectively. Similarly, CYP2A6/2A13 and CYP1A2 showed increased activity in HepaRG (870- and 6-fold). RT-qPCR analysis indicated limited gene expression in
BEAS-2B, with the exception of CYP1A1, CYP1B1 and CYP1A2 which showed a
significant induction (25-, 5- and 4-fold, respectively) following TCDD incuba-
tion. This correlated with the CYP1A1/1B1 activity induced in BEAS-2B.
1851 CHARACTERIZING THE ESTROGENIC POTENTIAL OF 1060 ENVIRONMENTAL CHEMICALS BY ASSESSING GROWTH KINETICS IN T47D CELLS.


In order to detect environmental chemicals that pose a risk of endocrine disruption, high-throughput screening (HTS) tests capable of testing thousands of environmental chemicals are needed. Alteration of estrogen signaling has been implicated in a variety of adverse health effects including cancer promotion, reproductive deficits, and vascular effects. Here we investigate the estrogenic potential of 1060 chemicals of environmental relevance using a real-time measure of growth kinetics by electrode impedance in the estrogen-responsive human ductal carcinoma, T47D cell line. Cells were treated in concentration response and measurements of cellular impedance were recorded every hour for six days. The anticipated exponential impedance was observed in response to known estrogen receptor agonists (17β-estradiol, genistein, bisphenol-A, nonylphenol, 4-tert-octylphenol). Several compounds, including bisphenol-A, and genistein caused impedance comparable to that of 17β-estradiol, although at much higher concentrations. Progestogens, an- drogens, and mineralocorticoids (progesterone, dihydrotestosterone, aldosterone) - estradiol, although at much higher concentrations. Progestogens, an- drogens, and mineralocorticoids (progesterone, dihydrotestosterone, aldosterone) were also tested giving an indication for potential endocrine activity. This abstract does not necessarily reflect Agency policy.

1852 IN VITRO ASSESSMENT OF SHAMPOOS EYE STINGING POTENTIAL USING TRANSIENT RECEPTOR POTENTIAL VANILLOID TYPE 1 (TRPV1).


Unmyelinated c-fibers are implicated in nociception and pain signal transduction. Transient receptor potential vanilloid type 1 (TRPV1) is described as a key integrator of nociceptive transduction and is expressed on sensory nerve terminals. TRPV1 is activated by heat, acidic conditions and capsaicin present in c-fibers. In order to evaluate the ability of 10 shampoos to activate TRPV1, we used a neuronal-cell line activated by heat, acidic conditions and capsaicin present in c-fibers. In order to evaluate the ability of 10 shampoos to activate TRPV1, we used a neuronal-cell line activated by heat, acidic conditions and capsaicin present in c-fibers. We then evaluated the ability of 10 shampoos to activate TRPV1, we used a neuronal-cell line activated by heat, acidic conditions and capsaicin present in c-fibers.

1853 COMPARISON OF THE DPRA WITH A THREE-TEST BATTERY FOR IN VITRO EVALUATION OF SKIN SENSITIZATION.


To protect people from allergic contact dermatitis (ACD), regulatory agencies require that the results from standardized animal tests be used for hazard labeling. Such labeling warns consumers and workers of the precautions necessary to avoid exposures to substances that may cause ACD. International legislation to ban animal testing of cosmetics has spurred efforts to develop in vitro replacements for ACD hazard tests that use animals. NIEHS retrospectively evaluated the performance of the direct protein reactivity assay (DPRA) against that of testing strategies using three in vitro assays: DPRA, the human cell line activation test (h-CLAT), and KeratinoSens. The murine local lymph node assay was used as the reference test for a set of 67 unique substances. The DPRA alone generated an accuracy of 85% (57/67), a false positive rate of 22% (5/23), and a false negative rate of 11% (5/44). Using the results from all three assays and the most prevalent result for each substance gave an accuracy of 82% (55/67), a false positive rate of 30% (7/23), and a false negative rate of 15% (5/44). To evaluate a sequential testing strategy for the three in vitro assays, all of the DPRA-positive substances that were classified nonreactive, and all of the DPRA-negative substances that were classified reactive, according to published reactivity criteria, were tested in the other two assays. The reactivity criteria identified 60% (3/5) of the DPRA false positives and 20% (1/5) of the DPRA false negatives. However, this sequential testing strategy gave the same performance as the DPRA alone. While it corrects one false negative result, no false positives were corrected, and one additional false negative was introduced by the two subsequent assays. Further efforts are being made to identify reliable indicators of discordant results for the in vivo tests (e.g., skin irritation potential) that would allow targeted retesting to improve performance. ILS staff supported by NIEHS contract N01-ES-35504.

1854 LIMITATIONS IN CHARACTERIZATION OF ACUTE DERMAL IRRITATION WITH REDUCED NUMBER OF TEST ANIMALS.


A worthy goal of toxicologists is to reduce the use of animals for standard toxicology testing. Current guidelines led to the reduction of group size for acute oral toxicity, primary eye and dermal irritation studies, with as few as three animals now considered acceptable. This reduction has had an impact on the assignment of Acute Toxicity Categories (I through IV) by the CA Dept. of Pesticide Regulation (CDPR) with respect to primary eye and dermal irritation. These categories determine signal words, precautionary language, first aid statements, farm worker restricted entry intervals (REI) and personal protective equipment (PPE) on product labels. One area of difficulty associated with reduced group size has been the demarcation between Toxicity Category I and II in dermal irritation studies. The use of the Primary Dermal Irritation Index (PDI) by registrants of pesticide products, which emphasizes numerical dermal irritation data from one to seventy-two hours after dosing, is sometimes in conflict with regulatory decisions at CDPR, which in addition to PDI, takes reversibility (FIFRA guideline, OCSP 870.2500), severity and depth of damage (i.e., suggesting necrosis) into consideration. A study is presented in which one of three animals showed more severe dermal irritation than the remaining two, with the study outcome considered reflective of Toxicity Category III by the registrant based on PDI score, while judged to be Category II by CDPR, based on the most severe reaction (i.e., lack of reversibility). In other words, the animal with the severe reaction could not be considered an outlier by CDPR because of the small group size. One consequence of reduced animal numbers has been a reduction in certainty in the establishment of toxicity categories, thus increasing the likelihood of conservative regulatory decisions by CDPR. Strategies that may be employed by testing laboratories to reduce this uncertainty are discussed.
Acute poisonings from chemicals and products continue to be a significant public health problem. Development of the Up-and-Down Procedure for acute oral systemic toxicity testing has reduced the number of animals used by 70% while continuing to provide accurate classification and labeling for human health hazards. U.S. regulatory agencies also require acute dermal systemic toxicity testing for chemicals and products to estimate their potential to cause life-threatening or fatal toxicity from skin exposures. The resulting estimated lethal dose (LD50) values are used for acute dermal exposure hazard classification and labeling in order to protect human health and the environment during the handling, transport, and use of chemicals. With the objective of reducing the number of animals used for acute dermal systemic toxicity testing while maintaining the protection of human health for acute dermal exposures, NICEATM analyzed acute oral systemic toxicity data to determine its usefulness for assigning acute dermal systemic toxicity hazard categories. Underclassification of substances is less protective of public health and would fail to appropriately notify material handlers of chemical hazards while overclassification could desensitize them to such hazards. Therefore, acute oral toxicity data should not be used for classifying dermal toxicity hazards because it would underclassify or overclassify a substantial proportion of substances. ILS staff supported by NIEHS Contract N01-ES-35504.

**1857 UPDATED NICEATM EVALUATION OF THE REDUCED MURINE LOCAL LYPH NODE ASSAY.**

1NTP/NICEATM, ILS, Inc., Research Triangle Park, NC and 2NTP/NICEATM, NIEHS, Research Triangle Park, NC.

To minimize the occurrence of allergic contact dermatitis (ACD), regulatory authorities require testing to identify substances that may cause ACD. Such substances must be labeled with the hazard description and precautions necessary to minimize the occurrence of allergic contact dermatitis. Compared to guinea pig tests, it requires fewer animals, less time, and eliminates pain and distress. The reduced LLNA (rLLNA), which uses only one high dose, further reduces animal use by 40% compared to the multiose LLNA. Furthermore, 136 substances were used in the ICCVAM evaluation of the usefulness of the LLNA for potency categorization of substances causing allergic contact dermatitis in humans.

**1858 EVALUATION OF TWO NONRADIOLABLED MURINE LOCAL LYPH NODE ASSAYS (LLNA) FOR POTENCY CATEGORIZATION OF SUBSTANCES CAUSING ALLERGIC CONTACT DERMATITIS IN HUMANS.**

1NTP/NICEATM, ILS, Inc., Research Triangle Park, NC and 2NTP/NICEATM, NIEHS, Research Triangle Park, NC.

The correct classification of strong skin sensitizers is critical since such substances are considered to have a significant potential for causing allergic contact dermatitis (ACD) in humans. Because the prediction of ACD is poor, sensitizing substances must be labeled with a description of the potential hazard and the precautions necessary for workers and consumers to avoid development of ACD. An accurate ICCVAM evaluation found that the LLNA correctly classified 52% (14/27) of the strong human sensitizers when an effective threshold concentration (EC) ≤ 2% was used as the criterion. Thus, ICCVAM recommends that the LLNA be used as a screening test to classify substances as strong sensitizers, but that the classification of substances as other than strong sensitizers requires additional information. The OECD recently adopted two test guidelines for nonradiolabeled versions of the LLNA that could be used to classify substances as sensitizers: the LLNA: BrdU-ELISA and the LLNA: DA. Although these LLNA methods use different decision criteria to classify substances for ACD hazard, their accuracies are comparable to the LLNA. Of the 136 substances used in the ICCVAM evaluation of the usefulness of the LLNA for potency categorization, LLNA: BrdU-ELISA data were available for 31 substances and LLNA: DA data were available for 30 substances. An EC ≤ 9% for the LLNA: BrdU-ELISA and an EC ≤ 2% for the LLNA: DA classified strong sensitizers at rates comparable to that of the LLNA. These results suggest that the LLNA: BrdU-ELISA and the LLNA: DA may also be useful for classifying substances as strong sensitizers. ILS staff supported by NIEHS contract N01-ES-35504.

**1859 PILOT ANALYSIS OF FOOD CONTACT NOTIFICATION OUTCOME.**

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The US Food and Drug Administration’s Office of Food Additive Safety (OFAS) in the Center for Food Safety and Applied Nutrition conducts assessments of food additives, including food-contact substances (FCs) that have the potential to migrate to food. Under OFAS, FDA’s food contact notification (FCN) program is a pre-market notification system and is the primary regulatory process for evaluating the safety of FCNs. To assist program development, the Center for Food Safety and Applied Nutrition conducted a retrospective assessment of FCN outcomes. A preliminary analysis of FCN outcome, we analyzed a random series of 52 FCNs. Subsets of FCN categories were identified and examined for effective or withdrawal outcome. All FCNs were completed in the mandated 120-day period. However, some FCNs, such as those containing a polymeric FCS, took longer to complete than non-polymeric FCNs. FCNs accompanied by a pre-notification consult (PNC) with OFAS scientists exhibited a greater likelihood of becoming effective than those without a PNC. This would suggest that the PNC process assists with FCN outcomes, especially for FCNs not previously assessed by OFAS. Our analysis of the FCN program may help guide current and future notifications and provide insight for future program planning.

**1860 SUGGESTED PROTOCOL FOR ESTIMATION OF HARMFUL AND POTENTIALLY HARMFUL CONSTITUENTS FROM MAINSTREAM AEROSOLS GENERATED BY ELECTRONIC NICOTINE DELIVERY SYSTEMS (ENDS).**

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In Sottera, Inc. v. Food & Drug Administration, 627 F.3d 891 (D.C. Cir. 2010), the Court of Appeals held that e-cigarettes and other products made or derived from tobacco can be regulated as “tobacco products” under the Act and are not drugs/devices unless they are marketed for therapeutic purposes. It is likely that the FDA will extend its authority over electronic nicotine delivery systems (ENDS, also known as e-cigs) and may also require the reporting of harmful and potentially harmful constituents in the mainstream aerosols produced with ENDS. Moreover, studies of the safety, abuse liability, and efficacy of ENDS will require demonstration that performance of any product to be studied performs as expected. However, equipment and protocols used to generate the aerosol need to reflect the conditions of use, including the effects of taking larger puff volumes than would be expected and ability to determine carbon monoxide (CO), a potential marker of ENDS overheating and abuse. Pending definitive internationally accepted puffing regimens for ENDS, we propose that ENDS be defined using a standard analytical smoking machine with 55 ml puff, 5 s puff duration, and 30 s puff interval and reporting of results on a constituent per liter of mainstream aerosol generated with the smoking machine and that any comparisons with conventional cigarettes (conv. cig.) be done with the latter smoked under the Health Canada Intensive smoking protocol (55 ml puff, 2 s puff duration, 30 s puff interval, with 100% blocking of filter ventilation). Typical data (mg/L except puffs) obtained for an ENDS versus full flavor conv. cig.: puffs 11, 10.3; TPM 11.6, 107; nicotine 0.1, 4.0; tar 10.5, 66.4; CO <0.3, 54.9; CH3CHO 0.02, 2.28; HCHO 0.01, 0.12., with similar results.
Rationale: To determine whether claims of reduced emissions from a smokeless electronic cigarette (electronic nicotine delivery system, aka ENDS) were justified.

Scope: The Ruyan® classic V8 electronic cigarette (ENDS) was tested against a very low tar (1.2 mg yield) cigarette (VLTC) and compared with published emissions for the US-style of Marlboro KS cigarettes.

Procedures: Product were smoke according to the ISO standard (35 mL puff, 2 s puff duration, 30 s puff interval), and the resulting mainstream aerosols analysed for 62 cigarette smoke toxicants by Labstat International and British American Tobacco, as per their library of methods.

Data: The Ruyan® ENDS emitted over 300 puffs of aerosol (10.5 L, mean TPM weight 0.88 mg) compris 82% propylene glycol, 15% water, 1% nicotine, 2% unidentified particulate matter and flavors. Of 62 cigarette-smoke toxicants 37 were measurable in VLTC smoke and 11 in Ruyan® vapor. Estimated relative toxicant emissions scores, adjusted for nicotine, were 0.4 for Ruyan; 55 for VLTC; and 137 for Marlboro KS. Three tobacco-specific nitrosamines in Ruyan® vapor were present at trace levels no greater than for medicinal nicotine; mercury was present at trace level. Ruyan®, VLTC, and Marlboro regular cigarette yielded 9 μg, 23 μg, and 101 μg mean nicotine, respectively, per 35 mL puff.

Conclusion: The Ruyan® aerosol as determined under ISO conditions for cigarettes is free of most toxicants found in cigarette smoke; and those measurable are in very low concentration. ENDS products are subject to frequent modications and should be retested at periodic interval using and machine smoking parameters that replicate actual human puffing behavior instead of those in the ISO standard.

1863 IMPLEMENTING BODY WEIGHT SCALING AS A DEFAULT APPROACH FOR DERIVING ORAL REFERENCE DOSES.

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The Minnesota Department of Health (MDH) evaluates the health risks of contaminated water and establishes human-based guidance values. The guidance values represent a water concentration that is without appreciable risk to human health. Mammalian animal data often forms the basis for dose-response assessment and extrapolation from laboratory animals to humans is typically required. Historically, oral reference dose (RfD) values were derived using the dose administered to the laboratory animal and an animal-to-human uncertainty factor (UFA). In February 2011 the U.S. Environmental Protection Agency (EPA) released the guidance document “Recommended Use of Body Weight %4 as the Default Method in Derivation of the Oral Reference Dose”. In this guidance EPA recommends deriving human equivalent doses (HEDs) from dosimetric adjustment factors (DAFs) based on body weight scaling as a default approach (i.e., the absence of chemical-specific toxicokinetic information).

The benefits of using body weight scaling over the default UFA are two-fold: 1) re-duction in the uncertainty by using a biologically-based interspecies toxicokinetic extrapolation factor and 2) harmonization with dose-response extrapolation methods utilized for the derivation of inhalation references concentrations and cancer potency values. A drawback to using this approach is the increased resources needed to calculate DAFs and HEDs. Calculating an HED requires specific information about the age, gender and species of the animals tested as well as the study duration. Driving an RfD may require that HED values are calculated for a large dose groups for multiple studies. MDH formulated a streamlined process to calculate HEDs by using general dura- tion, age, gender and species specific DAFs. MDH found that the use of HEDs could have a large impact on the selection of the point of departure and principal study for deriving an RfD. The expedited process will be presented along with ex- amples of how using HEDs, rather than the administered dose, affects the selection of the point of departure and principal study.

1864 PRACTICAL IMPLEMENTATION OF GREEN CHEMISTRY PRINCIPLES FOR EVALUATING SAFER ALTERNATIVES DURING PRODUCT DEVELOPMENT.

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Green chemistry initiatives are currently being driven by regulatory requirements, market drivers by retailers and corporate initiatives for sustainable product development. A number of methods have been put forward as tools for the evaluation of chemical hazards and the identification of safer alternatives for chemicals currently in use. To facilitate the adoption of green chemistry early in product development, rapid screening methods will be necessary. One framework, Green Screen for Safer Chemicals, an open source method by the Clean Production Action, was used to evaluate hazards for a subset of acrylic chemistries. This method was selected because it is recognized by EPAs DfE and aligns with GHS. Literature searches were conducted and hazard scores were assigned for the chemicals for which publically available hazard data could be located. For the acrylics, hazard data existed for 86% chemicals, with none having a complete data set. For these chemicals, fully 45% of the chemical-endpoint combinations remained as data gaps. Most alter-na-tives assessment methods suggest gaps should be modeled to allow for predictions of hazard. As an example, ToxTree was utilized to estimate acute toxicity, skin sensitization and carcinogenicity. Acute toxicity data was available for 86% of the chemicals evaluated; ToxTree accurately predicted toxicity for 38%, acute toxicity was over predicted in 23% and under predicted in 38% of the cases. Skin sensitization was accurately predicted for the 13 chemicals with test data. None of these chemicals were predicted to have carcinogenic potential, while 33% of these chemicals scored a moderate for these endpoints within the Green Screen (test data). For chemicals with existing data, a rank ordering is useful as one input within a broader weight of evidence approach to formulation selection. For those chemicals lacking in hazard data, incorporation of green chemistry principals requires efficient and accurate analysis of data gaps during the hazard review in order to accommodate early R&D during product development.

1865 THE ROLE OF CONFORMITY AUDITS OF GCP INSPECTIONS IN JAPANESE AUTHORITY AND THE QUALITY OF CLINICAL TRIALS IN JAPAN.

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The Pharmaceuticals and Medical Devices Agency (PMDA) is an incorporated administrative agency with non-civil servant status in Japan. It was established 1st April, 2004, and is located in Tokyo. PMDA’s mission is to help improve public health through activities, such as reviewing pharmaceuticals and medical devices for marketing authorization based on the Pharmaceutical Affairs Law of Japan, collection, analysis and dissemination of information related to the quality, efficacy and safety of pharmaceuticals and medical devices, and providing medical expenses, disability pensions, bereaved family pensions, etc., for people who have suffered from severe adverse events caused by pharmaceuticals or biological products. The ‘Office of Conformity Audit’ endeavored the protection of the patients’ safety and welfare of human research subjects involved in Japanese regulated clinical trials and verifies the accuracy and reliability of clinical trial data. The work involves site
visits to clinical investigators, pharmaceutical companies and nonclinical laborato-
ries in order to ensure consistency of raw data and records. The work of each office is
carried out in accordance with the regulations set forth in the Pharmaceutical
Affairs Law. We introduce the document based conformity inspections and the reli-
ability assessment in relation to new drug applications (NDA) for marketing ap-
proval by conformity audits. We compare the average of total inspection times, and
the number of GCP non-compliance or compliance with condition cases for NDA
between FY 2004 to FY2009. The average inspection time is becoming shorter,
while the number of NDA is increasing. The number of GCP non-compliance or
compliance with condition is decreasing year by year. Those data suggest that
the quality of clinical trials done in Japan has been improving. In conclusion, docu-
ment inspections ensure the scientific quality of clinical trials, the reliability of the
study results and the veracity of NDA documents.

1866 RETROSPECTIVE ANALYSIS OF THE ABILITY OF
INDUSTRY, ACADEMIA, AND ANIMAL RIGHTS
ORGANIZATIONS TO MEET THE TESTING AND
MARKETING BANS ESTABLISHED BY THE 7TH
AMENDMENT OF COSMETIC DIRECTIVE.

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In 2004, a timetable for phasing out animal testing under the framework of the 7th
amendment to the Cosmetics Directive (CD) was established. Based on an ad hoc
group of experts, the Scientific Committee on Cosmetic Products and Non Food
Products (SCCNFP) presented opinions regarding whether alternative tests could
be developed to meet the cut-off dates provided by the CD. At that point in time,
the SCCNFP predicted that non-animal alternatives to the toxicological endpoints,
skin irritation, eye irritation, skin absorption, and UV-induced toxic effects, could
be validated to meet the 2009 testing ban or the 2013 marketing ban of the CD (attain-
able group). At the same time, the SCCNFP did not foresee the validation of alterna-
tives to address acute toxicity, skin sensitization, subacute and subchronic toxicity,
genotoxicity, photo-allergy, toxicokinetics and metabolism, carcinogenicity, or re-
productive and developmental toxicity prior to the 2007 testing ban or the 2013
marketing ban of the CD (non-attainable group). Retrospective analysis of progress
in the development of alternatives was performed to determine the accuracy of the
SCCNFP predictions. For the attainable group, OECD guidelines utilizing normal
human cell based 3-dimensional (NHu-3D) tissue models have been established for
skin irritation and skin absorption, but only pre-validation studies for eye irritation
or photogenetotoxicity have been completed. For the non-attainable group, no ani-
mal alternatives have been validated but active programs utilizing NHu-3D and
other in vitro alternatives are ongoing in the areas of skin sensitization, genotox-
icity, photosensitization, and reproductive and developmental toxicity. Based on this
analysis, the SCCNFP predictions were partially accurate while the timetables set
forth in the CD were overly ambitious. It is anticipated that validation of alterna-
tive assays will continue but the realistic timetable for validation of these assays will
be longer than previously anticipated.

1867 DERIVATION OF ACCEPTABLE DAILY EXPOSURE
(ADE) VALUES—A GUIDANCE PROPOSED BY
PHARMACEUTICAL INDUSTRY.

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The Acceptable Daily Exposure (ADE) is defined as a substance-specific dose that is
unlikely to cause an adverse health event or undesirable physiological effect if an in-
dividual is exposed at or below this dose during lifetime. ADEs are now being in-
troduced as a measure of safe residual contamination of “multi-product” manufac-
turing equipment. Therefore, they will become the object of new regulatory scrutiny.
The ADE concept follows a well-established paradigm that has long been successfully
used in nutritional toxicology to describe acceptable levels of chemical food contamina-
tion (oral intake) and in occupational toxicology to derive accept-
able levels of workplace exposures (via inhalation). The ADE is typically based
on nonclinical and clinical data. Here, we describe a standard procedure of ADE set-
ting from nonclinical safety and clinical data: The reference effect level (e.g. NOAEL
observed in the most sensitive species) is multiplied with the human body weight (60 kg).
The resulting dose has to be divided by adjustment factors to compensate for uncertainties in the model applied. These include interindividual variability (F1), interspecies differences (F2), duration of the toxicity study (F3), severity of toxicity (F4), and the quality of the reference effect level (F5). While our guidance provides default values for F1 - F5, we recommend replacing them by substance-specific adjustment factors whenever some of this standard procedure are described for CMR substances. Whenever possible, an alter-
native ADE calculation is made based on clinical data. Here no defaults are used.

The derivation is made based on the quality of the compound-specific data and
professional judgement. Basic principles of the proposed procedure, different routes of
exposure, and differential sensitivity in human subpopulations are discussed. In conclusion,
we are presenting a standard approach implemented at Roche for the derivation of ADE values which may provide some guidance for other toxicologists
in charge of ADE setting.

1868 COMPARATIVE STUDY OF PLASMA MICROsampling
IN MALE RATS AFTER A SINGLE-DOSE OF
ACETAMINOPHEN.

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Small total blood volumes in animals, especially rodents, present a practical limita-
tion when serially sampling blood to obtain toxicokinetic data. This limitation can
increase the numbers of rats or mice used on a study and/or limit the number of
samples collected per animal. Microsampling strategies (e.g., dried bloodspots) can
effectively overcome this limitation and are increasingly used in the pharmaceutical
industry to reduce animal use and costs. Reducing blood volume need can also have
a positive impact by decreasing the number of animals used on a study and by al-
lowing for more robust serial sampling in an individual animal. Plasma microsam-
pling (~75 ul of blood per time point) significantly decreases the amount of blood
(~70%) collected per time point for toxicokinetic analysis. This study compared
data collected in male rats (n = 8) after a single 600 mg/kg dose of acetaminophen
using a plasma microsampling method (~75 ul of blood collected in EDTA-coated
capillary tubes with a thixotropic agent) and a standardized plasma collection
method (250 ul of blood collected into a 1.4 ml EDTA-coated Matrix tube per
time point). Drug exposure at 4 separate time points (1, 2, 5, and 8 hours) were
compared and found to be within 8% overall; the majority of samples (23 of 32)
were within 5%. Mean AUC and Cmax values were within 1% of each other using
the 2 different collection methods. Individual AUC or Cmax values within 5% or
6% of each other, respectively. These results demonstrate that this microsampling
technique is comparable to the standardized plasma collection technique.

1869 SPECIES COMPARISON OF INHALED SULFURYL
FLUORIDE PHARMACOKINETICS IN FISCHER 344
RATS AND NEW ZEALAND WHITE RABBITS.

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Sulfuryl fluoride (SO2F2) is a structural and post-harvest fumigant used to control a
variety of insect pests. Repeated daily 6h exposures (2 or 13 wks) to 100/300/600
ppm SO2F2 induces neurotoxicity in rabbits and rats and renal toxicity in rats. The
USEPA currently uses the results of a rabbit 90-day inhalation study for risk assess-
ment based on brain neuropathology. This study was designed to permit direct
comparison of the pharmacokinetics of SO2F2 in rabbits and rats by simultaneous 6
h exposures to the same SO2F2 concentrations (0, 3, 30 or 300 ppm). Plasma, cere-
brum and kidney samples collected immediately after exposure were analyzed for
the principle SO2F2 metabolites, fluoride (F) and fluorosulfate (FSO3-). Plasma F
levels were consistently higher (3x) in rabbits than rats, while plasma FSO3- levels
were similar in both species in each SO2F2 exposure group. F levels in rat cerebra
increased proportionately with exposure concentration. In rabbits, F above the
lower limit of quantitation (LLQ) was measured only in 300 ppm-exposed rabbits,
but at that SO2F2 concentration, rats and rabbits had similar renal F levels. No
FSO3- LLQ was detected in rat cerebrum in any exposure group. Only rabbits ex-
posed to 300 ppm SO2F2 had FSO3- in the cerebrum > LLQ (7% of F levels). These
results suggest the blood brain barrier in rats and rabbits limits FSO3- exposure to the
cerebrum. Kidney F levels > LLQ were measured in rats exposed to 30 or 300 ppm
and in all SO2F2-exposed rabbits. Exposure-response curves for kidney F differed
between species, but, 300 ppm-exposed rats and rabbits had similar kidney F levels.
Kidney FSO3- levels > LLQ were detected in rabbits exposed to 30 or 300 ppm but
only in rats exposed to 300 ppm. After exposure to 300 ppm the concentration of FSO3-
, in rat kidneys was approximately 6% of that measured in rabbits. These data
indicate that F, not FSO3-, is the principal toxinchic responsible for SO2F2-induced
neuro- and renal toxicity.
**SULFURYL FLUORIDE: PHARMACOKINETICS OF REPEATED INHALATION EXPOSURES IN FISCHER 344 RATS.**

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Sulfuryl fluoride (SO$_2$F$_2$) is a structural and post-harvest fumigant used to control a variety of insect pests. Rats repeatedly exposed (6h/d; 2 or 13eks) to 100/300/600 ppm SO$_2$F$_2$ develop neuro- and renal toxicity. This study was conducted to determine if repeated 6h exposures of rats to 0, 3, 30, or 300 ppm SO$_2$F$_2$ for up to 2wk alters the concentration, clearance or elimination of its metabolites, fluoride (F) and fluorosulfate (FSO$_3$), as compared to a single 4h exposure used in previous PK studies. Plasma, cerebrum and kidney were analyzed for F and FSO$_3$, immediately after 1 or 10 exposures (1x/10x). Serial plasma and urine samples were analyzed to determine plasma clearance and urinary elimination of F and FSO$_3$. Peak plasma F levels above the lower limit of quantitation (LLQ) were measured in rats exposed 1x to 300 ppm or 10x to 30 or 300 ppm. Plasma F levels of rats exposed 1x or 10x to 300 ppm were similar. Plasma FSO$_3$ levels increased proportionally with SO$_2$F$_2$ concentration and were similar in 1x- and 10x-exposed rats. Plasma elimination half-lives for F and FSO$_3$ were similar after 1x (2.3 h/1.7 h) or 10x (2.6 h/1.6 h) exposures. The levels of F in cerebrum and kidney and FSO$_3$ in kidney were similar in rats exposed 1x or 10x to SO$_2$F$_2$. FSO$_3$ levels > LLQ were not detected in the cerebrum of any rats exposed to any SO$_2$F$_2$ concentration. This suggests that the blood brain barrier limits FSO$_3$ exposure to the cerebrum and repeated exposure does not degrade this barrier. Urinary concentrations of F and FSO$_3$ were similar in 1x- and 10x-exposed rats and increased proportionally with exposure concentration. The results of this study show no accumulation of F or FSO$_3$ in plasma or tissues of rats repeatedly exposed up to 1x over 2 weeks. This suggests that the neuro-and renal toxicity observed in rats repeatedly exposed to high concentrations of SO$_2$F$_2$ are likely due to the cumulative effect of repeated daily insults to target tissues rather than a shift in SO$_2$F$_2$ pharmacokinetics.
1874 METHOD FOR THE ANALYSIS OF 8:2 FLUOROTELOMER ALCOHOL IN RAT PLASMA, LIVER, KIDNEY, AND BRAIN AND ITS APPLICATION TO A SINGLE DOSE ORAL GAVAGE TOXICOGENIC STUDY.

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8:2 Fluorotelomer alcohol (8:2 FTOH) was one of a series of perfluorinated compounds selected by the National Toxicology Program (NTP) for toxicity evaluation based on the compounds' widespread environmental presence. Quantitative bioanalysis of 8:2 fluorotelomer alcohol has been previously limited to the use of radio-labeled material that resulted in studies and methods that are costly and time consuming. A rapid analysis method was developed based on on-line solid phase extraction (SPE) with liquid chromatography tandem mass spectrometry (LC/MS/MS) and validated for rat plasma, brain, kidney, and livers. A key benefit of this method is that it requires little to no sample preparation and the total analysis time is approximately 10 minutes. Plasma can be directly analyzed without preparation and tissues require only a simple digestion in methanolic potassium hydroxide prior to analysis. The method had suitable ruggedness to analyze at least 140 samples per batch permitting the analysis of multiple treatment groups in a single run. Dilution of spiked matrix samples demonstrated that the total dynamic range for the method was 10,000 for plasma and 500 for tissues. The detection limit of this method were 0.5 ng/mL in plasma and 10 ng/g in liver, brain and kidney. The sensitivity of the method permitted the determination of plasma concentrations out to 24 hours following a 12 mg/Kg oral gavage administration. The 8:2 FTOH level could be measured in liver, kidney, and brain at 12 hours post-dose following a 24 mg/Kg oral gavage. 8.2 FTOH was rapidly distributed into the liver, kidney, and brain achieving a ratio greater than one for all time points with general trends of increasing tissue:plasma ratios over time. There were no apparent gender effects on tissue distribution of 8:2 FTOH. This work was supported by NTP Contract N01-ES-55551.

1875 TOXICOGENETICS OF PERFLUORODECANOIC ACID (PFDA) AFTER A SINGLE INTRAVENOUS OR GAVAGE ADMINISTRATION TO HARPAN SPRAGUE DAWLEY RATS.

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PFDA is considered a global contaminant due to detection in humans and wildlife in various geographical locations. To better understand the kinetics of PFDA in a rodent toxicity model, this study determined the concentrations of PFDA in Sprague Dawley rat plasma, liver, kidney, and brain after a single IV or gavage administration of PFDA in 2% Tween 80 in deionized water at dosages of 2 mg/kg (IV) and 2, 10, or 20 mg/kg (gavage) (n = 3/timepoint). PFDA IV and gavage TK profiles were characterized by a two-compartment kinetic model. After IV administration, male and female elimination (k₁) half-life values were 14.8 ± 1.8 days and 21.1 ± 3.4 days, respectively. Male and female beta half-life values were 35.6 ± 2.5 days and 37.7 ± 3.5 days, respectively. Male and female AUC∞ values were 3,750,000 ± 220,000 and 613,000 ± 450,000 ng/mL/hr. After gavage administration, elimination half-life values ranged from 19.9-24.1 days for males and from 30.7-35.5 days for females. Male and female urinary excretion increased in a nearly dose proportional manner from 6,440,000 to 59,200,000 and from 10,400,000 to 104,000,000 ng/mL/hr, respectively. PFDA was distributed to the liver with a liver/plasma ratio greater than one for all time points with a trend of increasing ratios over time in males. The kidney/plasma ratios remained near or below one for all time points. PFDA was poorly distributed to the brain in both males and females.

The results of this study provided TK parameters for PFDA in rats to correlate toxic effects with systemic availability and to improve the usefulness of toxicity study results in risk assessment. [Supported by NIH, N01-ES-55551]

1876 INCREASING DIETARY SATURATED FAT IS NEGATIVELY ASSOCIATED WITH PLASMA CONCENTRATIONS OF NONHIGH DENSITY LIPOPROTEIN CHOLESTEROL AND PERFLUOROOCANTANOATE (PFOS) IN APOE³- LEIDEN.CETP MICE AT CONSTANT LOW-LEVEL DIETARY PFOS.

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Cross-sectional (C-S) studies have observed positive associations of serum perfluoroocantanoate (PFOS) and perfluorooctane sulfonate (PFOS) with nonhigh density lipoprotein cholesterol (non-HDL-C) which weaken substantially at serum PFOA and PFOS concentrations ≥50 ng/mL. Laboratory investigation has established that PFOA and PFOS would be expected to reduce serum cholesterol, and a recent longitudinal occupational study has found no association of increasing serum PFOA and PFOS with non-HDL-C over the concentration range where previously observed in C-S studies. Dietary fat was hypothesized as a potential noncausal factor in the associations observed in C-S studies. To test this, male APOE³-Leiden.CETP mice (10/group) were fed diets designed to deliver isocalorically-adjusted, near-constant, low-level (~20-30 µg/kg diet) PFOA and PFOS simultaneously with saturated fat concentration adjusted with cocoa butter to yield 5, 15, 25, and 35% fat diets. Mice were run-in on diets for several weeks prior to adding PFOA and PFOS. Body weight and plasma lipids, lipoprotein profile, PFOA, and PFOS were determined at initiation of run-in, prior to introducing PFOA and PFOS, and after two and four weeks with PFOA and PFOS. Plasma PFOA and PFOS were also determined after one-week of exposure. Food consumption was recorded weekly. Dietary fat consumption was associated negatively with total cholesterol, serum non-HDL-C, and serum PFOA concentrations. No associations with PFOS were found. The positive associations observed with plasma PFOA concentration and plasma total and plasma non-HDL-C, as well as the negative association observed between plasma PFOA and plasma HDL-C are all likely noncausal with respect to PFOA and are more likely related to a hypothetical effect of dietary fat consumption on absorption of PFOA and cholesterol at some points on the pathway to plasma distribution.

1877 PLASMA PROTEIN BINDING OF PYRETHROID INSECTICIDES.

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Relatively little is known about the toxicoxicokinetics (TK) of pyrethroids, the most widely-used class of insecticides in the U.S. Plasma protein binding can influence the TK of highly-bound chemicals, by limiting the amount of compound free to reach target organs and sites of metabolism and elimination. Since the extent of plasma protein binding of common pyrethroids is unclear, this study was undertaken to determine whether deltamethrin (DLM), cis-permethrin (CIS) and trans-permethrin (TRANS) bind to human and rat plasma and to characterize their binding kinetics. A solvent extraction method was developed employing radiola beled pyrethroids to quantify the binding of these highly hydrophobic compounds. A 10-1 ul aliquot of 14C-DLM, CIS or TRANS was mixed with 100 ul of plasma and cold compound yielding concentrations of 25-100,000 nM. Samples were incubated and shaken at 37°C for 3 hr. The unbound fraction was extracted with 200 ul of isooctane: 1.4 dioxane (95:5). The remaining sample was then extracted with 200 ul of acetonitrile to determine the bound fraction. Since rat plasma is known to contain esterases that metabolize pyrethroids, samples were treated with 10 µl of a 1 mM solution of tetraisopropylpyrophosphate, an inhibitor of carboxylesterase. Pyrethroid concentrations were determined by radioactive counting against reference counts. DLM, CIS and TRANS binding to human plasma (~ 50% bound) was linear over the concentration range of 25-10,000 nM. Binding of pyrethroids to rat plasma was linear (~ 42% bound) for 25-2500 nM. The kd values were similar for humans and rats, however, Bmax for humans (19430-23140 nM/mg protein) was significantly higher than for the rat (7769-13549 nM/mg protein). These binding constants will be input into human and rat physiological TK models being developed for DLM, CIS, and TRANS. Supported by the Council for Advancement of Pyrethroid Human Risk Assessment.
1878 TOXICOKINETICS OF THE PYRETHROID INSECTICIDE BIFENTHRIN IN BLOOD AND BRAIN OF THE RAT.


Bifenthrin is a pyrethroid insecticide and human exposures occur by oral, pulmonary and dermal routes. Pyrethroids are neurotoxic agents and it is generally believed that the parent pyrethroid is the toxic entity. The objective of this study was to assess the toxicokinetics of bifenthrin in blood and brain of the rat. Adult male Long Evans rats were administered bifenthrin by oral gavage in corn oil (1 ml/kg) at dose of 0.3 or 3 mg/kg. Animals were sacrificed from 0.25 to 48 h after dosing and blood and brain were removed. Tissues were extracted and analyzed for bifenthrin by HPLC/MS/MS. For 0.3 mg/kg bifenthrin in blood, the maximal concentration (Cmax) was 86.1 ng/ml, the time of maximal concentration (Tmax) was 1 h and the area under the curve from 0 to 48 h (AUC0-48h) was 208.5 ng·h/ml. For 3 mg/kg bifenthrin in blood, the Cmax was 946.5 ng/ml, the Tmax was 1 h and the AUC0-48h was 2118.6 ng·h/ml. For 0.3 mg/kg bifenthrin in brain, the Cmax was 11.9 ng/g, the Tmax was 2 h and the AUC0-48h was 78.4 ng·g·h/g. For 3 mg/kg bifenthrin in brain, the Cmax was 142.8 ng/g, the Tmax was 6 h and the AUC0-48h was 2729.8 ng·g·h/g. There was a proportional increase in blood bifenthrin AUC0-48h with dose. However, there was a disproportionate increase in brain bifenthrin AUC0-48h with dose. This suggests that there may be either greater uptake or decreased clearance of bifenthrin in brain, which may explain the dose-dependent neurotoxic response of this insecticide in the rat. (This abstract does not represent US EPA or NIEHS policy.)

1879 INTRAVENOUS PHARMACOKINETICS OF MEROPENEM IN AFRICAN GREEN MONKEYS.

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Drug exposure of the antibiotic meropenem was examined in male and female African Green Monkeys following intravenous administration. Pharmacokinetic modeling was conducted following single dose treatment (20 and 28 mg/kg) and subsequent measurements of meropenem levels in plasma. Meropenem exposure (AUC0-inf) increased with the dose, but it is not clear whether it does it in a proportional manner. The dose-adjusted AUC0-inf values were 1378.05 hr*kg*ng/mL/mg (females) and 1516.27 hr*kg*ng/mL/mg (males) for the 20 mg/kg dose and 1100.03 hr*kg*ng/mL/mg (females) and 1436.85 hr*kg*ng/mL/mg (males) for the 28 mg/kg dose. The decrease in the value of this parameter as the dose increase might be indicative of lack of dose proportionality. AUC values were larger in males than in females for the same dose. Accordingly, systemic clearances were lower in males than in females for both dosing groups (662.12 mL/hr/kg vs. 738.00 mL/hr/kg and 698.53 mL/hr/kg vs. 910.34 mL/hr/kg). Likewise, half-lives were shorter (0.54 hr vs. 0.61 hr and 0.60 hr vs. 0.93 hr). The volume of distribution of the drug was low to moderate the extent of plasma exposure. The systemic clearance of meropenem may be lower in males, but further studies would be needed to confirm this.

1880 DOUBLE CROSS-OVER BIOEQUIVALENCE STUDY OF DERACOXIB FOLLOWING SINGLE DOSE ADMINISTRATIONS IN CANINES.


Bioequivalence is demonstrated by comparing total systemic exposure (AUC) and peak plasma concentration (Cmax) of similar drug formulations. A double cross-over design allows testing of two drug strengths within the same study. The 90% CI of both AUC and Cmax should lie between the accepted range of 80-125% to establish bioequivalence. This study was designed to determine the bioequivalence of two different formulations of deracoxib tablets to show therapeutic equivalence and establish interchangeability between the two formulations. A double cross-over bioequivalence study was conducted in 12 healthy, laboratory beagles of mixed-sex. The study consisted of two groups with six dogs per group and was conducted in four phases. Phases I and II compared two formulations of 12 mg tablets where dogs were dosed orally in a cross-over design using a test and reference item. Phases III and IV compared two formulations of 100 mg tablets where dogs were dosed orally in the same cross-over design. Dogs were fasted prior to dosing. Body weights were obtained prior to each dose. Dosing during phases I through III were followed by a wash-out period of at least 14 days. Blood samples were collected pre-dose and at 13 additional time points up to 24 hours post dose. Samples were analyzed by LC/MS/MS method. As the same dogs were used for the 12 and 100 mg phases, the dose for the 100 mg tablet was approximately 2x the labeled dose (3 mg/kg). For the 100 mg test item, the 90% CI for bioavailability ranged from 82 to 114% (AUC(0-t)), and 82 to 103% (Cmax). For the 12 mg test item, the 90% CI for bioavailability ranged from 101 to 136% (AUC(0-t)), and 93 to 147% (Cmax). Thus, for the 12 mg tablets, Cmax extended above the therapeutic range. The 100 mg test item demonstrated bioequivalence to the 100 mg reference item. The 10 mg test item was not bioequivalent to the 12mg reference item. Dogs appeared to tolerate deracoxib without adverse effect.

1881 PRECLINICAL PHARMACOKINETICS AND INTERSPECIES SCALING FOR ST-246, AN ORAL ANTIVIRAL THERAPEUTIC FOR THE TREATMENT OF ORTHOPOXVIRUS INFECTIONS.


The oral pharmacokinetics of ST-246, an oral anti-orthopoxvirus therapeutic, were compared in rats, rabbits, monkeys at 300, 100 and 300 mg/kg, respectively, and in healthy human volunteers at a 400 mg dose level. Standard non-compartmental analysis was applied to the plasma concentration data following administration of the compound at the stated doses levels. Allometric scaling was then performed on this resulting preclinical data for the prediction of human pharmacokinetics. The time to reach peak plasma levels (Tmax) was similar for the two formulations. A double cross-over design was used to compare the bioavailability of ST-246 from 0.5 to 0.8 hr from rats to humans. The time course profile in each tested species displayed a biphasic pattern, characterized by an initial rapid distribution phase followed by a slower terminal elimination phase with a half-life ranging from 8.4 to 25.8 hr. The estimated volume of distribution ranged from 54.8 to 128.2 L and the systemic clearance 3.8 to 37.5 L/hr from rats to humans. The estimated oral absolute bioavailability for the preclinical species ranged from 16.5 to 49.8%.

Our predictions for human systemic clearance, apparent volume of distribution and terminal elimination half-life by simple allometric scaling of the preclinical pharmacokinetic data were within the 2-fold criterion limits for successful extrapolation along with a predicted human absolute bioavailability of 61.1%. In summary, ST-246 displays similar pharmacokinetic behavior in these species in terms of the absorption, distribution and elimination kinetics based on the short period of time to reach maximum plasma concentration, large volume of distribution and prolonged terminal elimination half-life. Also, this evaluation demonstrates that the preclinical oral pharmacokinetic data for this compound in these species supports the allometric prediction of human pharmacokinetics. Supported by Contract Title: NDA-Enabling Development for ST-246: A Smallpox Antiviral Drug; Contract Number: HHSN26620060014C; ADB Contract Number: N01 AI-60014.

1882 DISTRIBUTION, METABOLISM, AND EXCRETION OF 14C-ITX 7650, A NOVEL ANTI-HCV COMPOUND.

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ITX 7650 is a novel anti-hepatitis C virus entry inhibitor in nonclinical development with a favorable safety profile based on acute and chronic exposure studies. The study objectives were to determine the route of excretion, distribution and metabolite profiles of 14C-ITX 7650. Male and female Sprague Dawley rats were administered a single dose of 10 mg/kg intravenously (iv) or 30 mg/kg by oral gavage (po), and tissues (brain, kidney, liver, lung and spleen), urine and feces were collected up to 24 (iv) or 48 (po) hr post dose for analysis of total radioactivity. The cumulative percent of total dose recovered in the urine and feces was comparable for the iv group (7-16%). More radioactivity was recovered in the feces (36-45%).
than the urine (13-15%) for the po group. The general rank order of tissue concentrations was liver > lung > kidney > spleen > brain, and concentrations were commonly highest at the first time points (1 hr for iv and 2 hr for po). Peak liver concentrations were -31 and -71 μg/g for the iv and po groups, respectively, which represented ~10-10% of the total dose. Brain concentrations (< 5 μg/g) were ~50% less than those in plasma. Blood radioactivity was lower than plasma at all time points; however, the plasma to brain ratio was about 3.6 in the iv group. Total recovery of radioactivity was 83-87% and 79-83% for the iv and po groups, respectively. The primary metabolites in plasma, urine, and feces were M6, M1, and M8, respectively, out of the 14 putative metabolites identified. M1 is likely to be a highly polar, possibly conjugated, metabolite. 14C-ITX 7650 was also extensively metabolized in vitro with distinct species differences between rat, dog, Cynomolgus Macaque, and human liver microsomes. In summary, liver was important for the accumulation, extensive metabolism and elimination of 14C-ITX 7650. Radioactivity distributed well to other tissues, and was excreted as ITX 7650 and polar, possibly conjugated, metabolite. 14C-ITX 7650 was also extensively metabolized in plasma and females had greater plasma exposure. The recovery of radioactivity was 83-87% and 79-83% for the iv and po groups, respectively.

1883 ENHANCED DELIVERY OF INTRASNALEY-ADMINISTERED NUCLEOSIDE DRUGS TO THE CENTRAL NERVOUS SYSTEM.

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Delivery of therapeutics to the brain to treat neurological diseases is a challenge due to impenetrable nature of the blood brain barrier (BBB). Intranasal (IN) drug administration is a non-invasive approach for rapid direct delivery from the nasal cavity to the central nervous system (CNS), thereby minimizing systemic exposure. The current study focuses on the use of IN route for delivery of the nucleoside drug gemcitabine (GEM). GEM is similar in structure to zidovudine (AZT), and AZT can be delivered to the brain by the IN route. In order to enhance drug delivery to the CNS, we used papaverine (PV), which has been demonstrated to transiently increase the permeability of the blood-tumor barrier and BBB after systemic administration. We hypothesize that by transiently increasing the permeability of nasal epithelial tight junctions using PV, we will increase the concentration of GEM in the brain extracellular fluid (BECF) following IN administration, with the goal of delivering therapeutic concentrations of nucleoside drugs to treat brain tumors. Experimental methods include in vivo brain microdialysis for BECF collection, in-vitro GEM recovery, HPLC analysis to measure GEM concentrations in BECF, and IN administration of fluorescein isothiocyanate-dextran beads (FD4) to determine IN drug distribution. A non-toxic dose of PV, which enhanced delivery of GEM to BECF, was determined. BECF pharmacokinetics of GEM shows AUC=5.5±0.84 ng.h/ml for PV (1%) + GEM (50mg/kg) treated animals (n=4), compared to 1.5±0.2 ng.h/ml without PV treatment (n=4). The IN drug concentra
tion in BECF was comparable to the AUC values when GEM and another BBB permeabilizer (RMP-7) were administered intravenously. Preliminary studies with FD4 beads showed significant deposition in the olfactory epithelium (OE), indicating drug uptake through OE. Thus, it appears that transient permeabilization of nasal epithelial tight junctions provides a non-invasive means to enhance delivery of nucleoside drugs to the CNS.

1884 STUDY ON TRANSPORT KINETICS OF VENLAFAXINE INTO RAT BRAIN ACROSS BLOOD-BRAIN BARRIER.


Objective: To evaluate the kinetics of venlafaxine transport into brain across blood-brain barrier in rats. Methods: In situ bilateral brain perfusion technique was used to examine brain venlafaxine uptake in male in ar rats. The concentration of venlafaxine in the perfusate was 0.5, 2.5 and 12.5 mg/L, respectively. The brain was harvested and homogenized at the end of the set perfusion time of 2.5, 8 and 10 min, respectively. And the concentration of venlafaxine in the brain parenchyma was analyzed by HPLC. Results: Venlafaxine can get across blood-brain barrier into brain parenchyma. The concentration of venlafaxine in brain increases with perfusion time and venlafaxine concentration in the perfusate. Brain unidirectional transfer constant (K) values for venlafaxine at different perfusion concentration were 1.13, 0.53 and 0.31 ml/min/g, respectively. The values diminished with the increase of the venlafaxine concentration in the perfusate. Conclusion: The transport of venlafaxine across blood-brain barrier accords with the model of passive diffusion in a membrane-limited rate.

1885 PHARMACOKINETICS AND BIOAVAILABILITY OF RIFAMPIN IN RATS.

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Rifampin is one of the antibiotics being tested for use as a potential countermeasure for biological threats. Pharmacokinetic data of its high doses in rats is not readily available. Drug exposure of rifampin was examined in 6 female and 6 male cannulated Sprague Dawley rats following single oral or intravenous dose at 10 mg/kg. Blood samples were collected and analyzed by a validated LC-MS/MS method. The volume of distribution (Vd) of this lipophilic drug was large (1.39 L/kg) indicating distribution to many tissues. The terminal elimination half-life following IV and oral dose was about 3.6 hr and 4.2 hr, respectively. The oral absorption of the drug was relatively fast (1.5 hr in females, 3 hr in males). The bioavailability of rifampin after single oral dose was 90.5% in males and 74.5% in females. The average systemic exposure after oral administration was 26723 and 34758 ng/hr/ml in males and females, respectively (Cmax = 2753 and 4546 ng/ml, respectively). A multiple dose study was conducted with rifampin at 30 mg/kg, for 5 days with sampling on days 1 and 5. Cmax in males was higher on day 5 vs day 1 (12005, 5 mg/ml and 14467.9 mg/ml on day 1; 14510.2 and 11797.0 mg/ml on day 5 for males and females, respectively), and systemic clearance was almost equal, but Vd was lower on day 5 for the males. A reverse trend was noted for females, wherein the Cmax was lower on day 5 vs day 1, but the systemic clearance was higher on day 5. The AUC0-inf values were 102226.4 and 126705.7 ng/hr/ml on day 1 and 106766.9 and 89392.9 ng/hr/ml on day 5 for males and females respectively. Bioavailability following 5 days of dosing was 92.6% and 83.1% in males and females, respectively. Thus, multiple dosing did not extensively alter rifampin pharmacokinetics in rats and the three-fold higher dose administered for 5 days was not reflected in increased oral bioavailability of the drug. This information will be useful to design further efficacy studies with rifampin in rats.

1886 KINETICS OF LEUCINE AND OTHER AMINO ACIDS IN THE LONG EVANS MALE RAT.


One of the essential amino acids (AAs), leucine, plays a role in enhancing cognition and memory in animals and man. Leucine administration leads to cognitive improvement after 24 h in the rodent, suggesting enhancement of normal functioning under stress conditions. However, the kinetics of leucine is not well characterized. Most published studies involve uptake of leucine-containing drinking water or diet, and tissue concentrations are only measured at terminal time points. The objective of this study was to collect tissue concentrations of leucine in Long-Evans (LE) male rats in order to develop a physiologically-based pharmacokinetic (PBPK) model. Such a PBPK model, once validated, will allow extrapolation of leucine kinetics across dosing regimens (drinking water, diet) and across species. Ultimately the goal will be to predict potential brain leucine concentration changes in humans. In order to fill data gaps and develop a model, leucine was administered intravenously, and tissues from both time-course and dose-response studies were collected. Leucine (5 & 12.9 mg/kg) was dosed via jugular vein catheter and tissues were collected at a number of time points between 2min and 6h. Preliminary results revealed that leucine is eliminated from the blood very quickly after iv dosing (5mg/kg), with only 7% of injected leucine detected still in blood at 5 min, while at 6 hr post dosing it was down to base line levels. Leucine levels in the brain were higher than blood for both dosing levels, indicating active transport of leucine across the blood-brain-barrier (BBB). Of the 16 other AAs measured in brain, about 2/3 were increased, even at very early times (<2 min) after iv leucine injection. Most of the AAs that either remained unchanged or were decreased in brain are known to be transported across the BBB by the L1 large neutral AA transporter and therefore subject to competition with leucine.

1887 DISPOSITION AND METABOLISM OF β-N-METHYLAMINO-L-ALANINE IN SPRAGUE DAWLEY RATS AND B6C3F1 MICE.

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β-N-methylamino-L-alanine (L-BMAA) was nominated to the National Toxicology Program (NTP) by the National Institute of Environmental Health Sciences (NIEHS) for comprehensive toxicological assessment based on potential widespread distribution in the environment, possible presence in certain dietary

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supplements, and evidence that the chemical is neurotoxic. Data describing absorption, distribution, metabolism and excretion (ADME) are needed to support NTP toxicity studies. These initial studies were conducted by oral dose administration to male Sprague Dawley rats at 1, 10, and 100 mg/kg and to male B6C3F1 mice at 10 and 100 mg/kg. The majority of the 14C labeled L-BMAA radioactivity was measured in expired air with up to 61 % recovered as 14CO2 after 24h. This high level of expired 14CO2 was similar between both rats and mice and across the low, mid, and high doses. By 24 hr, approximately 8.3 % and 12.7 % of the 14C-L-BMAA radioactivity was excreted in urine by rats and mice, respectively. Excretion in feces accounted for only 2.3 % and 3.7 % for rats and mice, respectively. A higher % of the dose was recovered in tissues for rats (19%) than for mice (8%) by 24 hr (p<0.05). The majority of the 14C recovered in tissues was primarily found in liver (4.2 and 2.8%), muscle (4.0 and 2.4 %) and skin (3.0 and 1.1 %) for rats and mice, respectively. The tissue burden in the brain was less than 0.2% for both rats and mice. Accumulation of L-BMAA in tissues was investigated by 5 consecutive daily low doses (1 mg/kg). Initial assessments show evidence for tissue accumulation in multiple tissues including the brain; however, it is not yet clear if this accumulation is due to tissue binding or is clearance rate limited. Chromatographic profiles of 14C in urine showed only very polar Phase I metabolites with no unchanged L-BMAA present. This work was conducted for the NTP under NIEHS Contract N01-ES-75562.

1888 INHIBITION OF HUMAN BREAST CANCER-RESISTANT PROTEIN (BCRP) TRANSPORTER BY ENDOCRINE-DISRUPTING CHEMICALS.

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Over the last decade, the scientific community has voiced increasing concern regarding disruption of endocrine and reproductive systems as a consequence of in utero exposure to xenobiotics. These endocrine-disrupting chemicals (EDCs) are quite diverse and include plasticizers, mycotoxins, phyoestrogens, and pesticides. The breast cancer resistance protein (BCRP) is a chemical efflux transporter expressed in the placenta as well as the developing fetus. Inhibition of the fetotrophic function of BCRP may be a mechanism involved in developmental toxicities following EDC exposure. The purpose of this study was to characterize EDCs as potential substrates and inhibitors of the BCRP transporter. Two in vitro screening assays, the BCRP ATPase assay and inverted membrane vesicle assay, were used to test interactions between BCRP and five chemicals with potential for endocrine disruption: bisphenol A, genistein, methoxychlor, prochloraz and zearalenone. The order of inhibitory potency was genistein > zearalenone = methoxychlor > prochloraz > bisphenol A. Cell-based studies are needed to further characterize the inhibitory kinetics of EDCs on BCRP-mediated transport. These findings may assist in risk assessment of perinatal exposure to these chemicals. Supported by ES-005022, DK-080774, ES-020522.

1889 INVESTIGATION OF THE METABOLISM OF 1,3-DICHLORO-2-PROPANOL TO EPICHLOROHYDRIN IN VIVO IN MALE HARLAN SPRAGUE DAWLEY RATS.

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1,3-Dichloro-2-propanol (DCP) is an industrial intermediate used in the production of epichlorohydrin (ECH), 1,3-dichloropropene, and 1,2,3-trichloropropene. ECH is a carcinogen in rats and causes tumors in the nasal passages following inhalation exposure, and in the forestomach following oral administration. ECH has been proposed as an intermediate in the metabolism of DCP in vivo. The objective of this study was to evaluate the metabolism of DCP to ECH in vivo. DCP (50 mg/kg) or ECH (1 and 25 mg/kg) was administered by gavage to male Harlan Sprague Dawley rats. Serial blood samples were collected at various time points between 5 minutes and 6 hours after dosing, and analyzed by GC-MS to obtain the blood concentrations of DCP and ECH. Blood concentration time data were analyzed using non-compartmental analysis. Following gavage administration of DCP, the half life of DCP was 42 min with a Cmax of 335 μM, whereas for ECH, the half life was 33 min with a Cmax of 0.073 μM. Following oral administration of ECH at 4 mg/kg, ECH was not detectable in blood, with an LOQ of 0.01 μM. However, at 25 mg/kg ECH, blood levels of ECH were readily detectable between 5 and 60 min after dosing, with a Cmax of 0.76 μM, and a half life of 34 min. Following administration of 50 mg/kg DCP AUC0-∞ for ECH was 4.10 μM.min/mL. Following administration of 25 mg/kg ECH, AUC0-∞ Dose was 43.1 μM.min/kg/mol. The dose of ECH in DCP-treated animals can be estimated as 4.10/43.1 mmol/kg, which is equivalent to 0.095 mmol/kg. On a molar basis, the amount of ECH derived from the metabolism of a dose of 50 mg/kg (0.386 mmol/kg) DCP indicates that there is 24.6% conversion of DCP to ECH in vivo in this study. This work was conducted for the NTP under NIEHS Contract N01-ES-75563.

1890 DETERMINATION OF N-BUTYLPARABEN AND METABOLITES IN HARLAN SPRAGUE DAWLEY RAT PLASMA, AMINIOFLUID, FETAL TISSUE, AND PUPS.

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n-Butylparaben (butyl 4-hydroxybenzate, BPB) is one of the parabens widely used as antioxidants and preservatives in foods, pharmaceuticals, and cosmetics. BPB is under investigation by the National Toxicology Program for potential reproductive and developmental effects. The objective of this study was to develop an assay to quantitate BPB and its metabolite 3-hydroxybutylparaben and their glucuronide and sulfate conjugates in support of a study investigating the maternal transfer of BPB in Sprague Dawley rats. Samples were obtained from dams and offspring administered BPB in the diet at 0, 5000 or 4000 ppm. Samples were analyzed by liquid chromatography/mass spectrometry (LC/MS) and quantitated by a sensitive LC/MS-MS method. The purpose of this study was to characterize EDCs as potential substrates and inhibitors of the BCRP transporter. Two in vitro screening assays, the BCRP ATPase assay and inverted membrane vesicle assay, were used to determine the levels of free and total BPB and 3-hydroxybutylparaben. Samples were analyzed by LC-MS/MS with 13C2 BPB as internal standard. At 0 ppm exposure, analytes in all matrices were below the limit of quantitation of 1 ng/mL. In all samples, free and total BPB increased with increasing BPB concentration in the diet. In maternal plasma, the majority of the BPB was conjugated. Both free and total BPB and 3-hydroxybutylparaben were elevated in GD 18 fetal tissue and amniotic fluid from exposed dams. In PND 4 pups, free BPB was higher than conjugated BPB. BPB and its catechol metabolite, 3-hydroxybutylparaben were present in free and conjugated forms in maternal plasma, amniotic fluid and fetal tissue from GD 18 and PND 4 pup carcass. Samples were prepared for analysis with and without incubation with β-glucuronidase/sulfatase, to determine the levels of free and total BPB and 3-hydroxybutylparaben. Samples were analyzed by LC-MS/MS with 13C2 BPB as internal standard. At 0 ppm exposure, analytes in all matrices were below the limit of quantitation of 1 ng/mL. In all samples, free and total BPB increased with increasing BPB concentration in the diet. In maternal plasma, the majority of the BPB was conjugated. Both free and total BPB and 3-hydroxybutylparaben were elevated in GD 18 fetal tissue and amniotic fluid from exposed dams. In PND 4 pups, free BPB was higher than conjugated BPB. BPB and its catechol metabolite, 3-hydroxybutylparaben were present in free and conjugated forms in maternal plasma, amniotic fluid and fetal tissue from GD 18 and PND 4 following exposure of dams to BPB in the feed. BPB crosses the placenta during rat gestation and is transferred to the pup during lactation. This method will be used to quantify the maternal transfer of butyl paraben in Harlan Sprague Dawley rats. This study was conducted for the NTP under NIEHS Contract N01-ES-75563.

1891 ABSORPTION, DISTRIBUTION, METABOLISM, AND ELIMINATION OF DBNPA IN CD RATS.


2,2-Dibromo-3-Nitrilopropionamide (DBNPA) is widely used as biocide in a variety of industrial processes to control algae, bacteria, fungi and yeast. To better understand the overall pharmacokinetic and metabolic fate of this compound, a new ADME study was conducted in CD rats via both oral gavage dosing and dermal application. Overall, 14C-DBNPA was well absorbed following oral gavage, with 87.2% of the total dose recovered in urine. The elimination of test material-derived radioactivity from plasma was substantially faster than the bromide, indicating rapid hydrolysis of both bromine atoms of DBNPA either prior to absorption in the GI tract or following first pass hepatic metabolism. This is consistent with rapid de-bromination seen in vivo in blood. The pharmacokinetic data for both 14C (radioactivity) and bromide fit well to 2-compartment models. The major metabolite of DBNPA was the di-debrominated cyanooxacetamide which accounted for 69-74 % of the total dose. No brominated metabolites were detected via sensitive LC/MS-MS analysis. Dermal absorption was fairly low, with 11.4% of the applied dose was recovered in excreta, tissues and expired air. An additional 33.7% was recovered in application-site skin, which may represent absorbable test material. Final radioactivity in all tissues was less than 1% of the total administered dose via oral route and
1892 COMPARISON OF IN VITRO DERIVED AND SCALED IN VITRO METABOLIC RATE CONSTANTS FOR SOME VOLATILE ORGANIC COMPOUNDS (VOCs).

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The reliability of physiologically based pharmacokinetic (PBPK) models is directly related to the accuracy of the metabolic rate parameters used as model inputs. When metabolic rate parameters derived from in vivo experiments are unavailable, they can be estimated from in vitro data. In vitro data are scaled up for use in PBPK models on the basis of mg of microsomal protein per gram of liver, liver weight and body weight. We compared values of VmaxC derived from in vivo vapor uptake studies with estimates of VmaxC scaled up from in vitro hepatic microsomal metabolism studies for four VOCs for which data were available in male F344 rats. For two of the four VOCs, agreement between the in vitro and scaled up VmaxC estimators differed by less than 2-fold; for 1,1-dichloropropene the metabolism studies for four VOCs for which data were available in male F344 rats. For studies with estimates of VmaxC scaled up from hepatic microsomal in vitro metabolism, the corresponding VmaxC were 3.25 and 5.5 mg/hr-kg. For bromodichloromethane (BDCM), the vapor uptake studies (compared to 1,1-dichloropropene), the values of VmaxC were significantly higher (approximately 5-fold higher than the in vitro derived and scaled up VmaxC (12.8 vs. 2.65 mg/hr-kg). For bromodichloromethane (BDCM), the in vivo derived VmaxC was approximately 5-fold higher than the in vitro derived and scaled up VmaxC (12.8 vs. 2.65 mg/hr-kg).

1893 HUMAN PBPK MODELING OF BENZENE INHALATION BASED CHINESE WORKER URINARY METABOLITE DATA: COMPARISON OF HUMAN AND MOUSE METABOLISM.

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A physiologically based pharmacokinetic (PBPK) model of benzene inhalation based on a recent mouse model was adapted to include bone marrow and urinary bladder compartments. Empirical data on human liver microsomal protein levels and CYP2E1 activities per gram protein were incorporated into the model, and metabolite-specific conversion rate parameters were estimated by fitting to human biomonitoring data including measurements in breath, blood, and urine. Data in the same subjects on benzene levels in blood and breath, and phenol levels in urine following short-term benzene inhalation were used to calibrate the rate of human conversion of benzene to benzene oxide (BO). Recent biomonitoring data on average urinary benzene metabolites from Chinese worker populations with occupational benzene exposure were used to calibrate the rate of human conversion of benzene to muconic acid (MA) and phenylmercapturic acid (PMA), and of benzene oxide to phenol (PH), catechol (CA), hydroquinone (HQ), and benzenethiol (BT). The calibrated human model reveals that while liver microsomal protein levels on average are higher in humans compared to mice, the CYP2E1 activity per gram protein is far lower on average in humans. Relative to humans, the mouse shows far lower rates of benzene conversion to MA and PMA, and far higher conversion rates of benzene to BO/PH, and of BO/PH to CA, HQ, and BT. The Chinese worker data for unexposed controls show appreciable background contributions to urinary PH, CA, HQ and BT, but not MA or PMA; these findings obscure the metabolic rate trends at benzene exposures below 1 ppm. Human data on the range of microsomal protein levels and CYP2E1 activity provide an objective basis for evaluating uncertainties associated with inter-individual variability. Given the differences in mouse and human metabolism of benzene, a calibrated human PBPK model with a bone marrow compartment may be more useful than mouse-based models in understanding human pharmacokinetics of benzene inhalation.

1894 INVESTIGATING COMPLEX PHENOTYPES: HAPLOTYPE ASSOCIATION MAPPING BENZENE PHARMACOKINETICS IN ISOGENIC MOUSE STRAINS.

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A panel of 18 genetically diverse inbred mouse strains was used to determine the range of total exposure kinetics in blood and bone marrow following a single oral administration of benzene (100 μg/kg) to male and female mice. Large ranges of variations in both blood and bone marrow were observed in the pharmacokinetic parameters for total exposure to benzene and its metabolites. Pharmacokinetic parameters in blood were not necessarily predictive for bone marrow pharmacokinetics. Protein and mRNA expression data for primary benzene-metabolizing enzymes CYP2E1 and UGT1A6 showed very little strain-dependent variation. Differences in mRNA levels of NQO1 and MPO were small but statistically significant, as were those for GAPDH and β2-microglobulin. Enzymatic activity has yet to be determined for these proteins. Pharmacokinetic parameters in males were not necessarily predictive for those determined for females. Final clearance (CL_F) was found to be the most statistically robust pharmacokinetic parameter as it accounted for exposure of the matrix (AUC) and normalized for dose variations among the strains. The CL_F values in blood or bone marrow used for haplotype association mapping showed statistically significant (logP=4). Four loci were found to be shared between males and females QTLs were compared for bone marrow. No overlap was found among blood QTLs in males and females. These data are useful for in investigation of genes associated with host susceptibility to toxicity following benzene exposure. This research was supported in part by the NIEHS NTP Grant N01ES45529, NIEHS Toxicology and Toxicogenomics Training Grant (5T32ES007091-29), NIEHS/NTP Division of Intramural Research, and Southwest Environmental Science Center Grant P3ES06694.

1895 DISPOSITION AND METABOLISM OF THE RUBBER PEPTIZING AGENT, 2,2-DITHIOBISBENZYLIDINE, IN SPRAGUE DAWLEY RATS AND B6C3F1 MICE.

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2,2-Dithiobisbenzylidene (DTBBA) is a highly produced chemical used as a peptizing agent in rubber products, especially tires. Although DTBBA contains structural alerts for toxicity, data for a toxicological evaluation of the chemical are lacking. In support of such an effort, the present studies were designed to characterize the fate of DTBBA in animal models. Results demonstrated that [14C]-DTBBA was ab sorbed from the gut of Sprague Dawley rats and B6C3F1 mice following oral administration of up to 400 mg/kg. The urine was a major route of excretion of DTBBA-derived 14C (up to 70% total dose in rats), with most other 14C excreted in feces within 24 hours of administration. Comparison with an iv-dosed (4 mg/kg) group of male rats indicated that at least one-half of the 14C excreted in feces of gavaged rats had been absorbed. Approximately 1% of the total dose remained in tissues in rats and mice at 24 hours post-dosing. The highest concentration of DTBBA-derived 14C was detected in the thyroid of the male rat. In most animals, the liver also contained a tissue-to-blood ratio greater than 1. Reduction of the disulfide bond of the dimeric DTBBA molecule is of toxicological concern and this potential was demonstrated in vitro by the formation of 2-thiobenzanilide and 2-phenylbenzothiole acetone upon incubation of DTBBA with glutathione. Several polar oxygenated metabolites were identified by LC-MS/MS in urine of rats gavaged with 40 mg/kg, Cleavage of the amide bond(s), but not the disulfide bond, was indicated for some of these metabolites. In conclusion, the results of these studies will contribute to future toxicological and safety evaluations of DTBBA. This work was conducted for the NTP under NIEHS Contract N01-ES-75562.

1896 THE QUANTITATIVE IMPACT OF USING BOTH RODENTS AND NONRODENTS IN DEVELOPMENTAL TOXICITY RISK ASSESSMENT.


Since the thalidomide crisis of the 1960’s, the standard practice for evaluating developmental toxicity for chemicals with a high potential for human exposure in pregnant women has included data from more than one species (i.e., rodent and nonrodent). The value of the assumption that two species are needed for an adequate assessment of hazard is a topic of interest for environmental and pharmacutical risk assessment. For food use pesticides, testing in two species is mandated.
Reviews of developmental toxicity data sets for environmental chemicals have previously indicated that either rodent or nonrodent species may be more sensitive to developmental insult. Additionally, gene expression studies have shown that there is a differential profile across species (rat vs. rabbit) for genes expressed during development. This analysis confirms previous findings that the use of a single species, either rodent or nonrodent, in preclinical developmental toxicity testing, is not necessarily predictive of the species may be more sensitive in terms of the effects observed, the relative severity of the effects, or the doses at which effects are found. These differences can impact risk assessment decisions in a qualitative and/or quantitative manner. The quantitative impact on risk assessment of the absence of developmental toxicity data for one of the two test species was examined for a number of environmental toxicants. This information has potential implications for the application of uncertainty factors in risk assessment, when data from only one species are available for characterizing developmental hazard and risk. Additional information, such as mode of action or toxicokinetic data, may be useful in providing information on the relevance of specific animal models in predicting developmental hazard to humans. Disclaimer: The views expressed are those of the authors and do not necessarily reflect the policies or opinions of the US EPA or California EPA.

1897 INVOLVEMENT OF PGE, AND URINE VOLUME INCREASE IN THE ONSET OF DIOXIN-INDUCED HYDROPHRENEOSIS IN MICE.

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a potent teratogen which induces hydrophreonephrosis in the kidney of perinatal rodents. We have previously found that cyclooxygenase-2 (COX-2) has a pivotal role in the onset of hydrophreonephrosis in the neonatal mouse exposed to TCDD. COX-2 is an inducible form of cyclooxygenase, which catalyze the first step in the conversion of arachidonic acid to prostaglandins (PGD2, PGE2, PGF2α, and PGI2) and thromboxane (TXA2). Here, we investigated the role of PGE2, the major prostaglandin in the kidney, and an increase in urine volume as the potential causative factors of TCDD-induced hydrophreonephrosis. C57BL/6 and mPGES-1 (an inducible form of PGE synthase) KO mice were used in the present study. In Experiment 1, mPGES-1 null mice were produced by mating mPGES-1 (+/-) mice. Dams were exposed to 0 or 10 μg/kg b.w. on PND1. Urine volume, hydrophreonephrosis, and urinary PGE level of pups were examined on PNDs 7 and 14. In the absence of mPGES-1 gene, an increase in urinary PGE level and the occurrence of hydrophreonephrosis induced by TCDD were completely suppressed. Urine volume was increased in a TCDD dose-dependent manner. These results showed that mPGES-1 dependent overproduction of PGE, is the cause of TCDD-induced hydrophreonephrosis, and that the increase in urine volume could be another contributing factor for its onset.

1898 EFFECT OF PRENATAL PERFLUOROOCTANOIC ACID (PFOA) EXPOSURE ON POSTNATAL DEVELOPMENT IN HUMANIZED PPARα MICE.

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PPARα is required for perfluorooctanoic acid (PFOA)-induced postnatal lethality. To determine if there is a species difference in receptor activity that might influence this phenotype, the present study examined the effect of prenatal PFOA administration on pre- and postnatal development using wild-type, Pparr-null and humanized PPARα mice. Mice of the same strain were mated overnight, and the presence of a copulatory plug was indicative of pregnancy and considered gestation day (GD) 0. On GD 19, mice were treated daily with water or PFOA (10 mg/kg b.w.) orally until GD 17 and then either euthanized on GD 18 or allowed to give birth and then euthanized on postnatal day (PND) 20. PFOA did not affect maternal weight gain or relative uterine weight but did increase relative liver weight in all three genotypes on GD 18. No changes in average fetal weight, crown to rump ratio or placental weight were observed on GD 18. Expression of the PPARα target gene (Acy1 CoA oxidase [ACO] and cytochrome (P450) 4a10 [Cyp4a10]) mRNA in maternal and fetal liver was increased on GD 18 in wild-type and humanized PPARα but not Pparr-null mice. On PND20, relative liver weight was higher in wild-type mice but not in Pparr-null or humanized PPARα mice. The percentage of mice surviving postnatal insult but not in Pparr-null or humanized PPARα mice. No change in pup weight gain or the onset of eye opening was found between any genotype. Hepatic expression of ACO and Cyp4a10 mRNA was higher in wild-type mice but not in Pparr-null or humanized PPARα mice on PND20. Results from these studies demonstrate a significant difference in the postnatal effects observed following prenatal PFOA exposure in mice mediated by mouse and human PPARα.

1899 DEVELOPMENTAL AND SPECIES-SPECIFIC SENSITIVITY TO PPARα AGONIST-INDUCED HEPATIC EFFECTS.

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PPARα mediates PPARα agonist-induced liver cancer in rodents, but humans are refractory to this effect based in part on the observation that humanized PPARα mice do not develop liver tumors following long-term administration of Wy-14,645. However, the effect of a potent human PPARα agonist has not been examined in mice. Moreover, there is also evidence suggesting that neonates could be more sensitive to the effects induced by PPARα agonists. Thus, the present study examined the hepatic effects of a high affinity human PPARα agonist, GW7647, in wild-type, Pparr-null and humanized PPARα mice following exposure in adults and/or neonatal mice. Exposure to GW7647 caused enhanced hepatomegaly in wild-type mice. These effects were mitigated in Pparr-null and humanized PPARα mice. Hepatomegaly caused by GW7647 was enhanced following perinatal exposure as compared to mice exposed as adults only. GW7647 caused increased hepatic expression of PPARα target genes encoding lipid catalyzing enzymes in wild-type and humanized PPARα mice; these effects were not found in Pparr-null mice. Some differences in expression of lipid-related PPARα target genes were also noted between mice exposed perinatally as compared to those exposed as adults only. These data demonstrate that humanized PPARα mice are resistant to the hepatic effects induced by a potent human PPARα agonist suggesting the hypothesis that humans are refractory to some hepatic effects induced by a potent human PPARα agonist. Additionally, these data suggest that neonates may be more sensitive to these hepatic effects. (Supported by CA124533, CA126826, CA141029, CA140369, FE5017568A)

1900 EXPOSURE TO 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) CHANGES DEVELOPMENTAL REGULATION OF N-METHYL-D-ASPARTATE-RECEPTOR 1 (NR1) SPLICE VARIANTS IN THE DEVELOPING ANTEROVENTRAL PERIVENTRICULAR NUCLEUS (AVPV).

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Developmental exposure to TCDD interferes with masculinization of luteinizing hormone release patterns. Consequently, female-typical LH surge release can be elicited by ovarian hormones in exposed males. LH surge release is regulated by the AVPV, a nucleus that is significantly larger in females. Thus, it is likely that TCDD interferes with processes important for masculinization of this nucleus. The underlying molecular mechanisms responsible for AVPV masculinization are unclear, but various N-methyl-d-aspartate receptor (NR) subunits likely play a role. TCDD effects on expression of NR subunits in the AVPV have not been examined previously, but such exposure alters NR subunit gene expression in developing hippocampus and cortex. Using QPCR, we found that AVPVs of postnatal day 2 (P2D) males exposed to TCDD through lactation (600 ng p.o. to dams on P11) had higher levels of NR1 mRNA than controls. We then used in situ hybridization to confirm expression of NR1 mRNA in the PD2 AVPV, and to examine TCDD regulation of NR1 splice variants in the nucleus. We also examined regulation of splice variants in the sexually dimorphic nucleus of the preoptic area (SD-POA), a nucleus that develops oppositely to the AVPV and is larger in males. We found that the NR1 3A subunit was expressed in the AVPV, while NR1 2A subunit was expressed in SD-PA of control males. TCDD treatment abolished NR1 3A and stimulated NR1 2A expression in the AVPV, but had no effect in the SD-POA. These findings suggest that TCDD interferes with AVPV masculinization by both increasing NR1 expression and controlling splicing of NR1 mRNA. This work was supported by NHLG grant ES013885 to SLP.
The whole embryo culture (WEC) model serves as a potential screening tool for gene expression changes in zebrafish embryos.

**1901 DIRECT EXPOSURE TO DEEPWATER HORIZON CRUDE OIL EMULSIONS ELICITS MORPHOLOGY AND GENE EXPRESSION CHANGES IN ZEBRAFISH EMBRYOS**

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RATIONALE: The 2010 Deepwater Horizon (DH) oil spill, the largest of its type in the history of the petroleum industry, damaged marine habitats and halted commercial fishing in much of the Gulf of Mexico. As the spill continued, polynuclear aromatic hydrocarbon-rich emulsions washed up along the Gulf shoreline. Zebrafish embryos (ZFE), a well-established model for vertebrate development, are very sensitive to a variety of environmental pollutants. METHODS: We assessed short-term developmental responses of ZFE that were exposed directly from 0-48; or, 48-96 hours post-fertilization in ZFE medium to DH-derived emulsions collected from 4 sites along the Gulf (in MS, AL and FL). ZFE were examined for morphological and behavioral changes, and qRT-PCR was used to determine mRNA fold-change values in emulsion-exposed ZFE relative to controls. RESULTS: Similar morphological changes (axial malformations, pericardial and yolk sac edema), altered swimming patterns and enhanced levels of gene expression were observed in samples from all the sites. Among Phase I biotransformation-related genes, up-regulation of cytp1a predominated at all time points. Over time, levels of other Phase I genes (cyp1b1, cyp1c1, 3a65; plus zhr2 and sulubi1) increased. Phase I gene expression levels dropped rapidly after ZFE were removed from the emulsions. In contrast, up-regulation of oxidative stress genes (nqo1, prdx1, hmxox1 and pgmr1l) was enhanced later in development and persisted even if ZFE were placed in emulsion-free media. CONCLUSIONS: DH spill products from along a 200-mile stretch of Gulf of Mexico shoreline were embryotoxic.

**1902 PUBERTAL DELAY IN MALE NONHUMAN PRIMATES (MACACA MULATA) TREATED WITH METHYLPHENIDATE.**

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Juvenile male rhesus monkeys were treated with methylphenidate (MPH) to evaluate the genetic toxicity of the drug. Animals were approximately 2.5 years old at the initiation of the 40-month dosing period, and were dosed orally twice a day with (1) 0.5 ml/kg of vehicle, N = 10 (2) 0.15 mg/kg of MPH increased to 2.5 mg/kg (Low Dose, N = 10), or (3) 1.5 mg/kg of MPH increased to 12.5 mg/kg (High Dose, N = 10). Increases in dose were necessary to achieve clinically relevant serum levels and a five-to-ten-fold increase above the therapeutic range in Low and High Dose groups respectively. After 14 months of treatment, impaired testicular descent and reduced testicular volume were observed in treated animals. Continued evaluation revealed that testicular volume was significantly reduced (p < 0.05) at Months 15 -19 and Month 27 and that testicular descent was significantly delayed (p < 0.05) in the High Dose group. Lower serum testosterone levels were detected in both the Low (p = 0.0017) and High Dose (p = 0.0011) animals through Month 33 of treatment. Our findings indicate that treatment with clinically relevant levels of MPH, initiated prior to puberty, impaired pubertal testicular development until approximately 5 years of age. It was not possible to resolve whether MPH delayed the initiation of the onset or altered the tempo of puberty. Regardless, deficits in reproductive maturation based on the endpoints observed disappeared over the 40-month observation period.

**1903 A TRANSCRIPTOMIC APPROACH TO QUANTIFY DOSE-RESPONSE SENSITIVITY TO PHTHALATES IN WHOLE EMBRYO CULTURE.**

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The whole embryo culture (WEC) model serves as a potential screening tool for in vivo developmental toxicity testing. In this model, cultured rat embryos are exposed during early embryogenesis and evaluated for morphological adverse outcomes.

The integration of molecular-based markers may lead to improved predictability of WEC to determine developmental toxic properties of compounds. Additionally, a mechanistic approach to determine chemical class potency may complement traditional morphological tests due to higher objectivity and sensitivity. In this study, we investigated the effect of two phthalates differing in potency, MEHP and MMP (less toxic), on the transcriptome in WEC, to examine their dose-dependent effects on gene expression in relation with morphology. In a dose-dependent fashion, MEHP induced greater changes in gene expression than MMP in relation with greater changes on morphology. We observed significant enrichment of steroid/cholesterol/fatty acid metabolism and apoptosis pathways within MEHP-induced gene expression alterations associated with developmental toxicity. Specific, primarily upregulated, dose-dependent impacts on genes within steroid/cholesterol/fatty acid metabolism pathways represented the most sensitive markers of MEHP exposure. These pathways have been previously identified as mechanisms of development/reproductive toxicity in vivo and represent more sensitive biomarkers of phthalate exposure than classical morphological endpoints in WEC. This study supports the use of WEC to examine phthalate-induced gene expression and morphological responses relative to in vivo. Furthermore, our results assist in defining the applicability domain of the WEC in determining complementary windows of sensitivity for developmental toxicological investigations.

**1904 EXPLORATION OF POTENTIAL MECHANISMS OF PERFLUOROOCTANIC ACID-INDUCED DEVELOPMENTAL CARDIOTOXICITY IN AN AVIAN MODEL.**

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Perfluorooctanic acid (PFOA) is a persistent environmental contaminant that induces developmental toxicity in laboratory models. We assessed PFOA's effect on heart development in an avian model and observed thinning of the right ventricular wall, including total thickness and thickness of a dense layer of myosin staining. We also noted alteration of multiple morphological and functional parameters measured by echocardiography. One pathway involved in heart development is the bone morphogenetic protein (BMP)-Smad pathway, which may be modulated by an endogenous target of PFOA, the peroxisome proliferator activated receptor alpha (PPARα). We hypothesized that activation of PPARα by PFOA would disrupt inflammatory cytokines and BMP-Smad pathways and contribute to developmental cardiotoxicity. However, quantitative real time PCR in four-day-old chicken embryos treated with 0, 0.5, 1 or 2 mg/kg PFOA prior to incubation did not show changes to these markers. To further investigate the role of PPARα, we also treated fertile chicken eggs with 0, 5 or 25 mg/kg WY 14,643, a known PPARα agonist. Western blot analysis was used to measure pSmad1/5, second messengers of the BMP-Smad pathway, to determine if PFOA agonism by WY 14,643 induced effects similar to PFOA. pSmad1/5 was decreased in the cytoplasm of D4 embryos exposed to PFOA (29.1% in 1 mg/kg and 27.8% in 2 mg/kg, P < 0.05) and increased in the nucleus of D4 embryos exposed to WY 14,643 (18.5% in 5 mg/kg and 29.6% in 25 mg/kg, P < 0.05). The differential response between PFOA and WY suggests that the cardiotoxic effect induced by PFOA is not simply mediated by PPARα agonism or that the BMP-Smad pathway is not altered by PPARα agonism to result in the cardiotoxic effects that we observed. More work with morphological and functional parameters of WY-treated animals is planned to further elucidate the mechanism of PFOA-induced developmental cardiotoxicity in chicken embryos.

**1905 COMPARATIVE ASSESSMENT OF LISTER HOODED AND WISTAR HAN RATS IN REGULATORY DEVELOPMENTAL TOXICOLOGY EVALUATION.**

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The Wistar Han outbred rat is one of the standard strains of rats employed in regulatory developmental toxicity studies. On occasion, other strains are required for use in accordance with specific study objectives, for example, to test a substance in a non-albino strain. The purpose of this assay was to compare control data from pigmented Lister Hooded (LH) and non-pigmented Wistar Han (WH) dams and litters, collected and evaluated from studies conducted in compliance with the principles of GLP regulations and following the ICH S5 or OECD 414 guidelines.
also were not detectably affected by TCDD. Expression of other genes that can affect prostate development via an epigenetic mechanism. (Supported by NIH grants Dnmt1 most of these genes in the developing UGS, but the effects of TCDD were few. The modes of action of PFCs are poorly known. In mammals, have proved to be toxic in various experimental animal models, including chicken embryos. However, the modes of action of PFCs are poorly known. In mammals, some PFCs have been found to interact with peroxisome proliferator-activated receptors (PPARs), which might be a contributing factor to the observed hepatic toxicity. In our study, transcription of a set of PPARα-regulated genes was compared in chick embryos following in ovo exposure to two ubiquitously found PFCs, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), and a known PPARα agonist, GW7647. This study is the first to show transcription of all three isoforms of PPARs (alpha, beta, and gamma) in chicken embryo kidney and liver. Furthermore, we demonstrated for the first time that exposure to a potent PPARα agonist (GW7647) generated a PPARα-regulated response in chicken embryos. Acyl coenzyme A (ACOX) was the only PPARα-regulated gene that was significantly induced by PFOA and no significant induction was found in embryos treated with PFOS. Principal component analysis (PCA) illustrated that the hepatic transcription pattern was more similar between PFOS- and GW7647-treated embryos than between PFOS- and GW7647-treated. The results from our study do not support that activation of PPARα is important for the embryotoxicity of PFOS and PFOS found in experimental studies on chicken embryos. Chronic exposure to arsenic is linked to increased risk for cancers including lung, kidney, bladder, and liver. Non-cancerous ailments such as birth defects, metabolic syndrome, hypertension, and atherosclerosis are also associated with arsenic exposure. Many of the organ systems involved in these diseases require epithelial to mesenchymal cell transition (EMT) to form and maintain tissue homeostasis. For example, development of EMT contributes to neural tube and heart morphogenesis. Disruption in EMT programming caused by environmental toxicants such as arsenic can result in birth defects and predispose for disease in adulthood. However, the mechanism of how arsenic disrupts EMT is not known. We use a primary mouse cardiac epithelial cell line to study the effects of arsenic exposure on developmental EMT. We hypothesized that arsenic disrupts developmental EMT programming causing a deficit in tissue mesenchyme. The expression of defined EMT genes including TGF-beta2, TGF-beta receptor-3, Snail, and Has-2 are decreased in a dose-dependent manner following exposure to arsenic. Low dose arsenic appears to attenuate TGF-beta2 activity, as cells exposed to arsenic show a significant decrease in phosphorylated Smad2/3, which are downstream mediators of TGF-beta2 signaling. This coincides with decreased detection of vimentin-positive mesenchymal cells invading three-dimensional collagen gels. In conclusion, we show that arsenic exposure blocks the developmental EMT process at multiple levels attenuating TGFbeta-2 activity and mesenchymal cell differentiation.

1908 ARSENIC DISRUPTS TGF-BETA-2 ACTIVITY AND EPITHELIAL TO MESENCHYMA TRANSITION. T. Huang1, P. Allison1, D. Broka1, P. Parker1 and T. Camenisch1, 2, 3. 1Pharmacology & Toxicology, University of Arizona, Tucson, AZ; 2Southwest Environmental Health Sciences Center, University of Arizona, Tucson, AZ and 3Steele Children’s Research Center, University of Arizona, Tucson, AZ.

The cardiovascular system is one of the most characteristic and important targets for developmental toxicity by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in fish larvae. Involvement of the aryl hydrocarbon receptor type 2 (AHR2) and its dimerization partner, aryl hydrocarbon receptor nuclear translocator type 1 (ARNT1) in TCDD-induced circulation failure as well as other endpoints of toxicity are believed in developing zebrasfish. However, the following signaling pathway causing edema is largely unclear. This is due to lower reproducibility and sensitivity to chemicals of conventional image analysis of pericardial edema with lateral still image at 72 hours post fertilization (hpf) or later. As heart volume is large in peri-cardial cavity, rate of change is not conspicuous in conventional analysis. Now, we found small cavity between heart and body wall was markedly increased by TCDD in 55 or 60 hpf larvae using high-speed camera (forecardial edema). Concentration dependence of TCDD on forecardial edema formation at 55 hpf was comparable with pericardial edema at 72 hpf. The response to TCDD was sensitive to knockdown of AHR2 and ARNT1 as well as antioxidant. A selective inhibitor of cy-clooxygenase type 2 (COX2), NS398 and thromboxane antagonist (IC50=192,60%) markedly inhibited TCDD-induced forecardial edema. Knockdown of COX2 was also effective for preventing edema formation by TCDD. These results suggest the
common mechanism including of COX-prostanoid signaling pathway could be involved in TCDD-induced foetal cardiac edema and mesenechymal circuation failure in developing zebrafish.

**1910 A COMPARISON BETWEEN RAT EMBRYOS IN VIVO AND WHOLE EMBRYO CULTURE USING TRANSCRIPTOMICS: DEVELOPMENTAL CHANGES, MODEL DIFFERENCES, AND RETINOIC ACID RESPONSE.**

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The whole embryo culture (WEC) model serves as a potential replacement for in vivo developmental toxicity testing. In this model, cultured rat embryos are exposed during early embryogenesis and evaluated for morphological adverse outcomes. Prediction of developmental toxicity may be improved by incorporating toxicogenomic approaches which can be evaluated across multiple parameters (e.g. time, model). In this study, we compared retinoic acid (RA)-exposed and non-exposed Wistar rat embryos derived from WEC (RA, 0.5mg/ml) or in vivo (dams, RA, 50mg/kg, gavage). We assessed changes in expression at the gene and functional level across six timepoints (GD10 + 2-48h) in relation to morphological embryonic changes. In nonexposed embryos in WEC and in vivo, we observed similar changes in gene expression across time in terms of significance, directionality and functionality. Greater changes in the magnitude of specific genes related to development were observed in vivo as compared to WEC at later timepoints (GD10 + 24, 48h) associated with more advanced development in vivo. Additionally, we observed strong similarities in RA-induced response at the gene and functional (e.g. development, cell differentiation) level which associated with common developmental adverse outcomes. Temporal-dependent differences in the magnitude of RA-response in enriched functional groups, including genes critical for RA metabolism (Cyp26a1, Dlx5), suggest kinetic differences in exposure between models. This study supports the use of WEC to examine compound-induced responses relative to in vivo models during this timeframe in development and assists in defining the applicability domain of the WEC in determining complementary windows of sensitivity for developmental toxicological investigations.

**1911 THE EFFECTS OF TRIPTHYLITIN ON THE BONES OF F1 RATS BY EXPOSURE VIA THE PLACENTA AND THEIR DAMS’ MILK AND/OR THEIR FOOD.**

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Osteoporosis is a major public health concern to which environmental chemical compounds may be related. Tripthylitin (TBT), has been used as an antifouling agent and may inhibit the differentiation of osteoblasts in vitro. In our previous study, developmental inhibition among F1 rats exposed to TBT via placenta and their dams’ milk was observed. In this study, whether or not TBT alters bone metabolism of F1 rats by exposure via placenta, dams’ milk, and/or food was evaluated. Pregnant rats were administered 0 and 125 ppm of TBT chloride in their food. After weaning, female F1 rats were fed with normal rodent chow. At 9 weeks of age, the rats were reassigned to groups fed 0 or 125 ppm TBT and further divided into 1 of 4 groups: control-control (CC), TBT-control (TC) exposed to TBT via placenta and dams’ milk; control-TBT (CT) exposed to TBT via food; and TBT-TBT (TT) exposed to placenta, dams’ milk, and food. At 15 weeks of age, the rats’ body weights were checked. The rats’ femurs were sampled and photographed (Softex X-ray) with the standard aluminum scale. The photographs were analyzed by microdensitometry; and the indexes calculated were: bone mineral content, bone mineral density, and cortical thickness. Mean body weights in the TC and TT groups were significantly lower than those in the CC and CT groups. Mean values of bone mineral content, bone mineral density in the CT, TC, and TT groups were significantly lower than those in the CC group. Mean cortical thickness index in the TT group was significantly lower than those in the CC and CT groups. The highest dose reduced intensity of staining and the two lower doses increased α-smooth muscle actin (α-SMA). Compared to control glands, FPOA-treated glands displayed increased abnormal architecture and increased stroma as indicated by thick blue staining around endothelial ducts when stained with Masson’s trichrome. For IHC stains, glands were scored based on intensity of staining in mammary epithelial cells. PFOA did not affect cytokeratin but had a significant effect on α-SMA expression. The highest dose reduced intensity of staining and the two lower doses increased intensity of staining. Additionally, we observed dose-dependent trends for increases in PR and significant dose-dependent decreases in ERα. These preliminary data suggest that PFOA has endocrine disrupting effects on mammary cells and that the effects persist into late adolescence even with low-dose exposures. This abstract does not necessarily reflect NIHES policy.

**1912 DEVELOPMENTAL EXPRESSION OF THE NRF2-RELATED FACTOR (NRF) TRANSCRIPTION FACTOR FAMILY AND REGULATION BY AHR2.**

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The inductive response to reactive oxidants and electrophiles proceeds through a family of Cap’n’collar (CNC) basic leucine zipper (bZIP) transcription factors. Zebrafish have six CNC-bZIP proteins: nuclear factor erythroid-2 (Nfe2), Nfe2-related factor-1a and 1b (Nrf1), Nrf2-related factor-2a and 2b (Nrf2), and Nrf2-related factor-3 (Nrf3). To understand the role of these genes in development, their expression was profiled with qPCR from zero to 120 hpf. nfe2 was maternally deposited, but significant transcription did not begin until 48 hpf and remained relatively constant until 120 hpf. nrf1b, but not nrf1a, was highly expressed during development. nrf2a expression increased steadily during development, while nrf2b was highly expressed throughout, 10-100-fold higher than nrf2a. nrf3, like nfe2, was maternally deposited, but its embryonic transcription turned on earlier (6 hpf). The expression of the nrf gene family upon oxidative stress was also measured following exposure of embryos (48-96 hpf) to the pro-oxidant tBHQ. tBHQ expressed the expression of nfe2 and nrf2 at 96 hpf, but had no significant effect on expression of nrf1a, nrf2a, nrf2b or nrf3. The regulation of these genes during development by the Ahr2 transcription factor was tested using morpholino gene knockdown technology and TCDD as an Ahr2 activator. At 24 hpf, nfe2 was induced by TCDD and both basal expression and inducibility were reduced by knockdown of Ahr2. nrf1a (24 hpf) and nrf2a (48 hpf) were not inducible but their basal expression was reduced by Ahr2 knockdown. nrf1b (24 hpf) and nrf2b (48 hpf) were TCDD-inducible in an Ahr2-dependent manner. Treatment with TCDD or Ahr2 knockdown reduced the expression of nrf3 (48 hpf). These results indicate that members of the nrf gene family are expressed throughout development in zebrafish, are differentially responsive to oxidative-stress, and exhibit different abilities to engage in cross-talk with the Ahr2 signaling pathway.

**1913 LOW-DOSE PRENATAL PERFLUOROOCTANOIC ACID EXPOSURE REDUCES ESTROGEN RECEPTOR-α AND ALTERS α-SMOOTH MUSCLE ACTIN EXPRESSION IN LATE ADOLESCENT FEMALE OFFSPRING.**

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We have previously shown that prenatal low-dose perfluorooctanoic acid (PFOA) exposure delays mammary gland (MG) development in CD-1 female mice. To determine whether these low-dose PFOA-induced MG aberrations persist beyond puberty, time-pregnant CD-1 mice were orally dosed with 0, 0.01, 0.1, and 1.0 mg PFOA/kg body weight daily from gestation days (GD) 10-17 and MGs were collected from female offspring from postnatal day (PND) 7-56. MGs were removed by microdensitometry; and the indexes calculated were: bone mineral content, bone mineral density, and cortical thickness. Mean body weights in the TC and TT groups were significantly lower than those in the CC group. A positive control study was conducted with acetylsalicylic acid (ASA) to support training identification of fetal anomalies. Rats (10-12/group) were administered a single intraperitoneal dose of 0 or 500 mg/kg (5 mL/kg in 0.9% methylcellulose) on gestation day (GD) 9, 10, 11, or 12. A laparohysterectomy was performed on GD 21 and fetuses were weighed and examined for external, visceral,
and skeletal anomalies. Postimplantation loss was increased in the GD 9 and 10 groups (up to 35% vs. controls). Neural tube defects were only 2 live fetuses, which were normal. In the other 6-AN groups, the most severe effects, followed by GD 9, 10, 11, and 12.

Bisphenol A (BPA) perinatal exposure in mice suppresses processes critical to hepatic biotransformation and clearance. A. C. Donnepudi, T. P. Sidd, C. S. Rosenfeld and A. L. Slini, 1University of Rhode Island, Kingston, RI, 2Department of Biomedical Sciences, University of Missouri, Columbia, MO and 3Boud Life Sciences Center, University of Missouri, Columbia, MO.

BPA is a chemical used in plastic manufacturing. Multiple studies in rodents illustrate developmental exposure to BPA results in insulin resistance, adipogenesis, behavior, and physiological changes through multiple mechanisms – including epigenetic modifications. However, none of these studies document how developmental BPA exposure can affect liver function, which is crucial in determining circulating hormone concentrations, chemical detoxification and clearance. BPA is extensively metabolized to BPA-glucuronide and BPA-sulfate by Phase II enzymes and eliminated by ATP-binding cassette (ABC) transporters. Specifically in rodents, Ugt2b1, 2b35 and Sult1a1 metabolize BPA and Abcc2 eliminates BPA-glucuronide from liver. The purpose of this study was to investigate whether perinatal BPA exposure affects hepatic Phase-II and ABC transporter expression involved in BPA metabolism and clearance.


A positive control study was conducted with 6-aminoanidine (6-AN) to support training on identification of fetal anomalies. Rabbits (5/group) were administered a single dose of 6-AN orally by gavage at 0 or 8 mg/kg (8 ml/kg in 0.5% methylcellulose) on Gestation Day (GD) 9, 10, 11, 12 or 13. A lateral hysterectomy was performed on GD 29 and fetuses were weighed and examined for external, visceral, and skeletal anomalies. Aborted embryos were maintained in the groups dosed on GD 9, 11, and 13 (1, 1, and 3 rabbits, respectively). Postimplantation loss was increased in all 6-AN groups (58% vs. 19% in controls). This was primarily due to increased early resorptions in the GD 10 group (2.0 vs. 0.6 in controls). There was a resultant decrease in the number of live fetuses in the GD 9 and 10 groups (7.0 and 12.8 vs. 15.4 in controls). Neural tube defects from neural tube, abdominal wall, and tail anomalies were observed in the GD 9 group, and cleft lip/palate in the GD 9, 10, 11, and 12 groups. Cardiovascular, reproductive, liver, renal, adrenal, spleen, and diaphragm anomalies were observed with the overall group incidence in the order GD 9>10>11>12. The most common anomalies were great vessel transposition, absent aortic arch, ectopic testes/ovaries, abnormal liver lobation, absent diaphragm, and supernumerary kidney (GD 11>9). Axial skeletal development was markedly affected in the GD 9 group with numerous skull, rib, sternbral, and vertebral anomalies, most notably absent and fused structures. In conclusion, ASA administration to rats during major organogenesis resulted in embryolethality and/or malformations, which is consistent with the published literature. A single oral dose on GD 9 produced the most severe effects, followed by GD 10, and to a lesser extent GD 11 and 12.

1917 DEVELOPMENTAL TOXICITY OF LERSIVIRINE IN RABBITS WHEN ADMINISTERED THROUGHOUT ORGANOGENESIS AND WHEN LIMITED TO SENSITIVE WINDOWS OF AXIAL SKELETAL DEVELOPMENT.


Lersivirine is a second generation non-nucleoside reverse transcriptase inhibitor (NNRTI) undergoing clinical development for the treatment of HIV-1. An embryo-fetal development (EFD) study was performed to evaluate the maternal and developmental toxicity of lersivirine in pregnant rabbits. Pregnant New Zealand White rabbits were administered 0, 100, 250, and 500 mg/kg lersivirine by oral gavage once daily on Gestation Days GD 7-19, followed by cesarean section on GD 29 and fetal evaluation. Maternal toxicity was noted at all dose levels (decreased food consumption and body weight gain), with fetal toxicity at 500 mg/kg (decreased fetal weights, increased postimplantation loss). Equivocal findings for axial skeletal malformations were observed in 3 fetuses at 500 mg/kg. To better understand if these findings were related to treatment with lersivirine, a follow-up rabbit EFD study was performed with 1000 mg/kg/day lersivirine (500 mg/kg BID, 12 hour inter-dose interval) for 2 different 3 day windows, GD 8-10 or GD 11-13, which represent the sensitive windows of axial skeletal development in rabbits. Control rabbits were administered vehicle following the same dosing regimen from GD 8-13. Cesarean sections were performed on GD 29, and fetal skeletons were examined for the potential of lersivirine to cause skeletal malformations in rabbits. At maternal exposure levels higher than the initial study, lersivirine did not induce fetal skeletal malformations when administered in the sensitive windows of axial skeletal development. The results of these studies indicate that lersivirine is not teratogenic in rabbits.

1918 POLYCYCLIC AROMATIC HYDROCARBONS INDUCE DISTINCT mRNA EXPRESSION PROFILES IN DEVELOPING ZEBRAFISH.

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Poly cyclic aromatic hydrocarbons (PAHs) are ubiquitous in the environment as components of fossil fuels and by-products of combustion. Increasing levels of PAHs, some of which are carcinogens and developmental toxicants, are a growing health concern. Despite this, toxicological data is scarce for the hundreds of PAHs and substituted derivatives found in the environment. Some PAHs induce toxicity via activation of the aryl hydrocarbon receptor (AhR), while others act through uncharacterized AhR-independent pathways. We employed the zebrafish model to rapidly assess the developmental expression and AhR activation in embryos exposed to parent and oxygenated PAHs during development, from 6-120 hours post fertilization (hpf). CYP1A protein expression was analyzed in whole embryos with immunohistochemistry and served as a biomarker of AhR activation to identify PAHs that induced divergent toxicological responses. Seven oxygen- and parent PAHs (pyrene, benz[a]anthracene, 1,2-benzanthracene, benz[a]pyrene, benz[a]anthracene-7,12-dione, dibenzo[a,h]anthracene, 9,10-anthracquinone and 9-oxo-xanthene) were chosen for comparative mechanistic studies at concentrations that caused malformations, but not mortality at 12 hpf. Differential expression of the elicited (500 nM BDP, 12 hour inter-dose interval) PAH toxicity on the AhR was confirmed with a recently characterized AhR2-null zebrafish line, combined with morpholinos targeting the other two zebrafish AhR isoforms. To further unravel putative mechanisms of toxicity, mRNA expression changes in PAH-exposed embryos were examined with whole-genome microarrays at 24 and 48 hours post fertilization. Unique gene expression changes and ontology profiles were identified, which will be used to distinguish biomarkers of the multiple toxicity pathways induced by PAH exposure. This research is supported by NIEHS grants P30ES00210, P42ES016465 and T32ES07060.
During early vertebrate heart development the heart consists of two layers, the myocardium and the endothocardium. Further development of the heart relies heavily on the formation of the epicardium, which is the outer-most layer of cells surrounding the myocardium. The epicardium is derived from a transient mesothelial organ called the proepicardium (PE). In zebrafish (Danio rerio), static waterborne exposure to TCDD (1 ng/mL; 10 embryos/mL) at 24 hours post fertilization (hpf) blocks the formation of the epicardium. We hypothesized that failure to form the epicardium might be secondary to a TCDD-induced disruption of PE formation. To test this hypothesis we exposed zebrafish embryos to TCDD or vehicle control (Dimethyl sulfoxide; DMSO) and used bright field and video microscopy to score samples (n=15/treatment) for the presence or absence of the PE. From our experimentation it is clear that TCDD inhibits zebrafish PE formation (p<0.01). The loss of the PE is the first cardiac-related morphological alteration observed in the zebrafish. We then hypothesized that TCDD exposure perturbs the expression of genes that are required for normal PE development. In situ hybridization experiments revealed that exposure to TCDD alters the expression of acvrl1, tcf21, wnt1 and tbrx1 during PE development and PE outgrowth. We are currently conducting experiments to determine if zebrafish PE cell migration is altered following TCDD exposure. Cardiac malformations caused by TCDD exposure are not limited to zebrafish embryos. The developing chick (Gallus gallus domesticus) and mouse (Mus musculus) hearts are also targets of TCDD toxicity; therefore we have broadened our investigation and hypothesized that TCDD-induced defects in PE and epicardial development are conserved between the zebrafish, chick and mouse. This work was supported by NIH Grant ES012716 and UW Sea Grant.

Oxygenated polycyclic aromatic hydrocarbons (OPAHs) are produced by incomplete combustion and oxidation of parent PAHs. OPAHs are widely present in the environment and there is suggestive evidence that OPAHs may be more toxic than parent PAHs, making their timely evaluation important. We used a rapid throughput zebrafish developmental toxicity screen to evaluate the toxicity of a structurally diverse set of OPAHs. Dechlorinated embryos were exposed between 6 and 120 hours post fertilization (hpf) to serial dilutions of 40 different OPAHs. Zebrafish were evaluated for mortality and a suite of complex endpoints at 120hpf. In addition, HIC was conducted to evaluate AHR dependence through the induction of the downstream target, CYP1A. Structure-response observations indicated the most toxic class of OPAHs was those with adjacent diones on 6-carbon moieties while adjacent diones on 5-carbon moieties were among the least toxic OPAHs. Multi-ring structures with terminal, para-diones were the second most toxic “class” of OPAHs while the toxicity of multi-ring structures with more centralized para-diones varied considerably. Representative subsets of OPAHs were selected for further analysis based on differential toxicity and AHR-dependent CYP1A expression profiles. Evaluation of early molecular responses to these OPAHs was conducted at concentrations that induce malformations by 120hpf. RNA was isolated from 48hpf embryos and qRT-PCR was conducted using primers for a number of DNA repair and oxidative stress genes important in cellular detoxification and protection from oxidative damage. The Seahorse Extracellular Flux Analyzer was used to measure in vivo oxidative stress in 24hpf zebrafish embryos and preliminary results indicated an OPAH dependent decreased oxygen consumption rate (OCR). These results begin to reveal a structure toxicity relationship for environmentally relevant OPAHs. This research was supported by the NIEHS grant P42ES016465 and P30ES00210.
system and cause toxicity. These studies were aimed at investigating the potential developmental toxicity of quercetin and its molecular mechanism using the embryonic zebrafish model. Zebrafish embryos were exposed to quercetin starting at 6 hpf (0-50 μM). Embryos were evaluated for developmental abnormalities and/or mortality at 24 hpf and 5 dpf. Locomotor activity was recorded both in the presence and absence of visible light in 5 dpf visibly normal zebrafish larvae that were exposed to sub-lethal concentrations of quercetin (0.1 and 1 μM). There was a significant difference in motor activity in zebrafish exposed to 0.1 or 1 μM quercetin in the absence of visible light. In addition, there was a significant increase in developmental malformations and/or mortality following 50μM quercetin exposure. Malformations included curved body axis, reduced growth, pericardial edema and yolk sac edema. When co-exposed to both quercetin and an antagonist of the G protein-coupled estrogen receptor (GPER), embryos were indistinguishable from unexposed embryos and were therefore phenotypically normal. These results demonstrated that quercetin is developmentally toxic and that GPER plays a role in the toxicity mechanism. These studies were sponsored by NIH P30ES020210 and 1R21ES018970.

1924 MTBE ANTI-ANGIOGENESIS IN ZEBRAFISH EMBRYOS (DANIO RERIO) IS CAUSED BY THE DYSREGULATION OF THE HIF1-ALPHA-VEGF PATHWAY.

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Regulation of vascular endothelial growth factor (VEGF) in zebrafish embryos occurs primarily through hypoxia inducible factor 1 alpha (HIF1α), which is regulated by prolyl-4-hydroxylases (PHDs) and von Hippel-Lindau (VHL). Hydroxylation of HIF1α by PHDs under normoxic conditions is the marker for VHL binding, a component of the ubiquitination system. To test the hypothesis that methyl tert-butyl ether (MTBE) anti-angiogenesis is caused by a dysregulation of the HIF1α-VEGF pathway, we used plasmid containing VEGF (pVEGF) injections to rescue vegf expression [study 1], N-oxalylglycine (N-OG) to chemically inhibit PHD hydroxylase of HIF1α [study 2], and a VHL morpholino to knockdown translation and block HIF1α ubiquitination [study 3]. Embryos were injected at the 1-4 cell stage with 80ng of pVEGF for study 1, and exposed to either 0 or 5mM MTBE at 1k cell stage until 21somites. Embryos treated with MTBE exhibited a decrease in the mRNA expression of vegfa, vegfc, flk1, mmp9, mmp2, mmp9p, and wnt3a (ranging from 0.3-0.8 fold), while pVEGF injected embryos exposed to MTBE exhibited control-level expression or greater (1.07-4.86 fold). In summary, these studies suggest that the vascular lesions caused by MTBE are a result of a dysregulation of the HIF1α-VEGF pathway.

1925 METABOLOBICS-BASED COMPARISON OF HUMAN EMBRYONIC AND INDUCED PLURIPOTENT STEM CELLS TO PREDICT DEVELOPMENTAL TOXICITY.

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Birth defects are the largest cause of infant morbidity and mortality in the United States and teratogens are responsible for 5-10% of all birth defects. The majority of preclinical efficacy and toxicity testing of pharmaceuticals are currently performed using animal models, which show only 62% concordance to humans. We have developed the first all-human in vitro developmental toxicity screen that utilizes human embryonic stem cells (hESCs) and metabolomics to discover biomarkers of developmental toxicity. Induced pluripotent stem cells (iPSC) are being investigated worldwide as a more ethically attractive alternative to hESCs. We measured the secreted metabolites across three hESC and two iPSC lines using liquid chromatography mass spectrometry (LC-MS), to determine the metabolic differences between these cell types. Additionally, we exposed each cell line to 23 compounds of known teratogenicity to test the hypothesis that iPSCs exhibit a similar response to that of hESCs upon exposure. Cell viability assays were performed to measure the cytotoxicity of the dosed compounds and dose levels were adjusted to avoid overt toxicity. The secretomes of the iPSCs was identified and differential small molecule features were identified using statistical analysis. These features were annotated using Stemina’s in house metabolite database and were used to create a predictive model of developmental toxicity in iPSC line 19.9.7T. Both comparisons between three hESCs and two iPSC cell lines show little difference in the composition of their secretomes, indicating that extracellular environments produced by iPSC and hESCs are similar. The small molecules and pathways altered in response to teratogen treatments of line 19.9.7T are reported.

1926 COORDINATED DOWN-REGULATION OF HEPATOBILIARY TRANSPORTERS IN PREGNANT MICE.

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Xenobiotic transporters regulate maternal-fetal disposition of chemicals. Changes in maternal transporter expression may influence fetal drug exposure and susceptibility to developmental diseases. The objective of this study was to determine the temporal mRNA and protein profiles of uptake and efflux transporters in livers from pregnant mice and time-matched non-pregnant (non-P) control livers. C57BL6 mice (n=3 to 4/group/day) on gestational days (GD) 7, 11, 14, 17, and postnatal days (PND) 1, 15, and 30. As early as GD-7, mRNAs of Bcrp and Abcg8 in livers of pregnant mice were decreased 30-50% of virgin controls. The mRNAs of most transporters were reduced in pregnant mice between GD-11 to 17, with maximal mRNA down-regulation on GD-14. Compared to virgin controls, the mRNAs of uptake transporters Tnct, oatpl1α4, oatpl1b2, Oct1 and Ent1 were decreased by approximately 50%. During pregnancy, the mRNAs of both canaliculai efflux transporters (Bsep, Mrp2, Bcrp, Mdr2, Abcg5/8, Abt8b1, and Mate1) and sinusoidal efflux transporters (Mrp3, Mrp6, and Abca1) were decreased. Western blot and immunohistochemistry staining confirmed the down-regulation of Mrp2, 3, 6, Bsep, and Ntcp proteins. One day after parturition (PND-1), the mRNA expression of many uptake and efflux hepatobiliary transporters remained low. By PND-30, the mRNAs of all transporters returned to values comparable to virgin controls. Collectively, these data demonstrate a time-dependent down-regulation of many xenobiotic transporters in livers of pregnant mice. Further research is required to elucidate whether reduced expression of hepatobiliary transporters in mid to late pregnancy alters disposition and fetal exposure to chemicals. (Supported by ES-05022, DK-080774, ES-020522).

1927 EARLY EMBRYOGENESIS IS HIGHLY SUSCEPTIBLE TO TDCPP-INDUCED DEVELOPMENTAL TOXICITY.

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Following phase-out of PentaBDE in 2004, tris(1,3-dichloro-2-propyl)phosphate (TDCPP) - an organophosphate (OP) ester flame retardant (FR) - has been extensively used as an alternative FR for low-density polyurethane foams used in furniture. Similar to concerns about OP insecticides, exposure to TDCPP within residential and commercial indoor environmental media (e.g., air or dust) may pose a health risk to fetuses, infants, and children. However, little is known about the potential developmental effects of TDCPP. In this study, zebrafish embryos were continuously treated to water-borne TDCPP using the following exposure scenarios: 1) 2- to 64-cell-stage (0.75 to 2 hrs post-fertilization (hpf)), followed by incubation within vehicle control water to 24-h post-hatch (96 hpf); 2) 2-cell-stage (0.75 hpf) to 96 hpf; and 3) 50% epiboly (5.25 hpf) to 96 hpf. TDCPP and bis(1,3-dichloro-2-propyl)phosphate (BDCCP, a primary metabolite) concentrations within whole embryos were quantified using LC/MS-MS. While exposure to TDCPP from 50% epiboly to 96 hpf resulted in a concentration-dependent increase in teratogenesis (LC50 = 3 μM), TDCPP exposures initiated at the 2-cell-stage, or exposures restricted to the cleavage period (2- to 64-cell-stage) alone, resulted in a 3-fold decrease in the concentration required to induce a similar magnitude of teratogenesis (LC50 = 3 μM) compared to exposures initiated at 50% epiboly. This window of sensitivity was likely not due to differences in TDCPP uptake, yolk sac retention, or increased metabolism since (1) TDCPP concentrations within continuously
These findings suggest that lithium may protect against ketamine-induced neuroapoptosis. Lithium in-
termediates phosphorylation of an N-terminal serine, Ser9. Given these observa-
tions, we hypothesize that lithium protects against ketamine-neuroapoptosis by in-
mediating phosphorylation of an N-terminal serine, Ser9. Given these observa-
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mediating phosphorylation of an N-terminal serine, Ser9.
ethanol-induced teratogenesis in mouse embryos in vitro. We therefore tested the potency ofAsian ginseng (PG) as an antialcoholic agent using medaka (Oryzias latipes) as an animal model. Previously we have demonstrated that medaka embryos exposed to ethanol developed microcephaly with neurocranial cartilage deformities which are analogous to human FASD phenotypes. Moreover, chondrification in many cartilages, especially in ethmoid plate (EP), trabecular cartilages (TC), and polar cartilages were inhibited by ethanol. These inhibitions might be related to cleft palate development, a birth defect observed in a few cases of human FASD phenotypes. Further, we have observed that methanolic extracts of PG root can prevent some of these neurocranial deformities induced by ethanol in medaka. PG consists of many ginsenosides including Re, Rg1, Rb1, Rc, Rb2 and Rg3 as saponins. At present we are evaluating the effects of individual ginsenosides to find the most effective and appropriate compound that can effectively prevent the neurocranial cartilage deformities induced by alcohol in medaka. This study will be able to find an appropriate natural compound that can be used for preventing FASD in human.

1933 CHARACTERIZATION OF MOTOR ACTIVITY OVER THE LIFESPAN IN SPRAGUE DAWLEY AND Wistar HAN RATS.

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Motor activity is an important endpoint in various toxicology studies. Central aims of the present investigation were threefold: to assess the normal ontology of motor activity, to induce behavioral alterations using the known positive controls chlorpromazine (CHLOR; 5 mg/kg) and Methylazoxymethanol (MAM; 20 mg/kg), and lastly, to elucidate possible differences between the Wistar Han (WH) and Sprague Dawley (SD) strains which are preferentially used in Europe and the US, respectively. Three groups were used for each strain. F0-females received saline (Groups 1 and 3) or MAM (Group 2) i.p. on gestation Day 15. Animals littered, and their activity of offspring were tested on postnatal Days (PNDs) 13, 17, 21 and 60. On days of testing, animals were given saline (Groups 1 and 2) or CHLOR (Group 3) prior to testing. Basic, fine and ambulatory movements were automatically quantified over 60-minute sessions.

Control animals had a normal activity profile,habituating over each test period, and with the most activity at PND 60. CHLOR significantly attenuated activity over all ages tested. Conversely, MAM treatment had differential effects on activity depending on the age tested. Activity for MAM-treated animals was below controls at PND 13. However, activity increased over each age, far surpassing controls at PND 60. Finally, CHLOR and MAM treatments had similar effects in both strains. This is particularly interesting since the same animals demonstrated clear differences in MAM sensitivity in a learning and memory test where MAM was ineffective for WH rats, but very effective for SD rats. The similarities in MAM response for both strains supports that differential sensitivity may be mediated by neurobiological systems that were not actively recruited in this behavioral task.

Collectively these data demonstrate the sensitivity ofthe system to capture the ontogeny of normal motor activity over the lifespan, and further characterize potential differences between the two rat strains most commonly used in Europe and the US.

1934 POSTNATAL TRICHLOROETHYLENE MODULATES REDOX STATUS AND OXIDATIVE STRESS IN MOUSE HIPPOCAMPUS.

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A significant proportion of the maternal and pediatric population is exposed to the organic solvent trichloroethene (TCE). The brain, among the best characterized targets of TCE exposure, is immature at birth and highly vulnerable to environmental stress. However, the effects of TCE on developmental neural toxicity in specific brain regions have not been fully characterized. Glutathione (GSH) plays an important role in the detoxification of reactive oxygen species in the brain. Alterations in brain GSH metabolism mediated by xenobiotics might contribute to oxidative stress implicated in the pathogenesis of neurologic disorders including autism. Our goal was to study the impact of the postnatal TCE exposure ‘window’ in mice on mediators involved in oxidative stress and anti-oxidant capacity in the hippocampus, a region of the brain that is abnormal in autism and is involved in many important brain functions. Male offspring were exposed to TCE in the drinking water from postnatal day (PND) 0-42; birth until the juvenile period. Hippocampus was examined for metabolic biomarkers of oxidative stress and anti-oxidant responses to TCE exposure, with emphasis on the ability of TCE to induce a higher percentage of oxidized glutathione associated with a decrease in the intracellular GSH redox status (GSH/GSSG). Neuronal GSH precursors were also altered in TCE exposed mice. Consistent with impaired anti-oxidant capacity, 3-nitroproline, a marker of oxidative stress, was also significantly increased with TCE exposure. This alteration in redox status was associated with behavioral alterations as well as a significant decrease in neurotrophic factors thought to modulate inflammation and oxidative stress. The results suggest that the loss of GSH homeostasis and chronic oxidative stress in the brain may contribute to TCE neurotoxicity following low-level postnatal and early life exposure and may play a role in the development of certain neurodevelopmental disorders.

1935 SUBTHRESHOLD DOSES OF CADMIUM AND ARSENITE COMBINE TO PRODUCE NEURAL TUBE DEFECTS IN C57BL/6J MICE: IMPACT OF THE SPLOTCH ALLELE.


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To test for additivity in the production of neural tube defects (NTDs) by multiple environmental teratogens, the present study established threshold doses for several known teratogenic metals/metalloids and then tested combinations of these compounds at supra- and subthreshold doses. We have previously shown in mice that the sploch (Sp) mutation increases sensitivity to NTDs produced by arsenite (As3+) and cadmium (Cd) -induced birth defects. This study examines gene-environment interactions in the production of NTDs by introducing the sploch allele to observe changes in sensitivity to teratogenic agents. C57Bl/6J wildtype (+/+), mice were mated with other wild types or with C57Bl/6J (Sp/+), in order to introduce the sploch allele. Animals were treated with teratogenic agents i.p on gd 8.0. Supra- and subthreshold doses were determined for arsenite (As3+) and cadmium (Cd). Subthreshold doses did not cause NTDs, significantly reduce fetal weight or significantly increase resorptions. Combination experiments were conducted with supra- or subthreshold doses. The combination of As3+ and Cd was additive with respect to the production of NTDs at both supra- and subthreshold doses. Currently, studies are underway to evaluate the effect of the sploch mutation on NTDs induced by combined subthreshold doses of As3+ and Cd. We have previously shown that As3+ and Cd exhibit the opposite strain sensitivity in C57Bl/6J and SWV mice compared to other NTD-inducing agents. This finding, along with the observation that some teratogens, but not others, display additivity, support the hypothesis that, like strain sensitivity, additivity of teratogenic agents 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.

1936 MICRORNA EXPRESSION PROFILING AFTER DEVELOPMENTAL EXPOSURE OF ZEBRAFISH DANO RERIO EMBRYOS TO VALPROIC ACID.

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Congenital malformations are a prevalent cause of infant mortality in the United States and their induction has been linked to a variety of factors, including exposure to teratogens. However, the molecular mechanisms of teratogenicity are not fully understood. MicroRNAs are an important group of small, non-coding RNAs that regulate mRNA expression. MicroRNA roles in early embryonic development are well established, and their disruption during development can cause abnormalities. We hypothesized that developmental exposure to teratogens such as valproic acid (VPA) alters microRNA expression profiles in developing embryos. Valproic acid is an anticonvulsant and mood-stabilizing drug used to treat epilepsy, bipolar disorder and migraines. Zebrafish embryos were continuously exposed to valproic acid (1 mM) or vehicle control (DMSO) starting from 4 hours post-fertilization (hpf) and sampled at 48 and 96 hpf to determine the miRNA expression profiles prior to and after the onset of phenotypic defects. At 96 hpf, 95% of the larvae showed skeletal deformation, abnormal swimming behavior, and pericardial edema. Microarray expression profiling was done using Agilent zebrafish miRNA microarrays. Microarray results revealed changes in miRNA expression at both the time points.

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Thirteen miRNAs were differentially expressed at 48 hpf and 22 miRNAs were altered at 96 hpf. Among them, six miRNAs (miR-16a, 18c, 122, 132, 457, and 724) were common to both time points. Bioinformatic target prediction revealed that these miRNAs target several genes involved in neurotransmitter synthesis and secretion, histone modifications and liver carcinogenesis. In conclusion, these results suggest that the teratogenic effects of valproic acid could be partly due to altered miRNA expression. [Supported by NIH R21ES017304 and the WHOI summer fellow program]

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Maternal ethanol consumption during pregnancy can produce teratogenicity in offspring, collectively termed fetal alcohol spectrum disorders (FASD). Ethanol can produce metabolic teratogenicity, including insulin resistance and impaired glucose metabolism. Voluntary exercise (VE) has metabolic benefits, including reversing insulin resistance and improving glycemic control. This study tested the hypothesis that VE mitigates ethanol metabolic teratogenicity in the guinea pig. Pregnant Dunkin-Hartley-strain guinea pigs received ethanol (4 g/kg maternal body weight/day throughout gestation) or isocaloric-sucrose-pair-feeding (nutritional control). On postnatal day (PD) 21, offspring were randomly assigned to VE or no intervention. VE animals were placed in a dry-land maze for 30 min daily for 21 days (PD 24-44). Body weight was measured from birth until euthanasia. Fasting blood glucose concentration was measured prior to euthanasia at PD 150-200, when liver and pancreas were collected. Chronic prenatal ethanol exposure (CPEE), compared with nutritional control, produced growth restriction at birth. Female CPEE offspring had accelerated weight gain in puberty (PD 60-90) leading to increased body weight compared with female nutritional control offspring. This CPEE offspring had dysregulated fasting blood glucose concentrations and increased liver weight at PD 150-200. CPEE animals had increased intralobular fat and structural abnormalities in pancreatic islets. These effects were not mitigated by VE. The data demonstrate that this chronic maternal ethanol regimen produces metabolic teratogenicity in the guinea pig, and that VE mitigates CPEE-induced weight gain in female offspring. Supported by CIHR MOP84553 & ELA8022.


Chemical perturbation of vascular development is a putative toxicity pathway which may result in developmental toxicity. EPAs high-throughput screening (HTS) ToxCast program contains assays which measure cellular signals and biological processes critical for blood vessel development. By testing the Phase-I ToxCast chemicals in these assays, and comparing the results to prenatal DT study summary information derived from ToxRefDB, a vascular disruption signature was identified. This signature was correctly observed when the antiangiogenic thalidomide analogue, SHP2-33, was tested in a ToxCast assay subset. There is utility in using targeted in vitro functional assays to explore the potential consequences of chemicals that test positive in the ToxCast program, both for chemicals without DT data (SHP2-33) or as an intermediate tier for DT data comparisons. Therefore, SHP2-33 was tested in rat whole embryo culture (WEC) and in vitro rat aortic explant (AE) cultures. Mid-somite stage rat embryos were cultured in media containing 0, 1.6, 5, 15, 30, or 46 μM SHP2-33 for 48h followed by evaluation for developmental defects. To further confirm the direct effects of SHP2-33 on angiogenesis, rat AE were cultured in media containing 0, 0.46, 4.6, 46, 93, or 247 μM SHP2-33 for four days and the resulting inhibition of microvessel outgrowth was evaluated. In WEC, SHP2-33 caused developmental defects and embryolethality at 15 μM. Consistent with an antiangiogenic mode of action in embryos, SHP2-33 inhibited microvessel outgrowth in cultured AEs at 0.46 μM and completely abolished vesSEL outgrowth at 46 μM with a cell morphology similar to the outcome of cell-agent based in silico models informed by ToxCast data. Data from these targeted functional assays correlated with the in vitro HTS assay data for this vascular disrupting compound. This abstract does not necessarily reflect US EPA policy.

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It is crucial to develop therapeutic approaches for malignant mesothelioma, as well as to obtain information involving the possible mechanism involved in the development of mesothelioma. Thoracotomy was performed to infuse test particles directly into the thoracic cavity of A/J mouse (Experiment 1). Potassium octatitanate fibers, trade name TISMO, and the chemical formula K2OnTiO2, were supplied by Otuka Chemical Co., Ltd. (Osaka, Japan) with dimensions mostly <50μm in length and <2μm. Fiber-shaped particles of TISMO and granular-shaped micro- and nano-size order particles of titanium dioxide (TiO2) were employed (1.5 mg in 0.2 ml saline/inouse). The experiment was terminated after 21 weeks to assess responses. Only the fiber-shaped TISMO, morphologically similar to asbestos, induced a severe reaction of the pleura. Following Berlin blue staining, positive spots were observed around the TISMO, indicative of iron. These positive spots corresponded with mesothelial cells. The results indicate that the risk of mesothelial cell reaction does not depend on particle size, but may depend on shape. The next experiment for 52 weeks was employed (Experiment 2) to examine the long-term effects and weather inducing malignant mesothelioma or not by TISMO infused into the thoracic cavity of A/J mice. This experiment showed there were several TISMO fibers in the alveoli, indi-
Ozone is a highly pervasive environmental pollutant that threatens the health of children, a sensitive subpopulation. Recent studies have found that this may be mediated by disrupted airway growth and innervation. We hypothesized that exposure to ozone alters the normal expression of serotonin, its transporters and two key receptors involved in the mechanism of methanol embryopathies. (Support: CIHR, Rutgers University, Piscataway, NJ and Chemical Biology, Rutgers University, Piscataway, NJ).

Phase II enzymes, including Ugt's (UDP-glucuronosyltransferases), Sult's (Sulfotransferases) and Got's (glutathione S-transferases), are critical for the detoxification and biotransformation of nutrients and xenobiotics. In this study, the mRNA and protein expression of the major hepatic Phase II enzymes as well as key regulatory transcription factors were quantified in time-matched pregnant and (non-pregnant) virgin control C57BL/6 mice on gestational days (GD) 7, 11, 14, 17, and postnatal days (PND) 1, 15, and 30. Compared to virgin controls, the mRNA expression of Ugt1a1, 1a6, 1a9, 2a3 and 2b34 was decreased 40 to 60% in pregnant dams. Protein expression of Ugt1a1, 1a6, and 1a9 also decreased between GD14 and 17, with marked down-regulation of Ugt1a6. Similar to Ugts, levels of Gsta4, a1 and p1 mRNAs were reduced in pregnant dams in mid- to late-gestation. Coinciding with maximal decreases in Ugts and Gsts, the mRNA and protein expression of CAR, PPARα, and PXR and their target genes were down-regulated between GD14 and 17. Collectively, these data demonstrate a shift from glucuronidation and glutathione conjugation to sulfation in mice during pregnancy that is likely a result of reduced CAR, PPARα, and PXR signaling. These changes in Phase II metabolism may alter chemical disposition and increase the likelihood of fetal drug exposure during pregnancy (Supported by DK-080774, ES-020522, ES-005022).
suggesting that earlier in rat gestation the embryo may not be exposed to the Avastin antibody. These imaging experiments indicate that Avastin is detectable in the developing embryo as early as GD 15 (toward the end of organogenesis), but not as early as GD 11.

Nitrous oxide (N2O) and isoflurane (ISO) are anesthetics commonly used in the pediatric setting. It is known that developmental exposures to a variety of anesthetics can cause abnormal neuronal cell death in rodents and monkey models. Here, the effects of clinically-relevant concentrations of N2O and ISO were examined in a nonhuman primate model that closely mimics developing human infants. Injury to the central nervous system is often accompanied by activation of microglia at the site of injury and the peripheral benzodiazepine receptor (PBR), a marker of activated microglia, has been reported to be a sensitive biomarker of neuroinflammation. Here, we sought to monitor the suspected inflammation induced by the N2O/ISO combination using a novel PET tracer for the PBR, [18F]-FEPPA. On postnatal day 5 or 6, theus monkeys were exposed to 70% N2O, 29% oxygen and 1% ISO for 8 hours (n=4): control monkeys received room air only (n=4). One day, one week and three weeks later [18F]-FEPPA was injected intravenously and microPET/CT images were obtained over the next 2 hr. The radiotracer quickly distributed into the brains of all animals. One day after the N2O/ISO exposure the uptake of [18F]-FEPPA was significantly increased in the Temporal Lobe (TL) of treated monkeys. A week later, uptake was significantly increased in the Frontal Cortex of treated animals but not in the TL. No significant differences between control and treated animals were seen at 3 weeks. This preliminary study demonstrated preferential uptake of a PBR ligand in the FC and TL of young animals exposed to N2O plus ISO, suggesting microglial activation lasting up to one week in those areas. [18F]-FEPPA may serve as a translational biomarker of CNS inflammation.

Use of biofuels is increasing in the US automotive fleet. The primary alternative to petroleum fuels is ethanol, and the health risk associated with more than 10% ethanol in gasoline is uncertain. To address this uncertainty, we are assessing the effects of prenatal exposure to inhaled vapors of gasoline-ethanol blends. In this study, pregnant Long-Evans rats were exposed to vapors of ethanol (0, 5K, 10K and 21K ppm), 6 hr/day, on days 9–20 of gestation. Modeled blood ethanol concentrations in the 3 exposed groups at the end of a 6-hr exposure were 3, 8 and 195 mg/dL, respectively. We focus here on behavioral (n=10/sex/group), physiological (n=8/sex/group) and immunological (n=5 or 6/sex/group) evaluations of their offspring. No overt maternal toxicity was observed. No changes in litter size or weight, number, or weight gain of the pups were found. Motor activity (MA) was normal on postnatal days (PNDs) 13, 17, and 21. On PND29 and 62, offspring were evaluated with functional observational battery (FOB) and MA tests. Ethanol treatment altered the activity, neuromuscular, and sensorimotor domains of the FOB. We observed increased activity on PND62, reduced hind-limb grip strength in males on both days, and a trend towards increased sensory responsiveness in males on PND29. Not all effects were monotonically dose-related. Blood pressure (BP) and clinical chemistry were assessed on PNDs 90, 120, 150, and 180. BP was elevated in all treated males relative to controls at PND90. Smaller, non-significant increases were observed in all treated females at that age. No differences in body weight, hemate status, lipoprotein profile, liver function, or urinalysis were observed at any age. Maternal exposure to ethanol did not affect cell-mediated (delayed-type hypersensitivity) or humoral (primary antibody response) immunity in offspring of either sex at 6 or 10 weeks of age. Thus, even at very high exposure levels, prenatal exposure to inhaled ethanol affected some behavioral measures but little else. This abstract does not reflect EPA policy.

Due to the increased interest in ethanol blends as an alternative fuel source, there is a need to assess their possible health risks to sensitive populations. Specifically, ethanol is known to alter cortical functions such as attention, processing speed, movement, working memory, and response inhibition in the offspring of mothers consuming even moderate amounts of ethanol during gestation. Thus, dose-effect relationships are being assessed in the offspring of dams exposed to a range of gasoline-ethanol blend ratios. Beginning with ethanol alone we exposed 72 pregnant dams to inhaled ethanol at concentrations of 0, 5K, 10K, or 21K ppm for 6 hr/day from gestational day 9-20. Adult offspring (n=10/sex/group) received place training in the Morris water maze beginning on postnatal day (PND)76, and thereafter were tested using a matching-to-place paradigm in which the platform was moved to a different location each day. There were no significant differences in improvements in latency across each daily pair of trials. Treated rats showed a slightly slower swim speed and differences in spatial search strategy; however, these effects varied by sex and across days and were mostly obtained in the lower dose groups. Beginning on PND90 another set of male offspring (n=8/group) were trained to perform a choice reaction time (CRT) task. Preliminary results show no group differences on accuracy or movement times; however, a transient increase in decision time in the 5K and 10K ppm groups, and an increase in early nose removals, a measure of impulsivity, was observed during CRT performance in the 21K ppm group. These effects are consistent with effects of prenatal oral ethanol exposure in animals and humans on attention and impulsivity, but not on working memory. These findings suggest that inhalation of ethanol during pregnancy may impact some cortically-mediated behaviors in the offspring. This abstract does not reflect US EPA policy.

Recent legislation has increased national emphasis on the development of renewable fuels as alternatives to petroleum fuels. The toxicity of gasoline-ethanol blended fuels to the developing nervous system is of specific concern. The hippocampus, a brain region involved in spatial learning and memory, appears to be particularly susceptible to prenatal ethanol exposure. To investigate effects on these functions, pregnant Long-Evans rats were exposed to ethanol vapors (0, 5K, 10K and 21K ppm), 6 hr/day, on gestation days 9 through 20. Male and female offspring (n=10/sex/group) were trained on a delayed spatial alternation task (DSA) beginning on PND71. There were no effects of ethanol on acquisition of cued or non-cued alternation tasks. Females made fewer errors than the males at each delay of the DSA (0, 3, 6, 12, 18 s); however, no significant differences were observed between exposure groups. Finally, a third group of male and female offspring (n=16/sex/group) was assessed using a fear conditioning task. Female but not male ethanol-treated rats showed a statistically significant suppression of activity under the cue condition suggesting impaired learning of the cue; this effect was similar across dose groups. A similar dose-dependent suppression of specific trend was also seen on latent learning. In contrast to published literature on oral ethanol, these data show only minimal effects on hippocampal functional measures in offspring of rats that inhaled ethanol during pregnancy. This abstract does not reflect EPA policy.
system from exposure to ethanol vapors before examining gasoline mixtures. Because sensory dysfunctions are reported after developmental exposure to ethanol, we assessed neurophysiological measures of sensory functions as a component of a larger project involving developmental toxicity. Pregnant Long-Evans rats were exposed to 0, 5K, 10K, or 21K ppm ethanol vapors for 6 h/day over GD9 – GD20. Sensory evaluations of male offspring began around PND106. Peripheral nerve function (compound action potentials, NCV), somatosensory (cortical and cerebel-
lar evoked potentials), auditory (brainstem auditory evoked responses), and visual evoked responses were assessed. Visual function assessment included pattern elicited visual evoked potentials (VEP), VEP contrast sensitivity, and electroretino-
grams recorded from dark-adapted (scotopic) and light-adapted (photopic) flashes, and UV and green flicker. No consistent dose-related changes were observed for any of the physiological measures. All physiological responses had alterations related to stimulation intensity, and provided an estimate of detectable levels of change. The results show that gestational exposure to ethanol exposure did not result in large decrements in peripheral nerve, somatosensory, auditory, or visual function when the offspring are adults. Follow-up studies were conducted to evaluate the effects of exposure to evaporative condensates from gasoline and ethanol-blended gasoline. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

1950 US EPA BIOFUELS RESEARCH: BIOFUEL VAPOR GENERATION AND MONITORING METHODS.
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The interest in renewable fuels and alternative energy sources has stimulated development of alternatives to traditional petroleum-based fuels. The US EPA’s Office of Transportation Air Quality (OTAQ) requires information regarding the potential health hazards of these fuels regarding exposure to both evaporative emissions and to exhaust gases produced by their combustion. One particular concern is the potential for developmental neurotoxicity of evaporative emissions, given the known sensitivity of the developing nervous system to ethanol ingestion and solvent inhalation. The Inhalation Toxicology Facilities Branch (ITFB) developed novel inhalation exposure systems (vapor condensate generation and monitoring methods) to enable conduct of studies on pharmacokinetics and health outcomes of evaporative emission exposures. Initially we generated ethanol vapor condensates at stable atmospheres of 5K, 10K, and 21K ppm using dynamic countercurrent evaporation in both nose-only and whole-body exposure chambers. Then we generated stable atmospheres of vapor condensates manufactured from gasolines made to specifications from bio-fuel blends containing 0, 15%, and 85% fuel grade ethanol. Several monitoring methods were employed; real time analysis of the chamber was performed using Fourier Transforming Infrared Spectroscopy to check composition and concentration, chamber composition was verified using GC/FID analysis of grab samples, and dispersive infrared to verify concentrations. The composition of the vapor condensate was stable over time (percent difference < 2% vs. liquid vapor condensate) and provided a sufficient exposure scenario for pharmacokinetic and health outcome experiments. Abstract does not reflect US EPA policy.

1951 MECHANISMS OF BUTANOL-INDUCED DEVELOPMENTAL NEUROTOXICITY.

Butanols exhibit neurotoxic and potentially developmental neurotoxic effects. As part of evaluating the butanols in support of EPA’s Integrated Risk Information System (IRIS) Program, the literature on the mechanisms for neurotoxicity was reviewed. Studies with butanols have resulted in mixed findings with respect to developmental neurotoxicity (e.g. n-butanol) or relevant information is unavailable. In one study (Sitarek et al., 1994), it was observed that rat pups exposed to n-butanol during gestation had increased incidences of dilation of the subarachnoid space and the third lateral ventricle in the brain. It is widely known that alcohols, including butanols, interact with several types of channels and modulate the function of these targets both under acute and chronic exposures. In addition, butanols (including n- and t-butanol) inhibited fetal rat brain astroglial cell proliferation (Kotter et al., 2000; Kotter and Klein, 1999) more potently than ethanol. Pups exposed to n-butanol in utero were also reported to have significant increases in brain levels of dopamine and serotonin. t-Butanol was reported to inhibit muscarinic receptor-stimulated phosphoinositide metabolism which has been hypothesized to be a possible target for the neurotoxic effects of ethanol during brain development (Candura et al., 1991). The limited mechanistic data for the butanols support de-
velopmental neurotoxicity that has been observed in some of the studies and also suggest that butanols and ethanol both are able to produce developmental neurotoxicity. However, careful studies evaluating the neurobehavior of developing pups in sensitive strains, as well as characterizing the plausible mechanisms involved, need to be conducted in order to further elucidate the neurodevelopmental effects with butanols for risk assessment purposes. (Disclaimer: The views expressed are those of the authors and do not necessarily represent the views or policies of the US EPA.)

1952 A SINGLE NEONATAL EXPOSURE TO BISHENOL A CAUSES ADULT BEHAVIORAL DISTURBANCES IN MALE AND FEMALE MICE.
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Bisphenol A is widely used in polymer products in food and beverage packaging, baby bottles, dental sealants and fillings, adhesives, protective coatings, flame retardants, water supply pipes, and compact discs, and is found in the environment and in placental tissue, fetuses and breast milk. In recent years this chemical has caused great debate in the scientific and risk assessment communities. We have recently reported that neonatal exposure to other persistent organic pollutants can induce per-
sistent aberrations in spontaneous behavior and also affect learning and memory functions in the adult animal. Furthermore, several reports indicate that pre- and perinatal exposure to Bisphenol A can induce neurotoxic effects. In the present study we have exposed male and female mouse pups to a single dose of Bisphenol A (0.32-4.8 mg/kg bw) during the defined critical period of brain development, on postnatal day 10. At two months of age male mice showed an altered spontaneous behavior in a novel home environment, affecting cognitive function. Furthermore, these functional behavioral effects were dose-response dependent and long-lasting or irreversible since they were once again seen at 5 month of age. Spontaneous be-
havior in a novel home environment was also altered in adult female mice at 5 months of age. Earlier studies on neonatal exposure to persistent organic pollutants (POPs) in our animal model have shown the cholinergic system to be a target of neurotoxicity, but in the present study only minor effects on the nicotine-induced behavior was seen in male and female mice. Spatial learning and anxiety-like behav-
iors were not affected in Morris swim-maze and the elevated plus-maze, in adult male mice. The present findings show similarities with effects earlier reported after continuous pre- and perinatal exposure to Bisphenol A, and also with effects seen after a single postnatal exposure to other POPs, such as PBDEs, PCBs and PFCs.

1953 A SINGLE NEONATAL EXPOSURE TO BISHENOL A ALTERS THE LEVELS OF IMPORTANT NEUROPROTEINS IN MICE.
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Bisphenol A (BPA) is an industrial chemical commonly used in the production of plastics and resins for baby bottles, food and beverage containers, protective coats,
digital and electronic materials. It is widely found in the environment as well as in human placental tissue and breast milk. BPA is a well-known endocrine disrup-
ter, additionally it has been shown to cause persistent aberrations in sponta-
nous behavior and in learning and memory in mice. The purpose of the present study was to investigate if neonatal exposure to BPA can affect calcium/calmodulin- dependent protein kinase II (CaMKII), growth-associated protein-43 ( GAP-43) synaptophysin and tau levels in the neonatal and/or adult mouse brain. On postna-
tal day 10, male and female NMRI-mice were exposed to a single oral dose of 0.32, 3.2 or 6.8 mg BPA/kg body weight and control animals received the 20% fat emul-
sv. The animals were sacrificed 24h or 5 months after the BPA exposure and the brains regions cerebral cortex and hippocampus were analyzed for CaMKII, GAP-43, synaptophysin and tau with Slot-Blot analysis. No significant differences in protein levels were seen in the neonatal mice 24h after exposure. In contrast, in both adult male and female mice the protein analysis showed increased levels of synaptophysin in the cerebral cortex. Furthermore, decreased levels of CaMKII were seen in both cerebral cortex and hippocampus of adult female mice, an effect not seen in adult male mice. These results support recent findings, showing that a single oral exposure to BPA during a defined critical period of brain development can cause irreversible neurotoxic effects. The mechanisms behind the neurotoxic ef-
ffects seem to be different from other persistent organic pollutants (POPs) recently studied in the same animal model. The present results call for further investigations of the developmental neurotoxic effects of BPA.
1954  THE IMPACT OF BISPHENOL-A (BPA) EXPOSURE ON NEURODEVELOPMENT AND SUBSEQUENT VISUOSPATIAL LEARNING AND MEMORY.

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Exposure to exogenous agents during neurodevelopmental stages may be associated with the onset of neurological disorders. The emerging contaminant bisphenol A (BPA) is a widely used ingredient in the production of plastics and resins utilized in food and beverage packaging. This chemical pollutant has become ubiquitous in our environment and studies have shown that it may have a deleterious impact on developing organ systems when exposure occurs in utero. Our hypothesis is that gestational and lactational exposure to BPA will alter the neurological development of C57BL/6 mice, which will affect their performance on the Barnes maze, a task to assess visuospatial learning and memory. C57BL/6 female mice were orally exposed to 5, 25, or 50 mg/kg of BPA or a corn oil vehicle beginning at pairing with males and continuing through weaning of pups. Performance on the Barnes maze was initiated at weaning (postnatal day 21) in male and female offspring. Initial errors in finding the escape hole, total errors, time to initially reach the escape hole, and time to escape were measured. Time to reach the escape hole decreased with increasing doses of BPA. Although this trend was not statistically significant, this preliminary study indicates that BPA may impact visuospatial learning and memory. In addition, females performed significantly better than male offspring when comparing initial errors and time to initially reach the escape hole, which may indicate sex-related differences in the task or sensitivity to BPA exposure. To determine if behavioral performance correlates with altered brain morphology induced by BPA, evaluation of dendritic arborization in the hippocampi of exposed offspring is ongoing.

1955  IN UTERO BISPHENOL A-MEDIATED OXIDATIVE STRESS CAUSES NEOCORTICAL ABNORMALITIES IN MICE.

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Bisphenol A (BPA) is an additive in polycarbonate plastics and epoxy resins that has raised concerns about its safety, particularly with respect to brain development. Despite numerous BPA safety studies, there is little information about the potential mechanisms of toxicity, making risk assessment difficult. We investigated the role of BPA-initiated reactive oxygen species (ROS) formation on neurodevelopmental deficits during the fetal period of development. Time-mated CD-1 mice were dosed intraperitoneally on gestational day 15 with a single injection of 100 μg/kg BPA in corn oil, and fetal brains were evaluated 4 and 8 hours post-injection for oxidatively damaged DNA and morphological changes, respectively. Using high-performance liquid chromatography with tandem mass spectrometry, we found elevated levels of the DNA lesion 8-oxo-2’-deoxyguanine (8-oxodGuo), which was blocked by pretreatment with the free radical spin trapping agent phenylbutylnitrone (PBN), implicating ROS in the mechanism of macromolecular damage. Immunohistochemical analysis using TuJ1, a neuron specific β-tubulin marker, revealed more dispersed staining in the neocortex in the BPA-exposed brains than in controls. This BPA effect was prevented by pretreatment with PBN, suggesting a role for ROS in the pathogenesis. Further investigation of neocortical development is underway to determine the mechanism behind the abnormal TuJ1 staining pattern in BPA-exposed brains. These results suggest novel macromolecular and neurondevelopmental consequences of in utero BPA exposure that may broaden our understanding of the potential human risk. [Support: Canadian Institutes of Health Research]

1956  MILD DEVELOPMENTAL HYPOTHYROIDISM AND TRACE FEAR CONDITIONING: ROLE OF GENDER AND SHOCK DURATION.

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Rodent models of developmental thyroid hormone (TH) deficiency aptly reflect the deleterious effects of severe TH deficiencies on brain structure and function in humans. However, the impact of moderate TH insufficiencies on neurodevelopmental outcomes has proven more difficult to model. Trace fear learning was examined in adult male and female offspring following low level developmental TH disruption. Trace fear conditioning requires the involvement of the hippocampus by interposing a "trace" interval of time between the conditioned (CS) and the unconditioned stimulus (US). Using a standard trace fear paradigm, additional demands were placed on the animals by 1) limiting the number of CS (tone/light neutral stimulus) - US (footshock) pairings; and 2) addition of a noncontingent visual 'distur- dractor' stimulus throughout training. The latter requires the engagement of the prefrontal cortex as well as the hippocampus. 0, 1, 2, 3, 10 ppm Propylthiouracil (PTU) was administered via drinking water to the dam from early gestation until weaning on postnatal day 21 (PN21), resulting in graded levels of T4 reduction in the dams and pups. Independent groups of adult male and female offspring were examined using one of two trace fear training protocols that differed in saliency of the US. Deficits in context fear learning were seen in male but not female offspring exposed to 1, 2 and 3ppm PTU relative to control (0ppm). PTU did not impact cue learning in either sex at these dose levels. At the highest dose of PTU (10 ppm), deficits were seen in both males and females in context as well as cue learning. When US saliency was increased by increasing shock duration from 0.5 to 2 sec, cue and context learning impairments were limited to high dose mice of both genders. These results suggest that subtle differences in learning result from mild developmental TH insufficiency and males may be more susceptible to these insults. (Does not reflect EPA policy).

1957  ASSESSMENT OF THE ADULT TOXICITY OF PROPYLTHIOURACIL (PTU) AND DEVELOPMENTAL NEUROTOXICITY TO HARLAN WISTAR RATS.

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This study was designed to assess the potential toxicity of PTU to adult animals and subsequent developmental neurotoxicity in their offspring up to Day 60 of age. Propylthiouracil (PTU) is a known anti-thyroid drug that inhibits the synthesis of thyroid hormones in the thyroid gland. The study also provided data to support a combined 2-generation reproduction toxicity test (OECD 416) and developmental neurotoxicity (DNT) test (OECD 426) on a new generation plasticizer (GRIND-STED® SOFT-N-SAFE).

PTU was administered via the drinking water to the parental animals, for a ten week pre-pairing period and, for females, throughout, mating, gestation and lactation. For assessment of general toxicity in the parental animals, clinical signs, body weight and food and water consumption, mating performance, offspring survival and growth and macroscopic pathological change were monitored. Assessment of developmental neurotoxicity in F0 F1 offspring included pre-weaning surface righting, air righting and motor activity plus post-weaning motor activity, grip strength, rotor rod and water maze and startle response.

Parental exposure to PTU was associated with reduced body weight gain and lower food and water consumption, however mating performance and fertility (including numbers of corpora lutea, implantations and offspring) were unaffected. Lower body weight gain, inferior air righting performance and reduced motor activity scores were observed for pre-weaning offspring and increased offspring mortality was observed in the immediate post-weaning period. For the adolescent offspring, lower body weight gains, delayed sexual maturation, inferior grip strength, water maze, rotor rod performance and increased motor activity were observed. For young adults, lower body weight gain, inferior water maze performance, increased startle response, higher motor activity and lower brain weight were apparent.

1958  IN UTERO AND LACTATIONAL EXPOSURE TO DIOXIN ALTERS GENE EXPRESSION IN THE DEVELOPING MOUSE BRAIN.

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In utero and lactational exposure to environmental chemicals has been reported to impair the advanced brain functions. We previously reported that maternal exposure to TCDD disrupts the advanced brain functions in adult mice (Haijima et al., 2010; Endo et al., 2011). In this study, we used the same dosing paradigm and studied changes in gene expression in the TCDD-exposed developing mouse brain. Pregnant C57BL/6 mice were orally administered TCDD at a dose of 0, 0.6 or 3.0 μg/kg b.w. on gestation day 12.5, and brains of male progeny were collected on
PCBs are a group of structurally related chemicals with widespread distribution in the environment. The developmental neurotoxicity of non-co-planar PCBs may be mediated, at least in part, by their potently active towards ryanodine receptors (RyRs), which are broadly expressed in the nervous system and contribute to Ca2+-dependent signaling events in neurons. Human polymorphisms in RYR genes increase sensitivity to halogenated compounds and are linked to environmentally triggered disorders including malignant hyperthermia (MH) and cardiac arrhythmias. In this study, we tested the hypothesis that a heritable RYR1 mutation increases susceptibility to the developmental neurotoxicity of PCBs. Primary cortical cultures dissociated from either transgenic mice that express the R163C-RyR1 mutation or congenic wildtype (WT) mice were plated at high density and exposed to vehicle or PCB 95 (2,2′,3,5,6-pentachlorobiphenyl) from 7 to 9 days in vitro (DIV). Following exposure, spontaneous Ca2+-oscillations were monitored in neurons loaded with the Ca2+ sensitive dye Fluo-4. Exposure to PCB 95 (200 nM) significantly increased the frequency of spontaneous Ca2+-oscillations in WT and heterozygous R163C-RyR1 cortical neurons relative to vehicle controls. This effect was more pronounced in neurons expressing the R163C gene mutation relative to WT. However, in contrast to parallel studies in cultured mouse hippocampal neurons, this PCB 95 exposure paradigm had no effect on dendritic arborization in either WT or R163C-RyR1 cortical neurons. In summary, our results indicate that expression of a RYR1 gene mutation that confers susceptibility to halogenated hydrocarbons exacerbates the effects of PCB 95 on the fidelity of intracellular Ca2+ signaling in neurons, but the cellular readout of this perturbation may differ among cortical neuronal cell types. Supported by NIH grants R01 ES014901 and P42 ES04699.
1963 BDE-47 AND BDE-49 INFLUENCE MORPHOLOGIC DETERMINANTS OF NEURONAL CONNECTIVITY IN PRIMARY CULTURES OF HIPPOCAMPAL NEURONS. 

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Polycbrominated diphenyl ethers (PBDE) are widely used flame retardants that bioaccumulate in human tissues. Developmental exposure to PBDE has been linked to cognitive and behavioral deficits in humans and experimental animals. It has been proposed that like the structurally related non-dioxin-like polychlorinated biphenyls (PCB), PBDEs alter normal patterns of neuronal connectivity in the developing brain. Axonal and dendritic morphology are critical determinants of neuronal connectivity, and perturbation of the rate or extent of axonal or dendritic growth during neurodevelopment has been linked to neurobehavioral deficits in animal models and humans. In primary cultures of hippocampal neurons derived from postnatal day 1 rat, exposure to BDE 47 or 49 at concentrations in the pM to nM range during the first 48 hours after plating significantly inhibit axonal growth. In contrast, exposure to either BDE congener from days 7 to 9 in vitro has no effect on dendritic growth. However, exposure to BDE-47 and -49 over days 12-16 in vitro alters synapse density. The effects of PBDE on axonal growth and synapse formation are independent of cytotoxicity as measured by MTT and LDH release assay. Our findings suggest that like PCBs, PBDEs may cause developmental neurotoxicity by interfering with normal patterns of neuronal connectivity in the developing nervous system; however, unlike the non-dioxin-like PCBs, PBDEs preferentially target axonal growth rather than dendritic growth. Exposure to both PCBs and PBDEs may impair neuronal connectivity in an additive or synergistic manner. This work supported by NIH grants R01 ES014901 and P42 ES04699 and by the JBF Foundation.

1964 PAH PARTICLES PERTURB PRENATAL PROCESSES AND PHENOTYPES. 

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In the present study, we addressed the in utero exposure effects of benzo(a)pyrene aerosol (B(a)P) on Sp4 and N-methyl-D-aspartate (NMDA) dependent systems towards ascertaining later life behavioral phenotypes. Results from in utero exposed WT Cpx/lax/los offspring mice were compared with in utero exposed brain-Cpx null offspring mice (that do not express NADPH oxidoreductase associated with CYP thereby substantially reducing their capacity to produce B(a)P metabolites. Subsequent to in utero [E14-E17] exposure to B(a)P aerosol at a concentration of 100mg/m3, Cpx/lax/los offspring exhibited: 1) significantly elevated B(a)P metabolite and F2-isoprostane neocortical tissue burdens, 2) significantly elevated concentrations of the neurotransmitter glutamate in cortex, 3) premature Sp4 developmental expression, 4) negative modulation of NR2B:NR2A subunit ratios and 5) deficits in a novelty discrimination phenotype that is completely absent in brain-Cpx-null offspring subsequent to in utero exposure to B(a)P aerosol. Collectively, these findings suggest that, in situ generation of metabolites by the CYP1B1 thereby substantially reducing their capacity to produce B(a)P metabolites. These findings are consistent with the hypothesis that exposure to B(a)P in utero alters synapse density. The effects of PBDE on axonal growth and synapse formation are independent of cytotoxicity as measured by MTT and LDH release assay.

1965 ASSESSING LATER-LIFE BEHAVIORAL PHENOTYPES SUBSEQUENT TO IN UTERO EXPOSURE TO BENZOPYREN. 

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To characterize the impact of early-life exposure to B(a)P on later-life behavioral phenotypes Long Evans Hooded rats were exposed to either peanut oil, 600 or 1200mg/kg BW B(a)P on embryonic days (E) E14-17. The results reveal no effect of in utero exposure on birth indices, pre-weaning growth curves or on the initial and final body weight ratios in the experimental groups relative to controls. However, rat offspring exposed in utero to 600 and 1200mg/kg BW B(a)P required significantly more sessions to 1) acquire the original discrimination and 2) complete the first reversal as compared to controls (14-days as compared to 6-days for controls). B(a)P-exposed offspring regardless of dose, made significantly more errors than controls on the first reversal, while maintaining a similar number of errors in the original discrimination. B(a)P-exposed offspring also maintained shorter choice latencies than controls during the initial reversal session. These findings lead to a strong prediction that early-life exposure to B(a)P during the peak periods of neurogenesis produce a strong negative effect on associative learning in the offspring in later-life.

1966 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) INDUCED DISRUPTION OF THE PERIPHERAL NERVOUS SYSTEM OF DEVELOPING RED SEABREAM (PAGRUS MAJOR) EMBRYOS. 


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2,3,7,8-Tetrachlorodibenzop-dioxin (TCDD) induces various toxic effects such as neural damages in developing fish embryos. Although many studies have focused on the TCDD-induced neurotoxicity in the central nervous system of vertebrates, the effects on the peripheral nervous system (PNS) are poorly understood. We investigated TCDD-induced effects on the morphology of peripheral nervous system (PNS) in the developing red seabream (P. major). The embryos at 10 days post-fertilization (hpf) were treated with 0, 0.1, 0.4 or 1.7μg/L of TCDD in sea-water for 80 minutes. The morphology of PNS was microscopically observed at 48, 78, 120 and 136 hpf with florescence staining using an anti-acetylated tubulin antibody. The craniofacial distribution and nerve fascicle of PNS were notably disrupted in TCDD-treated embryos. Since the growth cone at the end of growing nerve axons advances through the surrounding tissues, we hypothesized that TCDD exposure would affect 1) the muscle as an axon target, 2) the nerve cell proliferation/differentiation and 3) the axon guidance molecules in the embryos. Histological analyses indicated that the expression levels of Semaphorin 3A and Plexin A1 mRNAs were altered in TCDD-treated embryos, but no effects on the muscle structure and the differentiation/proliferation of neurons were detected. Our findings demonstrated that TCDD produces specific effects on the development of craniofacial PNS in red seabream embryos even at a low concentration (0.1 μg/L).

1967 EFFECT OF MIDAZOLAM ON DEVELOPING BRAIN: IN VITRO AND IN VIVO STUDIES. 


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Last decade data raise the concern that most anesthetics may triggers widespread apoptotic neurodegeneration during development, because they are the NMDA antagonists, e.g. ketamine and/or GABA agonists, e.g. midazolam. PND-1 rat forebrain cultures were treated for 12 hrs with ketamine and midazolam alone, and ketamine plus midazolam at various doses to study if that combination will promote or prevent each other's neurotoxic effects. Ketamine (10 and 20 μM) resulted in a substantial increase in DNA fragmentation as measured by cell death ELISA, increased number of TUNEL-positive cells, and a reduction in mitochondrial metabolism. No significant apoptotic effect was observed in midazolam-treated cultures. Co-incubation of midazolam (1 or 5 μM) with ketamine (5 or 10 μM) mildly reduced ketamine-induced apoptosis, suggesting that concomitant activation of the GABA system may serve to attenuate the neurotoxic effects of glutamatergic overstimulation. Some studies was repeated on PND-3 monkey cortical cultures. No significant neurotoxicity was observed in midazolam-treated cultures. Co-incubation of 10 μM midazolam with ketamine (10 μM) partially prevented ketamine-induced neuronal cell death. PND-7 rats were intraperitoneally administered with 9 mg/kg midazolam or saline (control) 3 times at 2 h interval. No significant apoptotic effect was observed. Brain frontal cortical areas were collected 6 h after the last dose and RNA was isolated. Gene expression profiling was performed using the Illumina Rat Ref-12 Expression BeadChip with 22,523 probes. The differentially expressed genes (fold change 1.4 and a FDR of 0.05) and for each cell type (treatment to control) were analyzed by using IPA (Ingenuity System). Molecular function analysis showed the only gene Exso3 directly involved to cell
death was down-regulated in midazolam-treated pups. 2 other genes Tnixp and Mxl1 found are oxidative stress-related. Further study should be done with midazo- 
lam and co-injections of ketamine and midazolam.

This work was supported by NCTR E-7405.01.

**1968** DKK1 AND NOTCH1 MEDIATE GLUCOCORTICOID- 
INDUCED CHANGES IN HUMAN NEURAL 
PROGENITOR CELLS PROLIFERATION AND 
DIFFERENTIATION.

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Glucocorticoids (GC) are critical for the development of the brain and essential for the stress response; however excess of GC has been linked to detrimental conse- 
quences on the nervous system. There is little information available on the effects of GC on human neural stem/progenitor cells (hNPC). In the present study we have 
investigated the effects of the synthetic GC dexamethasone (Dex) on hNPC grown as neurospheres, with special focus on their proliferation and differentiation capac-
ity and the underlying molecular mechanisms. hNPC (Lonza Verviers SPRL) from different preparations (gestational week 16, 16.5 and 19) were cultured as neuro-
 spheres in Dulbecco modified Eagle medium and Hams F12 (3:1) supplemented 
with B27, 20ng/ml EGF and 20ng/ml rhFGF at 37°C with 5% CO2.

Epithelial to mesenchymal transition (EMT) is a hallmark of pathological contexts in cancer progression and fibrosis. This study investigated the inhibitory effect of Platycodon grandiflorum root-derived saponin on transforming growth factor-β1-induced EMT in A549 cells. 

**1970** VALIDATION OF CANDIDATE GI CANCER GENES 
WITH ION CHAIN FUNCTIONS, IDENTIFIED 
FROM SLEEPING BEAUTY TRANSPONSO-MEDIATED 
MUTAGENESIS SCREENS IN APC+/− MICE.

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University of Minnesota, Minneapolis, MN.

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the US. Our lab has used Sleeping Beauty transposon-mediated mutagenesis screens in 
Apc−/− mice to identify a set of common insertion sites associated with candidate CRC genes. These include two ion-channel encoding genes: Kcnq1 and Clca1, which 
act together to promote chloride ion secretion in the normal colon. We hypothesize that these two genes, when dysregulated, contribute to the development of CRC.

The normal physiological functions of Kcnq1 indicate it may work with CFPRT to 
prevent inflammation in the GI tract. The function of Kcnq1 was tested by its ge-
netic knockdown in the Apc−/− model of GI cancer and in the human CRC cell line 
DLD-1. In the mouse model, Kcnq1 expression was abrogated by targeted germline 
mutagenesis, resulting in a null allele. In cell culture, expression of Kcnq1 was de-
pleted by siRNA knockdown, confirmed by quantitative real time PCR. We found that 
haploinsufficiency for Kcnq1 significantly enhanced tumorigenesis in Apc−/− 
mice. In support of this result, a 60% knockdown of Kcnq1 in DLD-1 cells re-
sulted in an 1.4x increase in cell viability measured by MTT assay, compared with a 
control siRNA treatment at day seven after transfection. Colon tissues of Kcnq1+/− and 
Kcnq1−/− mice were compared for expression of the inflammatory mediator 
Nfkb and its mRNA level was increased by 1.6x in Kcnq1−/− mice. In summary, our 
results of both in vivo and in vitro studies confirm a tumor suppressor role for 
Kcnq1 in the GI tract. Current work is focused on investigating the potential con-
nections between Kcnq1 and CFPRT and the model that their genetic alterations 
promote oncogenesis by a common pathway, possibly by an inflammatory mecha-
nism. This knowledge should also further an understanding of how exogenous tox-
ics such as formaldehyde that promote inflammation in the GI tract may con-
tribute to human CRC.

**1969** SEX-RELATED DIFFERENCES IN HUMAN AND 
RODENT NEURAL STEM CELL SUSCEPTIBILITY TO 
METHYLMERCURY (MEHG).


Epidemiological and experimental data have generated a growing awareness about possible sex-related differences in the susceptibility to developmental neurotoxi-
cants. We have used human and rodent neural stem cells (NSCs) to evaluate whether in vitro models may be feasible to investigate sex-dependent differences in regard to developmental neurotoxicity. Human (h) NSCs were cultured as neu-
ropheres or dissociated cells. Neurospheres from different developmental stages (gestational week 8.5 and 16) were exposed to 10μM-100μM MeHg and alter-
ations in proliferation, differentiation and migration were examined and compared to untreated controls. The proliferation assay, performed in dissociated hNSCs ex-
posed to MeHg (10 - 25μM) for 24 hrs showed a significant decrease in prolifera-
tion as evaluated by quantification of Ki67 positive cells. Moreover, we observed a 
significant decrease in spontaneous neuronal differentiation, as determined by Tuj1 
quantification in 7 day-old cultures, similar to what we previously reported in ro-
dent NSCs. To identify the gender of the donors, we employed two independent 
PCR analyses using two Y-chromosome-loci (ZFY and SRY) or a X-Y homologous 
target NSCs. To identify the gender of the donors, we employed two independent 
PCR analyses using two Y-chromosome-loci (ZFY and SRY) or a X-Y homologous 
target NSCs. To identify the gender of the donors, we employed two independent 
PCR analyses using two Y-chromosome-loci (ZFY and SRY) or a X-Y homologous 

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**1971** INHIBITORY EFFECTS OF PLATYCODON 
GRANDIFLORUM ROOT-DERIVED SAPONIN ON 
GROWTH-CONTROLLING FACTOR-β1-INDUCED 
EPITHELIAL TO MESENCHYAL TRANSITION 
IN A549 CELLS.

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Epithelial to mesenchymal transition (EMT) is a hallmark of pathological contexts in cancer progression and fibrosis. This study investigated the inhibitory effect of Platycodon grandiflorum root-derived saponins (Changkil saponins: CKS) on 
transforming growth factor-β1 (TGF-β1)-induced alterations characteristic of EMT in A549 cells. CKS inhibited TGF-β1-induced proliferation in A549 cells. CKS 
blocked TGF-β1-induced E-cadherin downregulation and vimentin upregu-
lation, as well as retaining epithelial morphology. Furthermore, CKS inhibited 
TGF-β1-induced Snail expression. CKS attenuated TGF-β1-induced phosphoryla-
tion of smad2/3 and restoring of downregulation of smad7. CKS attenuated TGF-
β1-induced phosphorylation of PI3K/Akt and ERK1/2. Inhibition of PI3K/Akt and 
ERK also blocked TGF-β1-induced glycogen synthase kinase-3β (GSK-3β) 
phosphorylation. Also, LY294002, PD98059 and lithium chloride inhibited TGF-
β1-induced Snail expression. These results suggest that CKS inhibits the TGF-β1-
induced EMT process and prevent TGF-β1-induced acquisition of a myofibroblast phenotype in A549 cells.

**1972** ENHANCED INHIBITION OF CELL PROLIFERATION 
IN HUMAN COLON CANCER DLD1 CELLS OVER-
EXPRESSING PPARγ.

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Peroxisome proliferator-activated receptor gamma (PPARγ) is a nuclear hormone receptor that regulates many important functions, including adipogenesis, glucose homeostasis and cancer. Loss of function of this protein has also been associated with increased incidence of human colorectal cancer. The function of this nuclear
receptor was examined using stable human DLD1 colorectal adenocarcinoma cells over-expressing PPARγ. Over-expression of PPARγ was confirmed at both the pro-
tein and mRNA levels by western blot analysis and real-time quantitative PCR. Enhanced activation of a PPARγ target gene was also observed in response to ligand
activation of PPARγ as compared to control cells. The xCELLigence System from Roche® was used to monitor cell proliferation in real-time, providing quantitative
assessment of cell number over a period of 120 hours. Ligand activation of DLD1 cells over-expressing PPARγ caused enhanced inhibition of proliferation as com-
pared to controls. Results from this study show that activating PPARγ in DLD1 cells that over-express this receptor can effectively enhance inhibition of prolifera-
tion as compared to controls. This suggests that approaches that increase expression
of PPARγ in colon cancer cells could be developed as a new strategy for colon can-
cer chemoprevention. (Supported by CA124533, CA126826, CA141029, CA140639).

1973 THE ANTIDIABETIC DRUG METFORMIN INHIBITS PANCREATIC CANCER CELL GROWTH AND TARGETS DOWNREGULATION OF SPECIFICITY PROTEIN (SP) TRANSCRIPTION FACTORS.

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Metformin (1,1-dimethybiguanide hydrochloride) is one of the most widely pre-
scribed drugs for the treatment of type 2 diabetes. In addition to its antidiabetic
property it also exhibits antineoplastic effects which include inhibition of angio-
genesis and cell cycle arrest. In this study we evaluated the effects of metformin on specificity protein (Sp) transcription factor which is normally overexpressed in can-
cer cells compared to their low levels of expression in normal cells. We report for the
first time that metformin downregulates specificity protein (Sp) transcription fac-
tors (Sp1, Sp3, Sp4) and Sp dependent genes associated with cancer cell survival
(survivin), angiogenesis (VEGF) cell cycle progression (cycin D1) and proapop-
totic genes (bcl2, pPARP). However, when cells were pretreated with the protea-
some inhibitor - thioloxitin, there was a reversal of Sp downregulation. Enhanced Sp protein ubiquitination was observed when metformin treated cell lysates were
immunoprecipitated with Sp antibody and immunoblotted with Ubiquitin (Ub) anti-
body. Sp degradation was unaffected when cells were pretreated with leptomycin B
- a nuclear export inhibitor, indicating the nuclear degradation of Sp proteins. Fatty
Acid Synthase (FAS) a key enzyme of lipid metabolism is overexpressed in cancer
cells compared to the normal cells. The differential expression of FAS between nor-
mal and neoplastic tissues makes it a potential tumor biomarker. Expression of FAS
was significantly decreased when pancreatic cancer cells were treated with met-
formin and knockdown of Sp1, Sp3, and Sp4 proteins by RNA interference de-
creased the expression of FAS indicating that FAS is regulated by Sp proteins. The
effects of metformin on other molecular pathways and the potential use of FAS as a
biomarker are currently being investigated.

1974 ATTENUATION OF THE UV-CINDUCED DNA DAMAGE RESPONSE IN TKG CELLS WITH THE TUMOR PROMOTING AGENT TPA.

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The DNA damage response (DDR) represents an intricate network of signaling
pathways that converge to decide cellular fate following an insult to the DNA struc-
ture. This critical pathway initiates a myriad of downstream events which funnel
into basic mechanisms (DNA repair, cell cycle arrest and apoptosis) to prevent
genecis and overall genomic instability. Therefore, the purpose of this study was to
investigate the effect of a known tumor promoter (12-O-tetradecanoyl-
phorbol-13-acetate, TPA) on the DDR initiated by UV exposure. UVC is effi-
ciently absorbed by DNA which results in bulky pyrimidine dimers and 6-4 photo-
products that cause replication stress and double stranded breaks. In this study, TK6
cells were exposed to various levels of UVC (2, 5, 10, 20, 50 and 100 J/m2), result-
ing in different profiles of viability (trypan blue), cell cycle arrest (propidium io-
dide), apoptosis (annexin V/PI) and DNA damage (micronucleus assay) at 24
hours post-exposure depending on the amount of DNA damage incurred.
Treatment with TPA altered all of these endpoints indicating an overall attenuation
of the DDR. At cytotoxic levels of UVC exposure, TPA increased the overall cell vi-
ability by approximately 10-15%. The effect of TPA on cell cycle arrest was most
notable at 20 J/m2 in which UV-induced G1 arrest was almost completely abro-
gated. A decrease in G1 arrest was also observed at the higher UVC exposure
levels. TPA also attenuated apoptosis while also decreasing the overall dead popula-
tion. The effect was most notable at the highest concentrations tested (50 and 100
J/m2) where an approximately 50% reduction in Annexin V positive cells was ob-
served. Lastly, TPA appeared to increase UVC induced micronuclei at cytotoxic
concentrations. Since the DDR is essential for preventing the carcinogenic trans-
formation of cells, we hypothesize that attenuation of this pathway by exogenous
chemicals could potentially create an environment for clonal expansion of cells in
the tumor promotion phase of cancer.

1975 ANILINE-INDUCED CELL CYCLE PROGRESSION OF SPLENOCYTES: EXPRESSION OF microRNAs AND CYCLIN mRNAs.

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Aniline exposure is associated with toxicity to the spleen leading to splenomegaly,
hyperlplasia, fibrosis, and sarcomas on chronic exposure in rats. However, early
molecular events in aniline-induced cell cycle progression in the spleen remain
unknown. Cell cycle progression plays pivotal role in cell proliferation and cyclins
play a central role in cell cycle regulation. Recent studies have shown that microRNAs
(miRNAs), which are small noncoding RNA molecules, regulate the expression of
target genes by specifically binding to and interfering with their mRNA, and play
important role in cell growth, proliferation and apoptosis. This study was, there-
fore, undertaken on the expression of miRNA and regulation of mRNA of cyclins
in the spleen, in an experimental condition that precedes aniline-treated tumor-
genic response. Male SD rats were treated with aniline (1 mmol/kg/day, by gavage)
for 7 days (controls received drinking water only), and miRNA (microarray and
qPCR) and mRNA expression of cyclins (pCyclin) were analyzed. Microarray and
qPCR analysis showed that aniline exposure led to significantly decreased miRNA
expression of let-7a, miR-94c and miR-125b in the rat spleens. The decreases in
these miRNA expression were associated with significantly increased mRNA ex-
pression of cyclin D3, cyclin E, cyclin A and cyclin B in the splenocytes of aniline-
treated rats, as compared to the controls. The data suggest that let-7a, miR-94c and
miR-125b could play a role in the regulation of cell cycle proteins. Our findings
thus, provide new insight into the role of miRNA in cell cycle progression which
may contribute to aniline-induced tumorogenic response in the spleen. Supported
by NIH ES06476.

1976 LIGAND-DEPENDENT ACTIVATION OF THE ORPHAN NUCLEAR RECEPTOR NR4A2 (NURR1) IN PANCREATIC CANCER CELLS.

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The transcription factor Nur1 belongs to the nuclear receptor 4A (NR4A4) sub-
family and recent studies show that Nur1 is a potential drug target for clinical
 treatment of tumors. Methylene-substituted diindolylmethanes (C-DIMs) are a
new class of potent chemotherapeutic drugs that exhibit promising in vitro and in
vivo anticancer activities. Using a GaL4-Nur1 chimera and a GaL4-luc reporter, we
have identified C-DIMs that activate Nur1 in pancreatic cancer cells, and our re-
sults show that a select number of C-DIMs are more potent than 6-mercaptop-
urine, the widely used chemotherapeutic agent and Nur1 activator. These prelimi-
nary results were further examined using NBRRE or NurRE response element
sequences linked to a luciferase reporter gene and there was a good correlation be-
tween these assays. While modifications on the indole rings of DIM lead to moder-
ate Nur1 activation, phenyl-substituted C-DIMs such as 1,1-bis(3'-indolyl)-1-(p-
bromophenyl) methane (DIM-C-pPhBr) result in more than a 60 fold activation of
Nur1 in the functional assays. The structure-related activities of a group of phenyl
indole ring substituted C-DIMs (C-DIMs) are new class of potent chemotherapeutic drugs that exhibit promising in vitro and in vivo anticancer activities. Using a GaL4-Nur1 chimera and a GaL4-luc reporter, we have identified C-DIMs that activate Nur1 in pancreatic cancer cells, and our results show that a select number of C-DIMs are more potent than 6-mercaptopurine, the widely used chemotherapeutic agent and Nur1 activator. These preliminary results were further examined using NBRRE or NurRE response element sequences linked to a luciferase reporter gene and there was a good correlation between these assays. While modifications on the indole rings of DIM lead to moderate Nur1 activation, phenyl-substituted C-DIMs such as 1,1-bis(3'-indolyl)-1-(p-bromophenyl)methane (DIM-C-pPhBr) result in more than a 60 fold activation of Nur1 in the functional assays. The structure-related activities of a group of phenyl and indole ring substituted DIM-C-pPhBr analogs were compared and their activities as Nur1 activators were pPhBr > pPhBr > pPhBr > pPhBr (phenyl ring substituted) and 2-Me > N-Me (methyl groups on the indole ring). The activation of Nur1 by DIM-C-pPhBr is dependent on both N-terminal AC-GR and C-terminal C to F domains. Activation of the AR decreased activity of Nur1 by DIM-C-pPhBr is partially abolished by Mek1 inhibitor PD89509 and PI3K inhibitor LY294002. Expression of several genes that have Nur1 responsive NBRRE elements were induced as early as 4
hr after treatment with DIM-C-pPhBr and after knockdown of Nurr1 by RNA interference the induction effects were decreased. These results indicate that C-DIMs that activate Nurr1 inhibit pancreatic cancer cell growth and the Nurr1 dependent pathways are currently being investigated.

1977 STUDIES ON WILD YAM (DIOSCOREA VILLOSA) ROOT EXTRACT AS A POTENTIAL CHEMOPREVENTIVE AGENT FOR BREAST CANCER.

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Aberrant epigenetic alterations in the genome such as DNA methylation and chromatin remodeling are able to inactivate specific gene function which is considered as one of the mechanisms involved in breast cancer development. Attempts have been made to develop demethylating epigenetic drugs (epi-drugs) that are able to reactivate the silenced gene and prevent breast cancer. The current synthetic epi-drugs are non-specific and able to induce adverse side effects. In an attempt to search new and safe gene-specific demethylating agents (epi-drugs) from natural products, this study was aimed to evaluate the effects of wild yam (Dioscorea villosa) root extract in an estrogen receptor positive (ER+) breast cancer cell line MCF-7. Two genes, DNA methyl transferase 3B (DNMT3B) and GATA3 which are reported to be over expressed in breast cancer cells were used for evaluation. Additionally, by using Methyl Primer Express V1.0 software (Applied Bioscience) we were able to identify 3 CpG rich sites in the promoter region of GATA3 and 1 site in DNMT3B. MCF-7 cells at about 70 % confluency were exposed to various concentrations of wild yam root extract (0-50 \mu g/ml) for 72h. After cell count, total RNA was extracted and used for GATA3 and DNMT3B mRNA analysis by quantitative real-time PCR (qPCR). Moreover, the promoter methylation pattern of a specific region of GATA3 gene was also analyzed. It was observed that wild yam root extract significantly reduced the cell proliferation in a dose-dependent manner. The mRNA expression of DNMT3B was significantly enhanced in a dose-dependent manner as observed by qPCR while GATA3 mRNA expression was unaltered. From our preliminary data, it was evident that wild yam root extract has the potential to be used as a cancer chemopreventive substance, however, to establish its efficacy as an epi-drug more studies are needed.

1978 THE POTENTIAL ROLE OF ARYL HYDROCARBON RECEPTOR NUCLEAR TRANSLATOR 2 IN MOLECULAR RESPONSES TO XENOESTROGENS IN HUMAN BREAST CANCER CELLS.

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Recently, human studies found that the mRNA expression level of aryl hydrocarbon receptor nuclear translocator 2 (ARNT2), a member of the basic helix-loop-helix Per-ARNT-SIM family of transcription factors, was positively associated with the prognosis of breast cancer. In this study, we investigated the potential role of ARNT2 in molecular responses to environmental chemicals with estrogenic activities, known as xenoestrogens, in MCF7 human breast cancer cells. Xenoestrogens, such as bisphenol A, can significantly inhibit the mRNA expression of ARNT2 in MCF7 cells. The xenoestrogen-mediated decrease in ARNT2 expression was attenuated after the addition of the specific estrogen receptor 1 (ER\textsubscript{R}) antagonist, suggesting that ER\textsubscript{R} may play an important role in the mechanism by which xenoestrogens exert their effects on ARNT2 expression. Furthermore, the following function analysis revealed that knockdown of ARNT2 mRNA expression using small interfering RNA techniques significantly increased the expression of sensitive to apoptosis gene and decreased the expression of von Hippel-Lindau in MCF7 cells. The metabolite analysis revealed the contents of glucose, glycine, betaine, phosphocholine, pyruvate and lactate involved in the hypoia-inducible factor (HIF)-1-dependent glycolytic pathway were significantly decreased following knockdown of ARNT2 expression. Our results suggested that exposure to xenoestrogens may affect human cells by impacting ARNT2 function, including dysregulation of HIF-1-regulatory signaling and metabolism. This provides some concerns that xenoestrogens may reduce the natural defense of cells against cancer.

1979 EVIDENCE THAT SMAD5 DYSREGULATION IN HEMANGIOBLASTS MAY CONTRIBUTE TO HEMANGIOSARCOMA DEVELOPMENT IN MICE.


We have identified an intracisternal A particle (IAP)/an endogenous retroviral element insertion in intron 1 of the smad5 gene as a candidate hemangiosarcoma susceptibility locus in mice. SMAD5 is a critical signaling molecule in the bone morphogenetic protein (BMP)/transforming growth factor (TGF\beta) pathway involved in mesenchymal cell differentiation and plays an essential role in vasculogenesis. Smad5 KO is an embryonic lethal due to a lack of a well-organized vasculature in the yolk sacs. In the present study, we provide evidence that (1) spontaneous hemangiosarcomas from p53-/- mice express a combination of monocyte, leucocyte and endothelial cell markers (CD14, CD45, and CD31); and (2) genes in a branch of the BMP/TGFB signaling pathways that specifically regulate angiogenesis (TGFB\textgamma, ALK1, SMAD1/5, and ID3) are highly expressed in the hemangiosarcomas, and differentially regulated in MS1 mouse endothelial cells and C1C12 mouse myoblasts, e.g. BMP6 and TGFB\textgamma 1/2/3 induced phosphorylation of SMAD1/5/8 and ID1 expression in the endothelial cells, but only induced phosphorylation of SMAD1/5/8, not ID1 expression, in the myoblasts. The latter may explain why SMAD5 dysregulation affects hematopoietic hemangio blasts exclusively. Importantly we also found evidence of dysregulation of smad5 mRNA expression due to the presence of the IAP insertion; a splice variant of IAP-smad5 fusion mRNA is detected in RT-PCR reactions using an IAP-specific 5’ primer and a 3’ primer in exon 7 of the smad5 gene. The fusion mRNA is detected in all the hemangiosarcomas but not in control tissue from the same animal; it is also detected in embryonic stem cells that harbor an IAP in the smad5 gene. These studies support the hypothesis that smad5 dysregulation in hematopoietic progenitor cells, which suppresses endothelial cell differentiation, could contribute to the development of hemangiosarcomas in mice.

1980 SMAD5 AND ID1/ID3 PROTEIN EXPRESSION IN SPONTANEOUS HEMANGIOSARCOMAS WITH AN INTRACISTERNAL A PARTICLE (IAP) INSERTION IN THE SMAD5 GENE.

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Intracisternal A particle (IAP) sequences are endogenous retrovirus-like elements that can disrupt gene expression, and serve as species-specific tumor susceptibility factors in mice. A genotyping study found outbred CD-1 mice are heterogeneous (+/+, +/-, or -/-) for an IAP insertion in the smad5 gene, a key molecule in the TGFB/BMP signaling pathway, and all mice with hemangiosarcomas had at least one copy of the insertion. TGFB/BMP signaling pathways play roles as both inhibitors and stimulators of angiogenesis in endothelial cells. Inactivation of several molecules in TGFB/BMP signaling pathways have been shown to cause vascular abnormalities in gene knockout studies in mice, and in human hereditary haemorrhagic telangiectasia (HHT) is caused by mutations in ALK1 or endoglin (co-receptor) genes. Since dysregulation of the BMP/TGFB signaling pathways play role as both inhibitors and stimulators of angiogenesis in endothelial cells. Inactivation of several molecules in TGFB/BMP signaling pathways have been shown to cause vascular abnormalities in gene knockout studies in mice, and in humans hereditary haemorrhagic telangiectasia (HHT) is caused by mutations in ALK1 or endoglin (co-receptor) genes. Since dysregulation of the BMP/TGFB signaling pathways play roles as both inhibitors and stimulators of angiogenesis in endothelial cells, we analyzed the staining pattern, distribution, and intensity of Smad5, Id1, and Id3 in MS1 mouse endothelial cells and in spontaneous hemangiosarcoma from p53-/- mice. Protein expression of Smad5 was detected by western blot at the predicted MW of ~52 kDa, however, a variant of ~40 kDa was also detected in MS1 cells. The short variant protein may relate to the IAP insertion-induced deletion of exon 2, generating a new translation start from an ATG in exon 3. Immunohistochemistry revealed cytoplasmic expression of Smad5 and nuclear expression of Id1/Id3 in hemangiosarcoma in skin and muscle. These data confirmed the presence of Smad5, Id1, and Id3 in MS1 cells and in hemangiosarcoma tissue. Together with published data on the role of endostatin and DLL4/Notch signaling in regulation of Id1 and its downstream target, thrombospondin-1, our results provide further evidence that dysregulation of molecules in the BMP/TGFB signaling pathway leading to Id1 activation and thrombospondin-1 expression may play a key role in hemangiosarcoma development.
**1981** TAT-MEDIATED DELIVERY OF AN ARNT-INTERACTING PEPTIDE INTO CARCINOMA CELLS CAUSES SUPPRESSION OF THE HYPOXIA INDUCIBLE FACTOR-1 FUNCTION.


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The aryl hydrocarbon receptor nuclear translocator (ARNT) belongs to the basic helix-loop-helix Per-ARNT-Sim protein family. It heterodimerizes with the hypoxia inducible factor-1 alpha (HIF-1α) protein, which is essential for proper cancer cell growth. In addition, overexpression of HIF-1α has been associated with poor prognosis in many solid tumors. Our previous data showed that transient transfection of an ARNT-interacting peptide (Ainp1) expressing plasmid suppressed the HIF-1α signaling by an ARNT-mediated mechanism in Hep3B cells. Here we further examined whether delivery of the Ainp1 peptide directly into cells would be effective in suppressing the HIF-1α function. The bacterially expressed Ainp1 and TAT fusion of Ainp1 (TAT-Ainp1) were purified using 8M urea and then refolded by limited dialysis. Both Ainp1 and TAT-Ainp1 interacted with the basic helix-loop-helix domain of ARNT. After protein transduction, TAT-Ainp1 reached the maximum levels within two hours and remained detectable for up to 6 days in Hep3B cells. TAT-Ainp1 suppressed the cobalt chloride-dependent, HRE-driven luciferase expression in a dose-dependent manner. TAT-Ainp1 also suppressed the HIF-1α-dependent induction of the CAIX protein dose-dependently. TAT-Ainp1 caused the Hep3B cell death in the presence of cobalt chloride. We concluded that the Ainp1 peptide is capable of suppressing the HIF-1α function in Hep3B cells. This work is supported by NIH (WKC, R01ES014050).

**1982** IMMUNOHISTOCHEMICAL CHARACTERIZATION OF CELL PROLIFERATION-RELATED PROTEIN CHANGES IN CARCINOMA-MEDIATED LIVER TUMOR DEVELOPMENT.

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Key proteins in the process of CAR-mediated non-genotoxic hepatocarcinogenesis were evaluated by observing the expression of proliferation-related proteins in the foci of cellular alterations/adenomas produced by CYP2B inducers (piperonyl butoxide (PBO), phenobarbital (PB) and decabromodiphenyl ether (DBDE)) in C3H-derived wild and CARKO mice. Mice were treated with each chemical in diet for 13 or 27 weeks after diethylnitrosamine initiation. Cyclin D1, c-Myc, TGFβ receptors (TGFβR) 1 and 2 and phosphorylated (p-)Smad2/3 were selected as cellular proliferation-related proteins. After 13 weeks, eosinophilic foci were found in the wild PBO and PB groups, and basophilic foci were found in all groups including the CAR control and PBO groups, respectively, at week 13. At week 27, PB and PB increased eosinophilic foci/adenomas in wild mice but drastically reduced/eliminated them in CARKO mice. DBDE increased basophilic foci/adenomas in both groups. Immunohistochemically, cyclin D1 was positive in the cytoplasm and some nuclei only in eosinophilic foci at week 13. At week 27, the number of cyclin D1-positive nuclei had increased in the eosinophilic foci/adenomas, while it was primarily the cytoplasm that was positive in basophilic lesions. At week 13, c-Myc and TGFβRs were also strongly positive only in eosinophilic foci. The c-Myc signal was much stronger in the eosinophilic lesions at week 27. TGFβRs and p-Smad2/3 were positive in both types of lesions, but p-Smad2/3 staining was stronger in the basophilic lesions. These data suggest that cyclin D1 and c-Myc, which act downstream of CAR, might be involved in the development of eosinophilic lesions. In addition, CAR might be related to the expression of TGFβRs in the early process of hepatocarcinogenesis.

**1983** ANALYSIS OF MOLECULAR, CELLULAR, AND BIOCHEMICAL CHANGES IN THE LIVER OF MALE CD-1 MICE TREATED WITH THE HERBICIDE PRONAMIDE.

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To investigate potential modes of action (MoA) contributing to pronamide (3,5-dichloro-N-(1,1-dimethyl-2-propynyl)benzamide)-induced hepatocellular tumors at 250 mg/kg body weight/day (mld), male CD-1 mice were fed 0, 5, 20, 250, or 500 mld pronamide for 7, 14, or 28 days. Additional groups were given pronamide for 28 days then switched to a control diet for 35 days to investigate recovery. No alterations in clinical signs or body weight were noted in any of the treated groups. Animals administered 250 or 500 mld pronamide had statistically identified dose-related increases in liver weights, which corresponded to hepatocellular hypertrophy and cytoplasmic eosinophilia, and regressed during the recovery period. Analysis of hepatocellular proliferation via Bed1 incorporation indicated a clear dose-related induction of S-phase DNA synthesis in animals treated with 250 or 500 mld pronamide. Gene expression analysis of the liver indicated a robust, dose-related increase in Cyp2b10/CAR- and PPAR-t associated transcripts, consistent with direct activation of these nuclear receptors, but not AhR or PXR. Furthermore, a dose-dependent increase in peroxisomes was identified in the 250 and 500 mld dose groups, as determined by electron microscopy. Molecular and histopathologic analyses for the above endpoints in the recovery group indicated reversibility and were comparable to control. In summary, pronamide-induced effects are consistent with the causal, key events related to nuclear receptor-mediated robust activation of CAR and direct nuclear receptor activation, and were reversible on removal of the test material. Moreover, these data provided convincing evidence that key events and hepatocellular tumors do not occur at or below 20 mld. A Human Relevance Framework evaluation supports that this nuclear receptor-mediated MoA for mouse liver tumors associated with high doses of pronamide is not relevant to humans.

**1984** MITOCHONDRIAL UNCOUPLING PROTEIN 3 (UCP3) ANTAGONIZES EPIDERMAL TUMOR PROMOTION AND GROWTH SIGNALING.

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The propensity of tumors to exhibit increased glycolysis and decreased oxygen consumption compared to normal tissues is now considered a hallmark of malignancy. In contrast, mitochondrial uncoupling proteins drive futile mitochondrial respiration that is uncoupled from ATP synthesis. Our lab previously demonstrated that mice expressing a keratin 5 -uncoupling protein 3 (K5-UCP3) transgene in the epidermis were completely protected from the formation of skin carcinomas in response to a two stage chemical carcinogenesis regimen. To explore the mechanisms behind UCP3 induced cancer resistance, we inter-bred K5-UCP3 animals with Tg.AC mice that express a cutaneous oncogenic v-Ha-Ras transgene, allowing us to distinguish between effects of UCP3 over-expression on tumor initiation and tumor promotion. Whereas Tg.AC mice formed tumors in response to treatment with the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) alone, bigenic K5-UCP3/Tg.AC animals were protected from TPA-induced tumorigenesis, indicating that UCP3 likely inhibits tumor promotion. Moreover, K5-UCP3 mice exhibited a striking resistance to epidermal proliferation induced by TPA as measured using a bromodeoxyuridine (BrDU) based labeling assay. In agreement with these data, K5-UCP3 epidermis failed to show activation of Akt (Ser473 phosphorylation) and phosphorylation of downstream signaling molecules in response to TPA treatment. This effect corresponded to decreased expression of cyclins D1 and A, and increased expression of the cell cycle inhibitory proteins p21 and p27. Taken together, these data suggest that mitochondrial uncoupling can have pleiotropic inhibitory effects on cell growth and proliferation, and is a promising new avenue for cancer prevention research.

**1985** OVEREXPRESSION OF UBIQUITIN C INHIBITS EXACERBATED CHEMICALLY-INDUCED SKIN TUMORIGENESIS IN PPARβ/δ MICE.

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Previous studies have shown that 12-O-tetradecanoylphorbol-13-acetate (TPA) induces expression of ubiquitin C (UbC) in wild-type mouse skin but not in Pparβ/δ-null mice. Further, Pparβ/δ-null mice exhibit enhanced skin carcinogenesis due in part to decreased UbC mediated degradation of PKCδ. The present studies tested the hypothesis that increased expression of UbC in Pparβ/δ-null mice could reverse the enhanced tumor formation in Pparβ/δ-null mice. A tetracycline inducible transgenic mouse model in which UbC expression is driven by the keratin 5 promoter was generated and crossed with either wild-type or Pparβ/δ-null
The phosphorylated form (serine-209) of the eukaryotic translational initiation factor eIF4E exhibits pro-oncogenic activity and plays a critical role in cancer cell growth, transformation and metastasis. Ongoing studies in this laboratory demonstrate that the antitumorigenic activity of several agents is accompanied by induction of multiple phosphatases and some of the effects of these drugs are blocked by the phosphatase inhibitor sodium orthovanadate (SOV). For example, treatment of SW480 colon and LNCaP prostate cancer cells with synthetic cannabinoid WIN-55,212-2 (WIN) (7.5 μM) and 15 μM cannabidiol (CBD) inhibited cancer cell growth, induced apoptosis and multiple phosphatase genes and this was accompanied by decreased expression of several phospho-kinases and also phospho-eIF4E, in both cell lines. Treatment of SW480 cells with WIN or CBD alone decreased levels of phospho-eIF4E protein and co-treatment with 0.35 mM SOV significantly blocked cannabinoid-induced dephosphorylation of phospho-eIF4E in LNCaP cells. Protein phosphatase 2A (PP2A) has previously been linked to dephosphorylation of both eIF4E and Mnk-1, an upstream kinase that catalyzes phosphorylation of eIF4E. Treatment of SW480 cells with 7.5 μM WIN and knockdown of PP2A by RNA interference block dephosphorylation of phospho-eIF4E; however, the effects of CBD on dephosphorylation of phospho-eIF4E were PP2A-independent. Using a similar approach in LNCaP cells we showed that WIN and CBD also induced downregulation of Sp proteins that can be targeted by anticancer agents. The antitumor activity of CDODA-Me on Sp1, Sp3 and Sp4 proteins and Sp regulated genes was investigated in a panel of 4 colon cancer cells, SW480, RKO, HCT116 and HT29. Initial studies focused on the expression of several cancer stem cell marker proteins and the ability to form stem cell-enriched spheres. Results show that both HT29 colon cancer cell lines and four glioma cell lines which exhibited several cancer-stem-cell markers including increased expression of CD44, CD105, CD133, aldehyde dehydrogenase (ALDH1), HT29 cells formed significant number of primary and secondary spheres. The results with HT29 cells are characteristic of a population enriched in colon cancer stem cells. Results also show that CDODA-Me decreased HT29 adherent cell survival, expression of Sp1, Sp3 and Sp4 proteins and sphere formation. In the same cell line, CDODA-Me also decreased CD44, CD105 and ALDH1 expression which are cancer stem cell markers. Preliminary data using RNA interference studies suggest that the CD44 stem cell marker is also an Sp-regulated gene in HT29 cells. These results suggest that colon cancer stem cells also overexpress Sp1, Sp3 and Sp4 proteins that can be targeted by anticancer agents. The antitumor activity of CDODA-Me and Sp protein knockdown and effects on other cancer stem cell markers and sphere forming ability are currently being investigated.

Nuclear Respiratory Factor (NRF-1) is a redox sensitive transcription factor. The expression of 15-20% genes influenced by 17β-estradiol (E2) in breast cancer cells contains the binding sites for NRF-1. However, the role of NRF-1 in E2-mediated growth of breast cancer is unclear. The aim of this study is to test the effects of NRF-1 overexpression on the growth, migration and tumor formation of breast cancer cells. We have generated MCF-7 stable cell line with NRF-1 overexpression (MCF-7-NRF-1-OX). Stable expression of NRF-1 protein in MCF-7 cells was detected by western blot analysis and confocal fluorescence microscopy. Stable cells showed several fold higher NRF-1 expression compared to cells with vector alone. BrDu incorporation was the highest in stable cells with NRF-1 overexpression compared to cells with vector only control group. E2 treatment increased colony formation in MCF-7 cells with NRF-1 overexpression compared to cells containing vector. NRF-1 overexpression also increased in vitro cell migration and cancer cell aggregation. In summary, these data suggest that NRF-1 plays a role in regulating the proliferation, migration and in vitro tumor formation of breast cancer cells.
invasiveness. Two malignant head and neck tumor cell lines, OSC19 and HN30, were subjected to BaP (7.5 μM) for 24h followed by control: control, 2.3,7,8-tetrachlorodibenzop-p-dioxin (TCDD; 10 nM), a potent AHR agonist or CH233191, an AHR antagonist (500 nM). The greatest number of genes were altered by CH233191 compared to TCDD. Genes involved in various processes such as xenobiotic metabolism, apoptosis, signal transduction, angiogenesis and cellular proliferation were altered significantly. Notable among these was Angiopoietin-like 4 (ANGPTL4), an adipoctyokine, and RUNX2, an osteoblast differentiation factor both documented to be involved in various cancers. H3AS, involved in the production of hyaluronan and platelet derived growth factor A (PDGFα) implicated in cancers of the breast, prostate and pancreas, were also significantly up-regulated by TCDD, but mitigated by CH233191. Matrix metalloproteases (MMPs), epiregulin (EREG) and fibroblast growth factor (FGF2) were other significantly altered putative AHR target genes. Real time PCR analysis of these gene targets revealed a high level of correlation, with microarrays, for both OSC19 (r² = 0.96-0.99) and HN30 (r² = 0.91-0.99). Pathway analysis to map significantly altered genes with the pertinent pathways and/or diseases and functions was carried out. These studies point to certain previously unknown targets influenced by AHR, the regulation of which may represent a novel strategy to reduce the highly metastatic phenotype of these cells.

1991 CHARACTERIZATION OF HEPATOCELLULAR CARCINOMA-RELATED GENES AND METABOLITES IN HUMAN NONALCOHOLIC FATTY LIVER DISEASE.

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The worldwide prevalence of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH), the most advanced stage of NAFLD, are estimated to range from 9-37% and 5-17%, respectively. Hepatocellular carcinoma (HCC), the third most common cause of cancer related deaths worldwide, is primarily caused by hepatitis B infection, but retrospective data suggest that 4-27% of NASH cases will progress to HCC. Currently the connection between NASH and HCC is unclear. The purpose of this study was to identify expression changes of HCC related genes and metabolite profiles in NAFLD. Transcriptomic and metabolomic datasets from human liver tissue representing NAFLD progression (normal, steatosis, NASH) were utilized. For expression data, genes were divided into functional classes involved in carcinogenesis. Several classes of genes were over represented as upregulated in NASH that have also been reported to be upregulated in HCC. These include angiogenesis and fibrosis genes such as platelet derived growth factors and their receptors, and extracellular matrix and cell adhesion genes such as collagens, laminins and integrins. In contrast, genes involved in Wnt signaling and dose-dependent (p < 0.05). Based on the cytotoxic data, 20 μM of BaP were selected to treat BEAS-2B for 72 h for protein analysis. A total of nine different proteins involving in p53 function including total and phospho-p53 (phospho-Ser15 and Thr55), p53 nucleocytoplasmic shuttling factors (CRM1, Ran, RCC, and transportin1), and p53 downstream targets (p21 and survivin) were screened by multi-blot. Our data revealed that only phospho-p53 (Ser15) and p21 expression level were significantly up-regulated after BaP exposure as compared to the control (p<0.05). According to cytotoxicity assay and multi-blot test, 1, 5, and 20 μM of BaP and 0.05% DMSO (vehicle control) were chosen to treat BEAS-2B for 72 h. It showed a dose-response effect of up-regulation of phospho-p53 (Ser15) and p21 after BaP exposure as compared to the control (p<0.05). These results suggest that BaP could reduce cell viability possibly through increasing phosphor-p53 (Ser15) level and p21 expression. Further experiments are being pursued to elucidate the role of these protein(s)/pathway(s) in BaP transformed BEAS-2B cells. Findings of this study will facilitate the understanding of lung carcinogenesis after BaP exposure.

1994 1, 4-BENZOQUINONE-MEDIATED HISTONE MODIFICATIONS AND BENZENE MYELOTOXICITY.

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Redox-active and electrophilic quinone metabolites of benzene contribute to its myelotoxic effects, though the mechanism of carcinogenesis remains elusive. Administration of hydroquinone (HQ) and phenol (PHE) to rats reproduces benzene myelotoxicity. HQ oxidizes to 1,4-benzoquinone (1,4-BQ) and in the presence of glutathione (GSH) gives rise to multi-GSH substituted conjugates, which are present in bone marrow of rats or mice following PHE/HQ administration. HQ-GSH conjugates retain the ability to adduct proteins and to redox cycle. We now report that bone marrow malondialdehyde levels in PHE/HQ treated rats are 14-3-3 occurs in cancers of the lung, bladder, and liver. Adduction of 14-3-3 following exposure to benzene may cause chromatin remodeling abnormalities and genomic instability. Furthermore, metabolites of benzene bind to DNA and damage histones. This may lead to enhanced DNA breakage and the generation of DNA strand breaks. To
1995 PENTOXIFYLLINE DECREASES CYCLIN D1 THROUGH PROTEOSOMAL DEGRADATION AND ARRESTS RENAL CANCER CELLS IN THE G1 PHASE.

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Cyclin D1 is required for cells to progress from the G1 phase into the S phase of the cell cycle. Several tumors display elevations in cyclin D1 protein, concomitant with increased cell cycle progression and proliferation. QTRRE cells, a rodent cell model for renal cell carcinoma, are derived from the Eker rat (Tsc-2/−) which bears a germline mutation in the tuberous sclerosis-2 (Tsc-2) tumor-suppressor gene. QTRRE cells undergo spontaneous and chemically induced loss of heterozygosity and become tumorigenic when injected into nude mice. QTRRE cells also display elevated cyclin D1 protein levels. Pentoxifylline (PTX), a competitive non-specific phosphodiesterase inhibitor, has found recent use as an adjunct in chemotherapy for patients to help treat cachexia and capillary leak syndrome. We now report that PTX causes a time- (1-24 hr) and dose-dependent (35 μM - 3.5 mM) decrease in cyclin D1 protein levels. Subsequent analysis by RT-PCR revealed no significant changes in cyclin D1 mRNA suggesting that the PTX-mediated decrease in cyclin D1 protein are not regulated at the transcriptional level. However, PTX's ability to decrease cyclin D1 protein was abolished in the presence of a proteasome inhibitor, MG-132 (10μM). Consistent with decreases in cyclin D1, flow cytometric analysis revealed that 24hr PTX-treated QTRRE cells undergo cell cycle arrest in the G1 phase (85.9% relative to 50.6% in controls). Collectively the data suggest that PTX decreases cyclin D1 protein levels by stimulating proteosomal degradation, which promotes G1 phase cell cycle arrest in QTRRE cells. Moreover, because our findings indicate a novel anti-cancer chemotherapy property of PTX, the utility of PTX as an adjuvant therapy in the treatment of cancer, especially in tumors characterized by increases in cyclin D1 expression, should be further explored. The molecular mechanism underlying this effect of PTX is being investigated.

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1996 LOSS OF PPARγ IN ADIPOCYTES CREATES A PROTUMOURIGENIC ENVIRONMENT.

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One in 28 women currently dies from metastatic breast cancer. Improved understanding of genetic and environmental risk factors may aid in preventing deaths due to breast cancer. Previously, we showed using peroxisome proliferator-activated receptor (PPARγ) heterozygous (+/−) mice that PPARγ normally stops the in vivo progression of 7,12-dimethylbenz[a]anthracene (DMBA)-mediated breast tumours. Since PPARγ is expressed in adipocytes (A) and many cell types associated with breast tissue, each with unique signal patterns, we have also shown that A-specific deletion of PPARγ enhances susceptibility to DMBA-induced breast tumourigenesis. Here, we hypothesized PPARγ-A KO mice are more susceptible to multi-risk factor (DMBA + protumorigenic high fat (proHF)) diet-mediated breast tumourigenesis. To do this, we used previously generated PPARγ-A KO mice (n=11), and wild-type controls (WT) (n=25) treated with 1 mg p.o. DMBA once/week for 6 weeks, maintained on a proHF diet and monitored them for tumour outcomes for 25 weeks. Interestingly, overall survival was significantly reduced for PPARγ-A KO vs WT mice (100% vs 100%). The exquisite sensitivity of PPARγ-A KO mice to DMBA+proHF precluded breast tumourigenic evaluation. Nevertheless, serum analysis revealed that pro-tumorigenic leptin expression was 22.8% higher in PPARγ-A KO vs WT mice (p<0.05), and QRT-PCR showed that BRCA1 RNA expression was reduced 10-fold in PPARγ-A KO mice (p<0.01). Cell-specific immunofluorescent analysis of mammary glands indicated that BRCA1 reduction was specific to stromal adipocytes of PPARγ-A KO mice. Collectively, this data is the first to suggest that adipocyte-specific PPARγ protects against death associated with exposure to DMBA+proHF. Further, PPARγ activation within adipocytes may, in part, prevent breast tumourigenesis by maintaining reduced circulating levels of leptin and elevated levels of the breast tumour suppressor BRCA1.

1997 DEVELOPING A 3D CELL CULTURE MODEL OF DUCTAL CARCINOMA IN SITU.

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Purpose: To create a 3D model of human breast cancer progression in vitro in order to identify (1) molecular changes that occur in progression, (2) environmental toxicants that may stimulate progression, and (3) potential therapeutic targets for chemoprevention. Such a model is necessary because traditional 2D cell culture does not adequately represent the physiological factors that contribute to cancer development and progression. The bi-potent human breast cell line MCF10DCIS.com develops ductal carcinoma in situ (DCIS) in a mouse xenograft model. Hypothesis: We hypothesized that the DCIS.com cells would respond to a combination of growth factors and organize into 3D structures in a collagen matrix. Methods: A layer of collagen type 1 was laid down in the wells of 6 well tissue culture plates, topped with a collagen-DCIS.com suspension, followed by media. One week after plating, the growth factors CXCL12 (SDF-1) and TGFβ were added to the growth medium at every media change. Cell aggregation, including stellate structures and/or branching duct-like networks, was observed via light microscopy over the course of 1-2 weeks. Results: Immunohistochemical analyses confirmed the presence of both luminal and myo-epithelial cells the 3D/collagen cultures. Cytokine arrays revealed that the DCIS.com/3D collagen culture in the presence of growth factors secreted IL5 and IL6, whereas controls in the absence of growth factors did not. Also, exposure to the growth factors in 3D culture significantly increased the expression of 23 genes associated with extracellular matrix and adhesion compared to controls. Conclusion: Under the unique 3D culture conditions developed in our lab, we have demonstrated that the growth factor combination stimulates the DCIS.com cells to organize into duct-like structures. Pathological analysis is underway to verify that these structures do indeed recapitulate human breast cancer progression.

1998 DISTINCT CYTOCHROME P450 REGULATION BY PAH MIXTURES IN MOUSE SKIN: INSIGHTS ON CARCINOGENIC MECHANISMS FROM TRANSCRIPTIONAL PROFILING OF TUMOR INITIATION.

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The polycyclic aromatic hydrocarbons (PAHs) benzo[α]pyrene (BAP) and dibenz[def]chrysene (DBC) produce tumors in mouse skin, lung, liver and breast and were recently elevated to Class 1 known and Class 2A probable human carcinogens, respectively. However, most human PAH exposures result from chemical mixtures of multiple PAHs. Currently, little is known about the relative potency, carcinogenic potential and mechanisms of tumorigenesis for PAH mixtures. In this study, we measured gene expression profiles in skin of FVB/N mice collected 12 hours after initiation with BAP, DBC or one of 3 PAH mixtures (diesel exhaust, coal tar and cigarette smoke condensate). Overall, 922 probes were significantly (p<0.05) regulated compared to control across the study. Transcriptional signatures were determined for each PAH treatment and compared to tumor outcome to identify the molecular clusters responsible for the earliest events of carcinogenesis. Exposure of this mouse model to PAHs following a two-stage initiation/promotion skin tumor protocol resulted in tumor incidence and multiplicity profiles of BAP>BAP+Mix2=Mix3>Mix1-Control, based on statistical significance. The tumor profile was used to identify gene clusters and pathways that appropriately classified the PAH tumor outcomes. Transcript factor analysis of the predictive gene clusters further allows for identification of the upstream regulatory events associated with carcinogenesis. Of particular interest was a cluster of arylhydrocarbon receptor transcriptional targets, including Cyp1a1 and Cyp1b1, that were strongly up-regulated by BAP, Mix 2 and Mix 3 (p<0.01), but moderately down-regulated by DBC, in skin at 12 hours. These data provide a 'source-to-outcome' model that may be used to predict PAH interactions during tumorigenesis and provide mode-of-action based risk assessment of environmental PAH mixtures. Supported by P42_ES016465, P42_ES016465-S.
1999 THE ROLE OF ALDH1B1 IN ALCOHOL METABOLISM AND COLON CANCER.

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Aldehyde dehydrogenases (ALDHs) are a group of NAD(P)+-dependent enzymes involved in the metabolism of a wide spectrum of aliphatic and aromatic aldehydes. ALDH1B1 is a mitochondrial homotrameric enzyme, which is 65% and 72% identical to ALDH1A1 and ALDH2 proteins, respectively. In our in vitro studies have shown that human ALDH1B1 metabolizes acetaldehyde with an apparent Km of 55 μM, indicating an important role of this protein in alcohol metabolism. We have recently shown that ALDH1B1 expression in normal colon is sparse and confined to the crypt base where stem cells reside; whereas ALDH1B1 is extensively expressed in cancer cells of human colonic adenocarcinomas. Similar upregulation of ALDH1B1 was also observed in colon tumors from the multiple intestinal neoplasia (MIN) mice. These findings suggest a potential role of ALDH1B1 in colon carcinogenesis. To assess the in vivo role of ALDH1B1, we have generated transgenic Aldh1b1(−/−) null mice, in which the entire coding region of Aldh1b1 gene has been deleted. Aldh1b1(−/−) mice are fertile and have a normal growth pattern. ALDH1B1 messenger and protein were undetectable in examined tissues, including liver, colon, and small intestine. We also examined expression of ALDH2 and ALDH1A1 in these organs and did not find compensatory upregulation of these enzymes. Ethanol pharmacokinetics following a single injection of ethanol (i.p. 5 g/kg) did not reveal an significant differences between Aldh1b1(−/−) animals and Aldh1b1b1(−/+). Further studies using the Aldh1b1b1(−/) knockout mice that include comprehensive ethanol pharmacokinetics, alcohol drinking preference, and experimental colon carcinogenesis are currently underway in order to determine the role of ALDH1B1 in alcohol metabolism and colon cancer. This work is partially supported by NIH grants R01 EV011490 and R21AA017775.

2000 LATE-ONSET GENE EXPRESSION CHANGES IN THE LIVERS OF MALE C3H MICE MATERNALLY EXPOSED TO ARSENIC: IMPLICATIONS FOR AN INCREASE IN HEPATIC TUMOR INCIDENCE.

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Maternal arsenic exposure of C3H mice has been reported to increase hepatic tumors in male offspring (F1 mice) at 74 weeks-old. In order to characterize changes leading to tumor augmentation, we investigated gene expression changes in the F1 mice non-tumorigenic livers caused by gestational arsenic exposure. We also investigated L1 retrotransposon expression, which has been reported to be associated with chemical tumor promotion. We observed increased hepatic tumors in the arsenic-exposed males at 74 weeks-old as previously reported. Microarray and real-time PCR analysis revealed that more than 2-fold expression changes were induced in four genes in the non-tumorigenic livers of the arsenic-exposed mice compared to the control mice at 74 weeks-old. To trace the time course, expression changes of the four genes were also checked at 6 and 49 weeks-old. No significant changes were observed at 6 weeks-old, and two were detected at 49 weeks-old, indicating that the changes are late-onset. Among the four genes, one is implicated in apoptosis and another is involved in lipid metabolism. Changes in lipid metabolism are expected to increase oxidative stress. Consistently, the oxidative stress responsible gene HO-1 was significantly up-regulated in non-tumorigenic livers as well as the normal tissues in tumor-bearing livers in the arsenic-exposed mice compared to control livers. We also found that L1 expression was significantly increased in the tumorigenic livers from arsenic-treated mice compared to control mice. The involvement of late-onset gene expression changes and augmented L1 expression should be further investigated to delineate the mechanism of tumor promotion caused by gestational arsenic exposure.

2001 EFFECT OF STABLE OVEREXPRESSION OF PPARβ/δ IN HUH7 CELLS.

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Because there are conflicting reports in the literature suggesting that PPARβ/δ either promotes or inhibits tumorigenesis, there is a need for more studies to more definitively determine the role of this receptor in cancer models. In the present studies, Huh7 cells were used to generate a human hepatoma cell line overexpressing the bi-cistronic Migr1 retroviral system. Huh7 cells with stably integrated hPPARβ/δ-Mig1 (Huh7-hPPARβ/δ-Mig1) expressed markedly higher levels of both PPARβ and protein as compared to control Huh7 cells with stably integrated Mig1 vector (Huh7-Mig1) and parent Huh7 cells. Ligand activation of PPARβ/δ with GW0742 caused a markedly enhanced, dose-dependent increase in expression of PPARβ/δ target genes, angiopoietin-like protein 4 (ANGPTL4) and adipocyte differentiation-related protein (ADRP) in Huh7-hPPARβ/δ-Mig1 cells as compared to control Huh7-Mig1 and parent Huh7 cells. Real-time examination of cell proliferation did not reveal any change in growth between the three cell lines, in the presence or absence of ligand. Similarly, no changes in anchorage independent growth of Huh7 cells, Huh7-Mig1 cells, or Huh7-hPPARβ/δ-Mig1 cells was observed, with or without ligand activation, between the three cell lines. Ligand activation of PPARβ/δ did not protect against staurosporine-induced apoptosis in Huh7 cells, Huh7-Mig1 cells, or Huh7-hPPARβ/δ-Mig1 cells. Expression of PPARβ/δ mRNA and protein was not different between human tumor samples as compared to nontransformed control tissue. Combined, despite clear evidence of enhanced activity of PPARβ/δ in Huh7 cells overexpressing PPARβ/δ, the present studies indicate that PPARβ/δ does not modulate cell proliferation or apoptosis in Huh7 cells. Supported by CA124533, CA126826, CA141029, CA140369.

2002 FRY IS A NOVEL TUMOR SUPPRESSOR GENE CORRELATED WITH BREAST CANCER PROGRESSION.

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Quantitative trait locus (QTL) mapping in differentially susceptible rat strains identified the predicted mammalian FRY gene as a tumor suppressor gene conferring resistance to NMU-induced mammary tumorigenesis. Ectopic expression of FRY in human breast cancer cells significantly diminished tumorigenicity and invasiveness in vivo. Loss of FRY in normal mammary epithelial cells significantly altered cell morphology, polarity and adhesion. Our analysis of FRY in clinical breast cancer cohorts revealed that FRY gene expression was decreased in breast cancers and analysis of tissue microarrays by immunohistochemistry corroborated these findings further demonstrating that FRY protein is nuclear and significantly higher in normal epithelial cells than tumor cells. Further mining of gene expression data from >4,400 breast cancers demonstrated that decreased FRY is associated with loss of hormone receptors, undifferentiated histopathological subtypes, increased tumor grade and poor outcomes suggesting a role for FRY in tumor progression. These trends were then confirmed at the protein level in additional breast cancer cohorts. Research in lower eukaryotes, as well as our functional analyses of FRY in silico, in vitro, and in vivo, suggested that FRY is involved in epithelial mesenchymal transition (EMT) and as well as epithelial cell differentiation and polarity. We therefore also examined FRY levels in additional epithelial cell carcinomas (prostate, ovarian, lung and brain) and confirmed that FRY is decreased in these carcinomas as well. Our findings support the involvement of FRY in epithelial cell tumorigenesis and cancer progression.

2003 LOSS OF P53 ACCELERATES TUMOR FORMATION IN RAP80 MICE.

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The ubiquitin interaction motif (UIM)-containing protein RAP80 is a key player in DNA damage response (DDR). Our previous studies demonstrated that the expression of the RAP80 gene is regulated in a DNA damage-responsive manner by the master regulator p53. This regulation occurs at the transcriptional level through a noncanonical p53 response element in the RAP80 promoter. Although transcriptionally induced by p53, RAP80 regulates p53 through association with p53 and the E3 ubiquitin ligase HDM2, providing HDM2-dependent enhancement of p53 polyubiquitination and degradation. In this study, it was shown that loss of RAP80 increased the sensitivity of mouse embryonic fibroblasts (MEFs) to various DNA damage agents and promoted the transactivation of several pro-apoptotic p53 target genes. Loss of RAP80 enhanced the activation of p53-Chk2 signaling pathway.
and cell cycle arrest upon DNA damage. Immortalization of RAP80 null (RAP80-/-) MEFs by infection with SV40 large T-antigen, which suppresses p53, abrogated the p53 activation observed in primary RAP80-/MEFs. This study describes the generation and characterization of RAP80-/ and RAP80/p53 double knock-out mice. RAP80-/ mice are viable and no developmental defects were observed. RAP80-/ mice developed tumors spontaneously and upon IR. Loss of p53 accelerated spontaneous and IR-induced tumor formation in RAP80-/ mice. These data indicate that RAP80 functions as a tumor suppressor gene. Loss of RAP80 activates p53 signaling pathway upon environmental stresses and loss of p53 accelerates tumor formation in RAP80-/ mice.

2004 INVESTIGATING THE DUEL ROLES OF STRA6 IN CELL SIGNALLING AND VITAMIN A TRAFFICKING.
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The long-term goal of the present study is to understand how carcinogenesis can be reduced through the optimization of vitamin A trafficking and signaling in tissues prone to tumor development. However, little is known about vitamin A uptake, transport, or signaling in mammary, prostate, and colon epithelial cells. Retinol, the major circulating retinoid, travels to peripheral tissues in complex with retinol binding protein (RBP). Recently, a retinoic acid responsive protein with no previous known biological function, STRA6, has been identified as an essential transmembrane receptor that mediates retinol uptake in retinal epithelial cells. Recently, however, STRA6 has also been found to be a cell surface signaling receptor activated by the RBP-retinol complex. STRA6 contains an SH2 domain-binding motif in the receptor's cytosolic domain that can be activated through tyrosine phosphorylation after binding with the RBP-retinol complex. This activation leads to the recruitment and activation of Janus kinase 2 (Jak2) and Signal transducer and activator of transcription 5 (Stat5). Once activated, Stat5 is translocated to the nucleus where it regulates the expression of specific target genes related to differentiation, apoptosis, and anti-proliferation. In the current studies, we have tested our central hypothesis that phosphorylation of STRA6 recruits and activates the Stat5/Jak2 signaling cascade essential for the anti-proliferative effects of retinol in epithelial tissues. Our results have shown that treating cancer cells with all-trans-retinoic acid (ATRA) increased levels of STRA6. We investigated whether STRA6 phosphorylation of Jak2/Stat5 enhanced expression of PPARy and p21 and down regulated the expression of cell cycle regulators cyclin D1 in a dose dependent manner. Collectively these studies will offer new insight into the role vitamin A trafficking may have in the anti-proliferative actions of vitamin A derivatives.

2005 INVESTIGATION OF MOLECULAR MECHANISMS OF SUTENT-INDUCED BRUNNER'S GLAND CARCINOMA.

Sutent (sunitinib) is a multi-targeted receptor tyrosine kinase inhibitor with regulatory approvals for the treatment of second-line gastrointestinal (G3) stromal tumors, advanced renal cell carcinoma, and unresectable pancreatic neuroendocrine tumors. In carcinogenicity studies in both rash2 transgenic mice and Sprague-Dawley rats, Brunner's gland carcinomas, and unresectable pancreatic neuroendocrine tumors. In carcinogenicity studies in both rash2 transgenic mice and Sprague-Dawley rats, Brunner's gland carcinoma occurred in high dose-treated animals, with higher frequencies in males than in females. To investigate molecular mechanisms underlying Sutent-induced tumorigenesis, a study was performed in which C57/B16 mice were dosed orally for 8 weeks with vehicle control or 60 mg/kg/day Sutent. At study termination, no gross or microscopic lesions occurred in the GI tract, including the Brunner's glands. Global gene expression profiling was performed on RNA extracted and amplified from Brunner's glands in control and Sutent-treated mice. Statistical analysis revealed significantly perturbed genes attributable to gender or to effects of Sutent. Sutent treatment perturbed a greater extent of gene changes in males than in females, corroborating the gender differences in tumor incidence in carcinogenicity studies. Among significantly perturbed genes in males, there was enrichment in pathway genes of hypoxia signaling, energy imbalance (AMPK, fatty acid/cholesterol metabolism changes), DNA damage response, and pro-oncogenic signaling. These may lead to observed gene expression alterations in cell proliferation, cytokoskeletal reorganization, and cell cell communication, all of which are potential precursors to neoplastic transformation. Some of the same molecular signals could be discerned in Sutent-treated females, though to a much smaller extent. While the translatability of this carcinogenic finding to patients on Sutent therapy is unknown, future work to identify kinase(s) responsible for Brunner's gland carcinomas may provide value to other therapies that target specific kinases inhibited by Sutent.

2006 STUDIES CONDUCTED IN A MURINE MODEL OF BREAST CANCER METASTASIS REVEAL THAT AHR ACTIVATION INHIBITS THE SPREAD OF TUMORS TO SECONDARY SITES.
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Aryl hydrocarbon receptor (AhR) agonists can slow or reverse growth of primary mammary tumors in rodents, which has fostered interest in developing such compounds for the treatment of breast cancer. Despite promising evidence that AhR modulation may be useful in controlling primary tumor growth, a more important therapeutic goal for breast cancer treatment is to reduce or prevent metastasis, the primary cause of mortality in women with this disease. To test the effect of AhR activation on metastasis, 4T1.2 mammary tumor cells were injected into the mammary fat pad of syngeneic Balb/c mice treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Primary tumor growth was monitored for 4 weeks, at which time metastasis was determined. TCDD treatment suppressed metastasis by approximately 50%, as measured both in the lung, and in mammary glands at sites distant from the primary tumor. Growth of the primary tumor itself was not suppressed by TCDD exposure, nor was proliferation, migration, or colony formation of 4T1.2 cells affected by TCDD treatment in vitro. Our results suggest that the protective effect of AhR activation was selective for the metastatic process, and not simply the result of a direct decrease in tumor cell proliferation or survival at the primary site. These observations in immunologically intact animals warrant further investigation into the mechanism of the protective effects of AhR activation, and support the therapeutic potential of AhR modulators.

2007 ASPIRIN INHIBITS COLON CELL AND TUMOR GROWTH AND DOWNREGULATES SPECIFICITY PROTEIN (SP) TRANSCRIPTION FACTORS.
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Background and Aims: Acetylsalicyclic acid (aspirin) is a chemotherapeutic agent for colon cancer patients; however, the mechanisms are not well defined and were investigated in this study. Methods: Inhibition of colon cancer cell growth and induction of apoptosis by aspirin and sodium salicylate were investigated using cell counting and Annexin V staining, and modulation of specificity protein (Sp) transcription factors Sp1, Sp3, Sp4 and Sp-regulated gene products was determined by western blot analyses of nuclear, cytosolic and whole cell lysates. Mechanisms of aspirin- and sodium salicylate-induced downregulation were investigated using pathway-specific inhibitors and the in vivo anticancer activity of aspirin was determined in athymic nude mice bearing RKO cells as xenografts. Results: Aspirin and its major metabolite sodium salicylate induced apoptosis and decreased colon cancer cell growth and this was accompanied by caspase-dependent proteolysis of Sp1, Sp3 and Sp4 and decreased expression of Sp-regulated gene products including bel-2, survivin, VEGF, VEGFR1, cyclin D1, c-MET, p65 and b-catenin. Aspirin also inhibited colon tumor growth and decreased Sp1, Sp3 and Sp4 expression in tumors. Conclusions: Aspirin-induced repression of Sp transcription factors and Sp regulated genes play a role in the cancer chemotherapeutic effects of aspirin. Since patients administered aspirin exhibit high salicylate/aspirin serum ratios, results of this study suggest for the first time that salicylate may be the major contributor to the anticancer activity of aspirin in colon cancer.

2008 MODE OF ACTION FOR THE SYNTHETIC PYRETHROID PERMETHRIN-INDUCED MOUSE LIVER TUMORS: EVIDENCE FOR PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR ALPHA (PPARα) ACTIVATION AND ASSOCIATED LIVER CHANGES.
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Two-year treatment with permethrin produced benign liver tumors in both sexes of CD-1 mice. To understand the mode of action (MOA) by which the mouse liver tumors are produced, a series of studies conducted in mice examined the effects of
Cinnamon is a widely used spice in Norway, and can be used as topping on oatmeal porridge. Based on a study on coumarin concentrations in Norwegian foods, intake calculations were conducted to estimate a risk assessment of coumarin in the Norwegian population was performed. Intake estimates of coumarin shows that small children eating oatmeal porridge several times a week sprinkled with cinnamon had a coumarin intake of 1.63 mg/kg bw/day and therefore are at risk for exceeding the TDI with several folds. Adults drinking cinnamon-based tea and consuming cinnamon supplements can also exceed TDI. The coumarin intake could exceed the TDI by 7-20 fold in some intake scenarios. Liver toxicity may occur shortly after the start of coumarin exposure. Such large daily exceedances of TDI, even for a limited time period of 1-2 weeks, cause concern of adverse health effects.

Nutrition is a focal point of medical research as excess caloric intake is correlated with detrimental health outcomes, such as the metabolic syndrome; however in contrast, low-calorie diets and diets enriched in omega-3 essential fatty acids (EFAs) have beneficial health effects, such as lower cancer rates. However, our understanding of the causal relationship between diet and health is limited and largely elusive. We studied the molecular changes elicited by diets high in fat, sugar, or cholesterol, as well as diets deficient in calories or omega-3 and -6 fatty acids in C57BL/6 male mice. Microarrays were conducted on liver samples from 3 mice/diet and detected 20,449 unique genes of which 3,561 were responsive to diet. Correlational analysis found that diet restriction was the most unique diet because it correlated the least with, and affected more genes than the other diets. The majority of the diets affected several canonical pathways including the citric acid cycle, FXR/RXR activation, LPS/IL-1 mediated inhibition of RRX function, short chain fatty acid metabolism, and NRF2-mediated oxidative stress response. Of the 498 transcription factors, 87 were responsive to the diets with the majority belonging to the Zipper-Type (38%) and Zinc-Coordinating (31%) classes of transcription factors. Two-way hierarchical clustering of the nine diets identified three major patterns of transcription-factor expression: 1) most highly expressed in the diet-restriction group; 2) most lowly expressed in the diet-restriction group; and 3) most lowly expressed in the high-fructose and EFA-deficient diets. This study provides considerable insight into the molecular changes incurred by different diets and further our understanding of the causal relationships between diet and health.

Activation of Peroxisome Proliferator-Activated Receptor γ (PPARγ) has been associated with beneficial health effects like enhancing insulin sensitivity and lowering glucose and fatty acid levels in type 2 diabetic patients. The aim of the present study was to develop and validate a luciferase reporter gene assay to enable fast and low-cost measurement of PPARγ agonist and antagonist activity. Two reporter cell lines were obtained by stable transfection of U2OS cells with a PPARγ expression vector containing a selection marker and a luciferase reporter construct, respectively. pSG5-neo-PPARγ1 and pGL3-3xPPRE-tata-luc to generate the PPARγ1 CALUX (Chemical Activated Luciferase Expression) reporter line, pSG5-neo-PPARγ2 and pGL4-3xPPRE-tata-luc to generate the PPARγ2 CALUX line. In these PPARγ1 and PPARγ2 CALUX cells a range of known PPARγ agonists induced concentration-dependent luciferase activity. Their potency ranked in the following order: 1) highly expressed in the diet-restriction group; 2) most lowly expressed in the diet-restriction group; and 3) most lowly expressed in the high-fructose and EFA-deficient diets. This study provides considerable insight into the molecular changes incurred by different diets and further our understanding of the causal relationships between diet and health. [Support: NIH grants DK-081461, ES-019487, and ES-009649; CCHIR]

2010 RISK ASSESSMENT OF COUMARIN USING THE BENCH MARK DOSE (BMD) APPROACH: CHILDREN IN NORWAY, WHO REGULARLY EAT OATMEAL PORRIDGE WITH CINNAMON, ARE AT RISK FOR EXCEEDING TDI FOR COUMARIN BY SEVERAL FOLDS.


Cinnamon is a naturally occurring flavouring in cinnamon and many other plants. It is known that cinnamon can cause liver toxicity in several species and liver tumours in mice, and it is considered a non-genotoxic carcinogen in rodents. By using the bench mark dose approach we re-assessed coumarin toxicity and established a new TDI for coumarin of 0.07 mg/kg bw/day. Oral intake of coumarin is mostly related to consumption of cinnamon-containing foods and food supplements. Coumarin intake from food has previously not been estimated in Norway. Cinnamon is a widely used spice in Norway, and can be used as topping on oatmeal porridge. Based on a study on coumarin concentrations in Norwegian foods, intake calculations were conducted to estimate a risk assessment of coumarin in the Norwegian population was performed. Intake estimates of coumarin shows that small children eating oatmeal porridge several times a week sprinkled with cinnamon had a coumarin intake of 1.63 mg/kg bw/day and therefore are at risk for exceeding the TDI with several folds. Adults drinking cinnamon-based tea and consuming cinnamon supplements can also exceed TDI. The coumarin intake could exceed the TDI by 7-20 fold in some intake scenarios. Liver toxicity may occur shortly after the start of coumarin exposure. Such large daily exceedances of TDI, even for a limited time period of 1-2 weeks, cause concern of adverse health effects.

2013 TOXICOLOGICAL PERSPECTIVE OF NRF2 ACTIVATION.

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There is growing interest to identify chemicals activating the nuclear factor erythroid-derived 2-related factor (Nrf2) pathway because of their potential in cancer prevention. From a toxicological perspective, there is a broad diversity amongst Nrf2-inducers, some of them being potent while others weak toxicant. This raises the question of their safety in use. We have applied a multiparameter approach using transcriptomics, cytotoxicity and DNA-damage endpoints in primary rat hepatocyte cultures in order to evaluate the dose response effects and consequent biological significance of Nrf2 inducers. A number compounds covering a wide from low to high range of toxic potency (e.g. rosemary extract, carnosic acid, carnosol, genistein, alpha-lipoic acid coumarin, tert-butylhydroquinone (tBHQ), cadmium and acrolein), were selected. Dose response effect analysis applying a linear regression correlation of transcriptomics data reveals distinct subsets of transcripts correlating with either cytotoxicity or DNA-damage. Gene expression confirmation followed by linear regression of the selected subsets was performed with all test compounds confirming two highly statistically significant specific dose-related prediction fingerprints: cytotoxicity-fingerprint and DNA-damage-fingerprint. Specific data mining of Nrf2 pathway using Nrf2 binding sequence data was also allowed by linear regression of the selected subsets was performed with all test compounds confirming two highly statistically significant specific dose-related prediction fingerprints: cytotoxicity-fingerprint and DNA-damage-fingerprint. Specific data mining of Nrf2 pathway using Nrf2 binding sequence data was also performed. These data revealed an enrichment of exons directly correlated with increased cytotoxicity strongly suggesting a cytotoxic base effect when potentiating Nrf2 activity. Overall, the data suggest that dose-responses of all parameters studied are correlated and that Nrf2-activation cannot be easily discriminated from toxic/genotoxic responses. Activation of Nrf2-pathway cannot be considered as intrinsically innocuous and a thorough safety assessment is necessary prior to any application of Nrf2-inducers.

2014 THE NEPHROTOXICITY OF MYCOTOXIN CITRININ AND PATULIN DURING ZEBRAFISH DEVELOPMENT.

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Both citrinin (CTN) and patulin (PAT) are fungal secondary metabolites showing nephrotoxicity in various cellular and animal models. In the present study zebrafish embryos were used as vertebrate models for examining the early developmental effect of CTN and PAT. Various concentrations of mycotoxins were introduced to zebrafish embryos at 24 hour postfertilization (hpf), and the morphology and cell viability of embryos were observed at 72 hpf. CTN at the concentration of 50 μM caused the larvae showing significant aberrant morphology including heart tube malformation, pericardial and yolk-sac edema. On the other hand, the zebrafish embryos treated with PAT at concentrations up to 200 μM exhibited no evident differences in survival rates and morphological changes of larvae compared to the solvent-exposed group. A green fluorescent kidney line, Tg(wt1b:GFP), was used as a model to evaluate the kidney morphology after mycotoxin exposure. All the embryos, which have been treated with CTN or PAT for 24 or 48 h, did not demonstrate any abnormal kidney phenotypes under the fluorescent microscope. Since wt1a and Nsr4/Kr-ATPasce are regarded as the specific markers for the pronephric glomerulus and tubule/duct, respectively, we further carried out the whole mount hybridization/immmunostaining and found that only PAT, but not CTN, induced an apparent delay in the glomerular development; however, no pronephric tubule/duct malformation was displayed after either CTN or PAT treatment. It is known that glomerular filtration rate (GFR) is a quantitative measure of renal function, so the clearance rate of microalbumin, rhodamine 123 (10,000 Mz) was applied to assess GFR in larval zebrafish. Incubation of 24 hpf zebrafish with 7.5 μM CTN and 50 μM PAT for 48 h significantly decreased the their GFR (0.006-0.008 μM PAT for 48 h significantly decreased the their GFR) and 44.7% of the control level, suggesting low levels of CTN and PAT are able to damage the renal function of developing zebrafish. Potential drugs will be applied to see whether they can rescue the nephrotoxicity of mycotoxins.

2015 OIL SPILL CHEMICAL DISPERSANTS AND SEAFOOD SAFETY.


In 2010 the drilling rig explosion in the Gulf of Mexico resulted in a major oil spill. To minimize the environmental impact of the oil spill, chemical dispersants, such as COREXIT® EC9527A and COREXIT® 9500 were applied as remediation prod-

2016 LEVEL OF CONCERN FOR GLUTEN EXPOSURE IN INDIVIDUALS WITH CELIAC DISEASE.

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Celiac disease (CD) is a permanent hypersensitivity reaction in genetically predisposed individuals triggered by ingestion of certain grains or certain protein components of them. Gluten is the protein in wheat grain that elicits this disease state. Exposure to gluten in individuals with CD results in morphological damage to the lining of the small intestine. This exposure can also be associated with the emergence of a diverse array of clinical signs and symptoms in CD sufferers. Avoidance of gluten is the principal tool for management of these adverse effects. Thus, the determination of the background level of gluten exposure that is tolerable in sensitive individuals consuming a gluten-free diet is relevant. The toxicological level of concern (LOC) for gluten that is associated with CD-related morphological and clinical effects was derived from the identified gluten tolerable daily intake (TDI) values for each of these effects and estimates of the level of exposure to gluten-free food (GFF). The TDIs were previously determined from a health hazard assessment of dose-response data on adverse effects from oral food challenge studies in those susceptible to CD. They were derived from morphological and clinical effects of concern and were, respectively, 0.4 and 0.015 mg gluten/day. Food consumption estimates for GFFs that would replace foods containing the gluten-containing grains or their derivatives were determined from the 1994-1998 USDA Continuing Survey of Food Intake by Individuals (CFSII) database. This exposure assessment characterized daily food intake at the mean and 90th percentile levels for children (1-18 years: 0.4-0.7 kg food/day) and adults (>18 years: 0.4-0.9 kg food/day).

2017 POMEGRANATE POLYPHENOLS INHIBIT BREAST CANCER CELL GROWTH TARGETING miRNA-27A AND miRNA-155 IN THE REDUCTION OF INFLAMMATION AND CELL GROWTH.

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Several studies have demonstrated that botanical polyphenolics including extracts from pomegranate (Punica granatum L.) are potent inhibitors of cancer cell proliferation inducing apoptosis. There is growing evidences that specificity protein (Sp) transcription factors are overexpressed in tumors. Previous studies show that Pg extracts decreased inflammation in lung cell lines by inhibiting P38 dependent phosphorylation of AKT in lung and inhibited activation of NF-κB in vivo. The objective of the present study was to investigate anticancer and anti-inflammatory activity of Pg extracts in estrogen receptor positive (ER+) BT474 breast cancer cells and in nude mice and the effects on miRNA-27a-ZBTB10-Sp1 axis and microRNA155-SHPI1-AKT-NFκB axis.
The effects of Pg extract on cell viability were investigated in BT474 (ER+) MDA-MB-231 (ER-) cells. Gene expression was determined by q-RT-PCR and luciferase activity was used to determine activation of transfected constructs containing luciferase reporter genes. Proteins were identified by western blotting. The role of specific microRNAs was confirmed using specific inhibitors.

Results showed that cell proliferation was inhibited by Pg extract (2.5-25 μg/ml) in BT474 and MB-231 cells. Pg extract significantly decreased specificity protein Sp1, Sp3, and Sp4 and increased expression of the transcriptional repressor ZBTB10 and decreased miR-27a in BT474 cells. Pg extract also increased SHIP-1 protein expression and this was accompanied by down-regulation of miRNA 155 in the treated cells and inhibited PISK dependent phosphorylation of AKT. Similar results were observed in tumors from nude mice bearing BT474 cells as xenografts.

In summary the anticancer activities and anti-inflammatory effects of Pg in breast cancer cells were at least in part due to targeting microRNAs155 and 27a which play an important role in the proliferative/inflammatory phenotype exhibited by these cell lines.

2018 UPSN INCLUDED IN FOOD REDUCES AFLATOXIN BIOAVAILABILITY IN A CROSSOVER STUDY IN GHANA.

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Uniform Particle Size NovaSil (UPSN), a calcium montmorillonite, has high binding affinity for aflatoxin B1 (AFB1), a natural hepatocarcinogen produced by Aspergillus flavus and A. parasiticus species. Aflatoxin exposure occurs particularly in African and Southeast Asia due to a favorable climate for fungal growth and economic burdens. Chronic exposure to AFB1 in Ghana is an important contributor to the country’s high incidence of primary liver cancer. In this crossover study, participants (46) from the Ejura district were provided with common Ghanaian meals twice/day. Participants were split into two groups receiving 3g/day of either UPSN treatment or placebo mixed in their food. Participants were switched to the opposite treatment group halfway through the study (5 days). Palatability questionnaires and urine samples were collected each day. AFB1 biomarkers of exposure were analyzed using AflaTest® immunoaffinity columns followed by HPLC coupled fluorescence detection. At baseline AFB1 was detected in 100% of the population with levels ranging from 15.03-5,454.88pg/mg creatinine. During treatment, the placebo group had significantly higher AFB1 levels than UPSN treated participants with the data clearly illustrating the switch in treatment. No adverse events resulted from treatment and the application of UPSN in food was well received with taste, aroma and texture all scoring highly. These results suggest that the inclusion of UPSN clay in foods can considerably reduce aflatoxin bioavailability in a dose form that is easily administered, economical and culturally acceptable. This clay can positively impact the health and livelihood of populations in developing countries where staple crops are often contaminated with aflatoxin. (Supported by USAID LAG-G-00-96-90013-00 and NIH 1RO1MD005819-01).

2019 CHRONIC DIETARY TRYPPTOPHAN ENHANCEMENT AND DEPLETION IMPROVE MOUSE SOCIAL BEHAVIOR AND INCREASE BRAIN EXTRACELLULAR SEROTONIN LEVELS.


Social interaction deficits are prominent characteristics of autism that are modulated by serotonin (5-HT) signaling in the brain. While roughly 33% of autism patients have elevated platelet 5-HT, their brain 5-HT stores and signaling may be reduced. Clinical evidence of this includes behavioral improvement with 5-HT reuptake inhibitors, and worsening with tryptophan (TRP) depletion. Children with autism, particularly those on casein or gluten-free diets, tend to have low plasma TRP levels. Deficits in brain 5-HT vs. kynurenic metabolism are ameliorated in some with B vitamin supplements. We hypothesized that diets enhanced with 5% TRP and B-complex would improve sociability and boost brain 5-HT neurotransmission in mice (BTBR and 129S1/SvImJ strains) with low sociability. Conversely, chronic TRP depletion was expected to reduce brain 5-HT availability and worsen social behavior. Male mice (90-120 days old, N=8-10) were maintained on special diets (Research Diets Inc., NJ) for 28 days. B-vitamins did not affect any parameter measured. In social approach tests, surprisingly, both TRP enhancement and depletion improved sociability in both strains (p<0.001), but marble-burying was unaffected. Mice on TRP-depleted diets lost weight, despite normal food consumption, their serum corticosterone levels were higher (p<0.05, N=5), and 5-HT transporter levels in the hippocampus and caudate nucleus were higher (p<0.05, N=4-8). Higher serum 5-HT levels were found in all 129S mice, and oxytocin, a hormone associated with social bonding regulated by 5-HT, was higher only in TRP-depleted BTBR mice. The improved social behavior with both dietary TRP manipulations is a likely product of enhanced 5-HT neurotransmission, since dietary TRP supplementation increased brain 5-HT stores, and corticosterone can block 5-HT uptake via “uptake 2” transporters such as organic cation transporter 3. This study was supported by the Morrison Trust.

2020 CHRONIC EPITHELIAL NOD2 ACTIVATION TRIGGERS ANTINFLAMMATORY ATF3 AND PARADOXICAL SUPER-INDUCTION OF PROINFLAMMATORY CHEMOKINES BY CHEMICAL RIBOSOME-INACTIVATING STRESS.

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Mucosal experience of gut bacteria and bacterial products including Nod2 ligands triggers homeostatic regulation in response to various mucosal toxic insults. ATF3 is a negative modulator of proinflammatory responses via bacterial pattern recognition. On the assumption that ATF3 can be a critical regulator of epithelial inflammation, chronic epithelial exposure to Nod2 ligand was assessed for its effects on ATF3 and proinflammatory signals in response to mucosal ribosome-inactivating stress, which is a critical etiological factor of human intestinal inflammatory disease. Mucosal dipeptidylpeptidase (MDP) pre-exposure to Nod2 ligand, including muramyl dipeptide (MDP), is recognized by the Nod2 receptor, is an Nod2 ligand, and pre-exposure to it enhanced ATF3 expression in chemical ribosome-inactivating stress-exposed human enterocytes. In terms of gene regulation, Nod2-preactivation potentiated ATF3 induction by enhancing stability of the ATF3 transcript, which was particularly linked to the regulation of the 3' untranslated region of the human ATF3 gene. Moreover, chronic stimulation of Nod2 enhanced both the basal and chemical ribosome-inactivating-stress-stimulated cytoplasmic translocation of the HuR protein, which bound to and stabilized ATF3 mRNA. Functionally, chronic stimulation of Nod2 also led to superinduction of pro-inflammatory chemokine genes by the muco-active ribosome-inactivating stress. However, the chemokine superinduction was not affected by ATF3 gene regulation although Nod2-triggered ATF3 had suppressive effects on the pro-inflammatory NF-kB signal. This paradoxical superinduction of chemokines was also mediated by enhanced mRNA stabilization by HuR protein in spite of ATF3-mediated suppression of NF-kB signal in human intestinal epithelial cells. (This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (MEST) (No. 2009-0087028 and No. 2009-0065479)).

2021 EFFECTS OF AFLATOXIN B1 ON THE IMMUNE PHENOTYPES AND CYTOKINE SECRECTIONS OF SPLEEN LYMPHOCYTES IN FISCHER 344 RATS.

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Aflatoxin B1 (AFB1) has been linked to suppressed cell-mediated immune status in human populations. In this study we examined the effects of AFB on spleen lymphocyte phenotypes and the secretion functions of CD4+, CD8+ T cells and CD3-CD8+ NK cells in rats. Male Fischer 344 rats were randomly assigned to five groups and treated orally with 0, 5, 10, 25, and 75 μg AFB/kg body weight. Animals were sacrificed at 1-, 3-, and 5-week and their spleen tissues were dissected and lymphocytes were isolated for measuring surface markers and secretion of proinflammatory cytokines. While roughly 33% of autism patients on casein or gluten-free diets, tend to have low plasma TRP levels. Deficits in brain 5-HT vs. kynurenic metabolism are ameliorated in some with B vitamin supplements. We hypothesized that diets enhanced with 5% TRP and B-complex would improve sociability and boost brain 5-HT neurotransmission in mice (BTBR and 129S1/SvImJ strains) with low sociability. Conversely, chronic TRP depletion was expected to reduce brain 5-HT availability and worsen social behavior. Male mice (90-120 days old, N=8-10) were maintained on special diets (Research Diets Inc., NJ) for 28 days. B-vitamins did not affect any parameter measured. In social approach tests, surprisingly, both TRP enhancement and depletion improved sociability in both strains (p<0.001), but marble-burying was unaffected. Mice on TRP-depleted diets lost weight, despite normal food consumption, their serum corticosterone levels were higher (p<0.05, N=5), and 5-HT transporter levels in the hippocampus and caudate nucleus were higher (p<0.05, N=4-8). Higher serum 5-HT levels were found in all 129S mice, and oxytocin, a hormone associated with social bonding regulated by 5-HT, was higher only in TRP-depleted BTBR mice. The improved social behavior with both dietary TRP manipulations is a likely product of enhanced 5-HT neurotransmission, since dietary TRP supplementation increased brain 5-HT stores, and corticosterone can block 5-HT uptake via “uptake 2” transporters such as organic cation transporter 3. This study was supported by the Morrison Trust.
Deoxynivalenol (DON, vomitoxin) is a common type B trichothecene mycotoxin that naturally contaminates cereal grain products worldwide. Adverse effects of DON include acute gastroenteritis and vomiting in humans, and anorexia, growth suppression and immune system perturbations in mouse models. Regulatory levels of DON in food have been based on the dose that does not cause weight suppression in mice (1 ppm DON). Though DON-induced anorexia is likely responsible for subsequent weight suppression, the exact mechanisms of anorexia have yet to be clarified. Recently, published data (Flannery et al. Food Chem Toxicol. 2011) suggested that DON-induced anorexia was attenuated at 1 mg/kg bw by 180 min and at 5 mg/kg bw by 360 min. Here, using a mouse model, we tested the hypothesis that DON-induced anorexia was attenuated at 1 mg/kg bw by 180 min and at 5 mg/kg bw by 360 min. DON is a potent anorexigenic agent that increases the levels of proinflammatory cytokines and decreases the levels of proanorectic hormones, such as PYY and CCK. This study was conducted to evaluate the use of HPLC to measure the sorption of Aflatoxin B1 (AFB1) and Fumonisin B1 (FB1) onto NovaSil™ clay (NS). AFB1 and FB1 were analyzed with each isotherm and consisted of water, water plus clay, and AFB1 or FB1 spiked with concentrations ranging from 0.4-8.0 (ppm). Three controls were analyzed with each isotherm and consisted of water, water plus clay control. The samples were capped, shaken for 2 hours at 1,000 rpm, and analyzed with each isotherm. The NOAEL > 100 μM. This indicates an in vitro detoxifying phase II and antioxidant enzymes by binding of the nuclear factor E2-related factor 2 (Nrf2) to the electrophile-responsive element (EpRE). The aim of the present study was to determine the potency of phytotoxins present in tomato fruit, as well as extracts of tomato fruit to induce EpRE-mediated gene expression using the EpRE(KNOQ1)-LUX reporter cell line, based on an EpRE element from the human NADPH:quinone oxidoreductase 1 (NQO1) gene (Boeboem et al., Biochem Pharmacol 2006; 72: 217-226). Of the phenolic compounds known to be present in tomato, kaempferol, quercetin, naringenin and naringenin chalcone were able to induce EpRE-mediated luciferase activity. Of the isoprenoids known to be present in tomato, including lycopene, lutein, β-carotene, α-, β-, and γ-tocopherol and neoprenoester, only the latter was able to induce EpRE-mediated luciferase activity. An extract of ripe tomato fruits, enzymatically hydrolyzed to remove the glycosyl residues from the bioactive phenolic ingredients, was able to induce EpRE-mediated luciferase both on protein and mRNA level. PCA analysis of LC-MS data quantifying the chemical contents of extracts from tomato varieties with high and low inducing capacity suggested that quercetin was an important contributor to the induction of EpRE-mediated gene expression by tomato extracts. It was concluded that induction of EpRE-regulated genes, such as detoxifying phase II and antioxidant enzymes, may contribute to the beneficial health effects of tomatoes. Subsequent studies will focus on in vivo validation of these findings.
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The inclusion level of NS clay used in the present study for isothermal analysis was FB1 and AFM1 levels following intervention with NS in capsules at 1.5 or 3 g/day.

same concentration. Recent studies in Ghana reported a reduction in both urinary prothrombin and the resulting isotherm does not fit the Langmuir model. When combined, a lower percentage of each mycotoxin is bound to the clay. For instance, at 8 ppm AFM1, the clay bound about 10% less when AFM1 was present at the same concentration. For FB1, the clay bound about 67% less when AFB1 was present at the same concentration. Recent studies in Ghana reported a reduction in both urinary prothrombin and the resulting isotherm does not fit the Langmuir model. When combined, a

2027 DEVELOPMENT OF A MONOCLONAL ANTIBODY AGAINST OKADAIC ACID AND ITS APPLICATION IN ENZYME-LINKED IMMUNOSORBENT ASSAY AND GOLD NANOPARTICLE IMMUNOCHROMATOGRAPHIC STRIP.

Okadaic acid (OKA) is a toxin that accumulates in bivalves and causes diarrheal shellfish poisoning. A monoclonal antibody (mAb) specific to okadaic acid (OKA) was produced from a stable hybridoma cell line, 6B1A3, generated by the fusion of P3/NS1/1-A4G-1 myeloma cells with spleen cells isolated from a BALB/c mouse immunized with OKA-r-globulin. The 6B1A3 mAb binds to the immunoglobulin G1 (K chain) isotype. A competitive direct enzyme-linked immunosorbent assay (celELISA) and a competitive indirect ELISA were established for antibody characterization. The concentrations causing 50% inhibition of binding of OKA-horseradish peroxidase to the antibody by OKA were found to be 0.075 ng/mL in the celELISA. A sensitive and rapid mAb-based gold nanoparticle immunochromatographic strip was also developed using this mAb. This strip has a detection limit of 5 ng/mL for OKA and can be completed in 10 min. Close examining 20 seafood samples by celELISA revealed that 12 were slightly contaminated with OKA, with a mean concentration of 1.5 ng/mL. Analysis of OKA in seafood samples revealed that data obtained from immunochromatographic strip were in a good agreement with those obtained from celELISA. The mAb-based celELISA and immunochromatographic strip assay established in this study were sensitive and accurate for rapid screening of OKA in seafood samples.

2028 THE TOXICITY OF MONILIFORMIN—A COMMON FUSARIUM MYCOTOXIN.

Moniliformin (MON) detected worldwide and the highest detected values in human food have been close to 20 mg/kg. MON acts as an inhibitor of thiamine pyrophosphate depending enzymes and the induction of oxidative damage is also implicated over 92 hours with 11 toxicity ratings based on phenotype and mortality. Hence, our objectives in the present work were to develop a rapid in vivo assay to predict the combined toxicity of AFB1 and FB1, and to evaluate the protective effects of UPSN. The freshwater polyp, Hydra vulgaris, is a useful organism to utilize as an intermediate between in vitro and higher organism toxicity testing. A culture of cloned hydra was exposed to FB1 (100-400 ppm), AFB1 (5-30 ppm) and combinations of the two toxins with and without UPSN. Toxic response was documented over 92 hours with 11 toxicity ratings based on phenotype and mortality. Sorption assays were conducted to investigate competition of AFB1, and FB1, for binding sites to UPSN. Results showed that the minimum effective concentration (MEC) for AFB1, in hydra was 25 ppm, while the MEC for FB1 was not reached. When in combination, the MEC were 400 ppm FB1 + 10 ppm AFB1. UPSN protected the hydra from the toxic endpoint at all tested levels. Initial binding assays showed a possibility of site specific competition between AFB1 and FB1. When in combination (10 ppm AFB1, + 400 ppm FB1, + 0.4 mg/clay), UPSN bound 20% less aflatoxin, and 5% less fumonisin than when alone. This study demonstrates that the combination of aflatoxin and fumonisin results in a more toxic response to the hydra and that UPSN is able to confer protection by absorbing both mycotoxins. This research was supported by USAID LAG-G-00-96-90013-00 and NIH 1R01MD005819-01.

2029 MODIFIED HYDRA BIOASSAY TO EVALUATE COMBINED EFFECTS OF AFLATOXIN B1, AND FUMONISIN B1.

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Lack of national regulation systems and food security in developing countries often leads to chronic exposure of vulnerable populations to aflatoxin B1 (AFB1) and fumonisin B1 (FB1) with contamination levels of both mycotoxins often exceeding U.S. action limits. Montmorillonites (i.e. NovaSil, NS), have been suggested as natural enterosorbents for aflatoxins in animal feeds and recently in human food. NS and its refined form, Uniform Particle Size NovaSil or UPSN, have similar binding affinities for AFB1 and FB1, suggesting a potential dual sorption ability of the clay. Hence, our objectives in the present work were to develop a rapid in vivo assay to predict the combined toxicity of AFB1 and FB1, and to evaluate the protective effects of UPSN. The freshwater polyp, Hydra vulgaris, is a useful organism to utilize as an intermediate between in vitro and higher organism toxicity testing. A culture of cloned hydra was exposed to FB1 (100-400 ppm), AFB1 (5-30 ppm) and combinations of the two toxins with and without UPSN. Toxic response was documented over 92 hours with 11 toxicity ratings based on phenotype and mortality. Sorption assays were conducted to investigate competition of AFB1, and FB1, for binding sites to UPSN. Results showed that the minimum effective concentration (MEC) for AFB1, in hydra was 25 ppm, while the MEC for FB1 was not reached. When in combination, the MEC were 400 ppm FB1 + 10 ppm AFB1. UPSN protected the hydra from the toxic endpoint at all tested levels. Initial binding assays showed a possibility of site specific competition between AFB1 and FB1. When in combination (10 ppm AFB1, + 400 ppm FB1, + 0.4 mg/clay), UPSN bound 20% less aflatoxin, and 5% less fumonisin than when alone. This study demonstrates that the combination of aflatoxin and fumonisin results in a more toxic response to the hydra and that UPSN is able to confer protection by absorbing both mycotoxins. This research was supported by USAID LAG-G-00-96-90013-00 and NIH 1R01MD005819-01.

2030 EXCESSIVE CONSUMPTION OF CATECHIN-RICH BEVERAGE HAS NO IMPACT ON LIVER FUNCTION.


Background: Green tea has a long tradition of consumption in the East. The popularity of green tea is increasing in the West as extensive scientific studies have reported that the main ingredients, catechins, have potential health benefits in the management of obesity associated cardiovascular diseases (CVD) due to promotion of thermogenesis and fat oxidation. However, few studies have investigated effects of daily consumption of the beverages enriched with catechins on biochemical safety parameters as well as body weight in human. We aimed to assess the impacts of catechin- rich beverage in a large-scale population method. Methods: In this report, we have critically examined data from 354 subjects (M: 168; F: 186; Age: 20-64 y; BMI: 22-30 kg/m2) from eleven clinical studies, conducted as per the standard safety guidelines from Japanese Government on Food for Specified Health Use, to evaluate safety of consuming threefold serving size of the catechin-rich beverage. The study design was a randomized, double-blind, controlled, parallel study with two arms. Healthy Japanese men and women were divided into two groups who consumed either three bottles of catechin-rich beverage or three bottles of conventional beverage per day for 4 weeks. The subjects were reorganized into catechin group (n=276) consuming 1.8-1.9 g catechin/day or control group (n=78) consuming <0.2 g catechin/day derived from the test beverages, and then analyzed on intention to treat basis. Results: The results of this analysis revealed significantly lowered body weight (-0.64 kg vs. -0.25 kg, p<0.001), waist circumference (-0.6 cm vs. -0.1 cm, p<0.001), HbA1C (-0.04 % vs. +0.03 %, p<0.001) in the catechin group than the control group, and consistent with previous reports. No significant changes were observed in biochemical parameters related to liver function including AST, ALT and γ-GTP in both groups and between groups. Conclusion: Continuous, daily consumption of a catechin-rich beverage will be safe to consume and contribute to the management of obesity linked to CVD.

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Resistance of Maize Varieties to the Growth of Toxigenic Strains of Aspergillus Flavus


Afatoxins (Af) are naturally occurring mycotoxins which are produced by many species of Aspergillus, mainly A. flavus. They can colonize and contaminate grains before harvest or during storage. The Af are carcinogenic compounds which produce numerous alterations in humans and animals that ingest contaminated grains. Several strategies have been developed to control the presence of Af in food; one of them is the selection of grains and seeds which have a greater resistance to fungi which produce Af. This strategy seems to be one of the safest and most economical. The objective was to evaluate the resistance of several maize varieties toxigenic strains of A. flavus in laboratory, with controlled conditions of temperature, humidity and infective dose. Infective procedure was performed with spores from 2 Mexican toxigenic strains of A. flavus. Inactivated spores from A. flavus were added to immature grains of 11 maize varieties and then incubated for 14 days using 5 humidity levels. Af concentrations were measured via HPLC. The least sensitive varieties to A. flavus were: popcorn, 50G40 and Az910, which showed resistance to fungi growth and Af contamination. On the other hand, the criollo, Cal Oro, 3028W and 30R39 varieties, allowed the growth of the 2 strains of A. flavus to be rapid and abundant. The other varieties had an intermediate growth. The growth of fungi was associated with the production of AfS, which was found to be between 13.4 y 40.6 mg/kg. The range exceeds the allowable limit. These results demonstrate variability in the genetic resistance to A. flavus strain growth in the varieties tested. This variability suggests that selecting maize could contribute to control the fungi growth, as well as to decreasing food contamination by Af. This can help mitigate the carcinogenic and toxic effects on human and animal health.

Mitochondrial Dysfunction, Oxidative Stress and Mitochondrial Membrane Potential


Mitochondrial dysfunction is associated with numerous chronic diseases including metabolic syndrome. Environmental chemicals can impair mitochondrial function through numerous mechanisms such as membrane disruption, complex inhibition and electron transport chain uncoupling. Currently, high-throughput toxicity screening efforts by Tox21 utilize culture conditions that facilitate ATP production exclusively through glycolysis, not oxidative phosphorylation, and therefore these cells may be less susceptible to mitochondrial toxins. To demonstrate this, we cultured HER293T cells in media supplemented with galactose rather than glucose, thereby forcing ATP production via oxidative phosphorylation. To compare media conditions, both galactose and glucose-grown cells were exposed to fifty test compounds, including those with known mitochondrial toxicity (e.g., rotenone, antimycin and carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP)). In addition, cytochrome c oxidase (COX) activity was measured in cell-free COX assays to confirm mitochondrial membrane potential with little impact on apoptosis and cell death, longer time of treatment results on impact on all three parameters. Diamond, a thiol oxidant demonstrates early impact on mitochondrial superoxide levels, changes in mitochondrial potential and cellular vitality followed by a slower impact on cellular vitality, apoptosis and cell death. Organocmetricurials such as Thimerosal, shows rapid and simultaneous impacts on mitochondrial membrane potential, apoptosis and cell death. The study of the inter-relationships between mitochondrial potential changes, apoptosis and cell death may thus provide more sensitive and comprehensive methods to evaluate impact on mitochondrial function and cellular health and understanding the mechanistic basis for the action of compounds.

Monolyso-cardiolipin—biomarker of mitochondrial dysfunction induced by total body irradiation: LC-ESI-MS study

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Oxidized phospholipids and their hydrolysis products are important signaling participants of the mitochondrial stage of apoptosis. Although cardiolipin (CL) comprises a minor fraction of cell phospholipids, its regulatory function is central to cell activity. Previously, we demonstrated that total body irradiation (TBI) results in oxidation of CL in small intestine and lung. Characterization of individual oxidized molecular species of CL and their hydrolysis products is essential for further understanding of mechanisms of radiation damage and development of radioprotectors/radiomitigators. C57BL/6 mice were exposed to TBI at a dose of 10 Gy and sacrificed 0, 10 and 24 h thereafter. Oxidative lipidomics analysis of CL and its hydrolysis products isolated from lung, brain, bone marrow, small intestine and plasma was performed. We found that TBI results in significantly decreased content of CL in small intestine, bone marrow (5.3- and 2.4-times, respectively) compared to lung and brain tissues. We were able to detect a significant accumulation of non-oxidized monolyso-CL (MCL) in tissues from irradiated mice. In addition, we found that MCL was a major CL hydrolysis product containing exclusively linoleic acid. Moreover, a significant increase of CL molecular species containing four linoleic acids (m/z 1448) was found in plasma of mice 10 h after TBI. We suggest that appearance of mitochondrial phospholipid, CL, and its metabolites in plasma of irradiated mice may reflect tissue-specific irradiation-induced metabolic disturbances and serve as biomarkers of mitochondrial dysfunction induced by TBI. Supported by NIOSH OH008282; NIH U19 AI068021, HL70755, HL094488, ES020693.

Mitochondria Targeting of Nonperoxidizable Triphenyl phosphonium-conjugated oleic acid protects mouse embryonic cells against apoptosis: role of cardiolipin remodeling.

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The early stage of intrinsic apoptosis is characterized by the formation of cardiolipin (CL)/cytochrome c complexes in mitochondria that exhibit a potent peroxisome activity towards polyunsaturated CL. Accumulation of CL oxidation products in mitochondria of apoptotic cells has been found essential for the release of pro-apoptotic factors into the cytosol. We suggested that integration of mono-unsaturated octadecenoic acid (C18:1) into CL- via its remodeling pathways in mitochondria - will generate nonoxidizable CL species hence protect cells against apoptosis. We synthesized a nonperoxidizable triphenylphosphonium (TPP) C18:1 ester (TPP-C18:1) and used it for targeted delivery into mitochondria of mouse embryonic cells (MEC). Using oxidative lipidomics analysis we established that pro-apoptotic stimulation with actinomycin D (A(2)D) was accompanied by selective oxidative consumption of CL molecular species containing polyunsaturated oc-
2036 A MITOCHONDRIA-TARGETED IMIDAZOLE-SUBSTITUTED FATTY ACID INHIBITS CYTOCHROME C PEROXIDASE AND MITIGATES RADIATION-INDUCED DEATH.

J. Atkinson1,2, A. Kapralov1,2, N. Yanamala3, Y. Tyurina1,2, A. Amoscato1,2, L. Pierce1, J. Peterson1, Z. Huang4,1, J. Jiang4,1, A. Samahan-Arias1,2, A. Madsen2, W. Feng1, N. Belikova3,2, V. Tyurin3,2, H. Wang4,1, J. Fletcher4, Y. Wang4,1, V. Lasova4,1, J. Klein-Seetharaman2, D. Stoyanovskiy2, H. Bayir2, B. Pitt1, M. Epperly3,2, J. Greenberger3,2 and V. Kagan4 respectively) and Ru360, a specific MCU inhibitor, diminished I/R-induced death and 10 μM of EMC did not prevent against any parameter measured. CONCLUSION: Mitochondrial depolarization and MMP inhibition do not explain cytoprotection by minocycline and doxycycline because minocycline did not depolarize at cytoplasmic concentrations and no cytoprotection was observed with MMP inhibitors. Rather, inhibition of Fe2+ and/or Ca2+ uptake through MCU likely underlies cytoprotection by Ru360, minocycline and doxycycline.

2038 ETHYMERCURY INDUCES ER STRESS AND MITOCHONDRIA-MEDIATED APOPTOSIS.


Mercury is one of the most important environmental and industrial pollutants throughout the world. Exposure to mercury causes strong damage to organs including the brain, blood, liver, bone and kidneys. Renal proximal tubular cells represent the primary target site where mercury is taken up, accumulated, and expresses toxicity. We previously investigated the cytotoxicity of seven kinds of mercury compounds in human renal proximal tubule (HK-2) cells. Our experimental data demonstrated that ethymercury was shown the strongest cytotoxicity among them at 24h and 48h exposure in HK-2 cells. In this study, we explored the mechanism of ethymercury induced cytotoxicity in HK-2 cells. Ethymercury chloride (EMC) induced cytotoxicity with 2.4 and 0.76 μM of IC50 values exposed to 24h and 48h, respectively. For 24h exposure to 1 and 2 μM of EMC, the cells were dose-dependently undergone the apoptosis in FACs analysis. After 48h treatment of cells to 2 μM EMC, cleaved of caspase-9 and caspase-12 which are activated by ER stress, were markedly activated. EMC was up-regulated the mRNA of CHOP (transcription factor, C/EBP homologous protein), XBP1 (X-box binding protein-1), ERDj4 (endoplasmic reticulum-localized Dna) homologues) and GRP78 (78kDa glucose-regulated protein) expression. In HK-2 cells exposed to 2 μM EMC, phosphorylated eIF2 and CHOP protein were increased at 3h and GRP78 protein at 12h. To determine the EMC induced oxidative stress, we observed the changes in mitochondrial membrane potential with JC-1 and gene expression using RT-PCR analysis in HK-2 cells. EMC was increased the accumulation of JC-1 monomers in mitochondria from 0.5 to 2 μM concentrations in a dose-dependent manner. The expression of Hic (hydrogen peroxide-inducible clone)-5, which were related to oxidative stress, was also induced by EMC treatment. Collectively, these results suggested that EMC exposure induced apoptosis via ER stress and mitochondrial dysfunction in human renal proximal tubule (HK-2) cells. * This research was support by a grant (10182KFDA992-2101) from Korea Food & Drug Administration in 2011.

2039 CADMIUM-INDUCED OXIDATIVE STRESS DAMAGE CAUSES NEURON CELLS APOPTOSIS THROUGH JNK/MITOCHONDRIA-DEPENDENT/ENDOPLASMIC RETICULUM STRESS PATHWAYS.

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Cadmium (Cd), a well-known toxic metal, is an important pollutant throughout the world. In mammalian, exposure to Cd causing injuries of kidney, liver and osseous system has been demonstrated. Although some studies have shown the possible connections between neurodegenerative disorders and Cd exposure, the toxic effects of Cd on neuron cell are still unclear. In this study, we designed to investigate the effects and possible mechanisms of Cd-induced neuron cell death. Our results found that after exposed to Cd in cultured Neuro-2a cells for 24 h obviously decreasing the viable cells, mitochondrial membrane potential, and led to glutathione depletion in a dose-dependent manner with a range from 1 to 20 μM, which accompanied by a marked Cd accumulation in cytosol. Cd also induced the expression of Hic (hydrogen peroxide-inducible clone)-5, which were related to oxidative stress, was also induced by EMC treatment. Collectively, these results suggested that EMC exposure induced apoptosis via ER stress and mitochondrial dysfunction in human renal proximal tubule (HK-2) cells. * This research was support by a grant (10182KFDA992-2101) from Korea Food & Drug Administration in 2011.
Oxidative stress was demonstrated to promote the progression of diabetes mellitus. It has been suggested that copper may play a specific role in the progression and pathogenesis of DM. Pyrroline dithiocarbamate (PDTC), a widely apply to the medicine and pesticide, was known to be capable of enhancing copper accumulation. In this study, we investigated the effect of submicromolar-concentration Cu2+/PDTC complex on pancreatic β-cells damage and evaluated the role of oxidative stress in this effect. CuCl2 (0.01–300 μM) did not affect the cell viability in RIN-m5F cells. However, combination of CuCl2 (0.5 mM) and PDTC (0.3 mM) markedly reduced RIN-m5F cell viability. Cu2+/PDTC complex could also increase in oxidative stress damage, and display several features of mitochondria-dependent apoptosis signals, which accompanied with the marked increase the intracellular Cu2+ levels. These apoptotic-related responses of Cu2+/PDTC complex-induced could be effectively prevented by antioxidant NAC. Furthermore, Cu2+/PDTC complex was capable of increasing the phosphorylation of ERK1/2 and JNK, and its upstream kinase MEK1/2 and MKK4, which could be reversed by NAC. Transfection with ERK2-siRNA and MAPK8-shRNA could inhibit ERK1/2 and JNK activation and attenuate MMP loss and caspase-3 activity induced by the Cu2+/PDTC complex. Taken together, these results are the first report to demonstrate that the Cu2+/PDTC complex triggers a mitochondria-regulated apoptosis via an oxidative stress-induced ERK/JNK activation-related pathway in pancreatic β-cells.

Mycotoxins toxicity has been related with inhibition of protein synthesis, mitochondrial dysfunction, formation of DNA adducts, disruption of calcium homeostasis and ROS generation. Disruption of mitochondrial membrane potential (MMP) in the cell cycle and increase of apoptosis in Hep G2 cells of four emergent Fusarium mycotoxins, enniatin A (EN A), enniatin A1 (EN A1), enniatin B (EN B) and enniatin B1 (EN B1) using flow cytometry were studied. The data comprise the measurements of a dual staining for MMP using TMRM and for plasma membrane permeability by ToPro-3; and a single staining for DNA analysis and cell cycle phase distribution by PI. It was demonstrated for the first time that a depolarization of the mitochondrial membrane in HepG2 cells by the emergent mycotoxins treatment triggers the mitochondrial pathway leading to cell death. An altered mitochondrial response in HepG2 cells was observed after 24h incubation. A different decline of MMP was accompanied by decreasing cell viability. Arrest of cell cycle phases were affected by time exposure and concentration of mycotoxins studied. All the parameters studied varied depending on the ENs tested. Cells arrested in G2/M phase prevents cells from entering or completing division or cells may enter slowly, arrest of S phase slows the cell cycle possibly related with effects occurring during M phase. This work has been supported by the Science and Innovation Spanish Ministry (AGL 2010-17024/Ahl).

Polybrominated diphenyl ethers (PBDEs) are a class of flame retardants with evidence of toxic potential due to their high bioaccumulation potential and lipophilicity. These compounds have shown different toxic effects on health which are not yet understood. This work investigated the potential of BDE-47, BDE-99, and BDE-209 to modify the intracellular homeostasis of HepG2 cells assessing changes on mitochondrial membrane potential and reactive oxygen species accumulation which can lead to cell death. Cell viability was assessed by MTT assay, while mitochondrial depolarization and ROS accumulation were measured using the fluorescence dyes TMRM and CM-H2DCFDA, respectively. Cells were incubated at 37°C, in an atmosphere containing 5% CO2 and 96% relative humidity for 24 h before treatment. PBDEs congeners in concentrations ranging from 0.1μM to 25μM were then incubated with the cells for 24 and 48 hours. Our results showed that PBDEs congeners BDE-47 and BDE-99 can decrease cell viability after incubation with 10 μM or higher, while BDE-209 showed significant result only in the highest concentration evaluated. The change on cell viability was caused by a significant decrease of the mitochondrial membrane potential showed by all tested congeners. In addition, it was demonstrated that BDEs -47 and -99 had also potential to increase reactive oxygen species accumulation in a dose-dependent manner, while BDE-209 induced ROS accumulation for all tested concentrations. These results are evidences that the cytotoxic potential of PBDEs are demonstrated for all tested congeners, however the congeners that have fewer brominated subunits are more toxic on HepG2 cell; and their toxic potential is related to a disturbance on mitochondrial membrane potential and ROS accumulation.

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Background: Acrolein is a common environmental, food and water pollutant and a major component of cigarette smoke. Also, it is produced endogenously via lipid peroxidation and the metabolism of certain amino acids and drugs. Importantly, acrolein has been identified as both a product and an initiator of lipid peroxidation and oxidative stress. Acrolein is known to be cytotoxic to many cell types including hepatocytes; however the underlying molecular mechanisms are not fully understood. Methods: Primary hepatocytes and human hepatoma cells (HepG2) were used to study acrolein-induced hepatotoxicity; and to study endoplasmic reticularum stress (ERS), mitochondrial death pathways and the induction of apoptosis. Results and Conclusions: Acrolein was cytotoxic and caused a dose-dependent loss of survival of hepatic cells. Cell death was apoptotic at moderate and necrotic at high cytotoxic concentrations of acrolein. Acrolein exposure (i) caused a rapid and dramatic decrease in the intracellular antioxidant glutathione and overall antioxidant capacity; (ii) activated the signaling kinases JNK and p38 MAPK; (iii) induced endoplasmic reticularum stress and activated ERS proteins PERK, eIF2α, GADD153/CHOP and ATF-3, -4, and -6; and (iv) disrupted mitochondrial integrity and function as evidenced by fluorescence microscopy, High-Content Cellomics analysis, and depletion of cellular ATP. We postulate that acrolein triggers multiple cell death mechanisms which together contribute to its hepatotoxicity. Our study defines basic mechanisms contributing to liver injury caused by oxidative stress molecules and reactive aldehyde pollutants such as acrolein. This study was funded by grants from NIH-NIAAA, NIH-NIEHS and VA.

We have shown that 2,3,5-tris(glutathion-S-yl)hydroquinone (TGHQ), a metabolite of benzene, catalyzes the generation of reactive oxygen species (ROS) and caspase-dependent apoptosis in HL-60 cells. We now report that TGHQ induced severe DNA damage, as evidenced by DNA ladder formation and H2AX phosphorylation, and the subsequent activation of the DNA nuc sensor enzyme, PARP-1, leading to rapid ATP and NAD depletion and the presence of poly(ADP-ribosylated) proteins (PARs). PJ-34 (a PARP-1 inhibitor) completely prevented the
formation of PARs, partially attenuated TGHQ-mediated ATP depletion, but had no effect on NAD depletion. Intriguingly, the IC50 for EGFR inhibition of Z-VAD-fmk and Nec-1 (20 μM, non-caspase inhibitor) attenuated TGHQ-induced apoptosis, co-treatment with PJ-34 led to a further decrease in apoptosis. The findings suggest that PARP participates in caspase activation during the apoptotic response to DNA-damage. Indeed, PARP-1 inhibition appears to reduce TGHQ-induced caspase-3,-7, and -9 activation by at-
tenuating cytochrome C translocation from mitochondria to the cytoplasm. In contrast, PJ-34 potentiated TGHQ-induced caspase-8 activation, suggesting that PARP-1 plays a dual role in regulating TGHQ-induced apoptosis via opposing ef-
fects on the intrinsic (mitochondrial) and extrinsic (death-receptor) pathways. Finally, TGHQ-induced cell death was accompanied by the nuclear accumulation of PARP-1, a specific inhibitor of receptor interacting protein-1 (RIP-1) - a key protein in necroptosis - and assessed their protective effects against irradiation-induced (4 Gy)

death of embryonic carcinoma NCCIT cells (5 days after irradiation). We found that the inhibition of Z-VAD-fmk (100 μM plus Nec-1 (20 μM) plus wortmanin-1 (20 μM)


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dant and metal chelator, augmented HgCl2-induced cytotoxic effects by facilitating
decreased oxidative stress caused macrophage cell death via a mixed type of apoptosis
and necrosis. These findings imply for first that PDTC may enhance the uptake of
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2049 TARGETING THE ANAPHASE PROMOTING COMPLEX/CYCLOSUME TO INHIBIT CELL CYCLE AND TO INDUCE APOPTOSIS IN TUMOR CELLS.

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Inhibition of cell division is a major facet of chemotherapy and is the mechanism of action of mitotic spindle function disrupting drugs such as Taxol. Resistance to these types of drugs is a major impediment to successful therapy. The anaphase-promoting complex/cyclosome (APC/C) is an E3 ubiquitin ligase that is the major regulator of both M-phase progression and G1 to S phase transition. Inhibition of APC/C is expected to inhibit mitotic arrest in spindle assembly checkpoint proficient (“Taxol-sensitive”) cells, and pseudo-G1 arrest and apoptosis in spindle assembly checkpoint deficient (“Taxol-resistant”) cells. We have derived homology structure models for key interacting components of the APC/C. ANAPC11 is the zinc RING finger protein and catalytic subunit that binds to the cullin subunit ANAPC2. We have used these models to perform in silico screening of two sites on ANAPC2 to identify compounds that will interfere with ANAPC11 binding to ANAPC2. Testing of 9 compounds indicates that one induces mitotic arrest and apoptosis in Taxol-sensitive human cervical carcinoma cells and a second induces cell cycle arrest and apoptosis in Taxol-resistant human melanoma cells. These data suggest that inhibition of APC/C may be an effective approach to developing new anti-cancer drugs. Supported in part by a NIEHS grants R01ES011314 and T35ES001459.

2050 INDUCTION OF HUMAN STEROID SULFATASE BY INSULIN-LIKE GROWTH FACTOR II THROUGH NUCLEAR FACTOR KAPPA B SIGNALING PATHWAY IN HUMAN PROSTATE CANCER CELLS.

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Steroid sulfatase (STS) is responsible for the hydrolysis of aryl and alkyl steroid sulfates and therefore has a pivotal role in regulating the formation of biologically active estrogens. There are correlations between increasing level of active estrogens and hormone-dependent cancers, including prostate cancers. Therefore STS is considered as a new promising drug target for treating estrogen-mediated cancer. It has been demonstrated that induction of STS by TNF-α is mainly through PI3K/Akt signaling pathway. In this article, we found that insulin-like growth factor (IGF) II significantly induces STS expression both mRNA and protein level in concentration- and time-dependent manners in PC-3 human prostate cancer cells. To elucidate the signaling pathway of STS gene induction by IGF-II, we have determined the effect of NF-κB inhibitors in PC-3 cells. When the cells were treated IGF-II with NF-κB inhibitors such as Bay 11-7082 or NBD (Nemo Binding Domain) binding peptide, STS expression induced by IGF-II was significantly blocked. Moreover, we found that proteasome inhibitors such as MG-132 and bortezomib, which are known to prevent NF-κB maturation also strongly blocked IGF-II-induced STS expression. Furthermore, IGF-II induced 17β-hydroxysteroid dehydrogenase 1, 3 and reduced sulfotransferase. One of the sulfotransferase is estrophine induced estrope sulfotransferase. These means IGF-II induces forward direction enzymes like STS and 17β-hydroxysteroid dehydrogenase 1, 3 that would produces estradiol and reduces reverse direction enzymes like estrone sulfotransferase. Taken together, these data strongly suggest that IGF-II induces STS expression through NF-κB signaling pathway in PC-3 cells and may cause induction of estrogen production and estrogen-mediated carcinogenesis.

2051 FGFR21 EXPRESSES IN THE DIABETIC HEARTS AND PROTECTS FROM PALMITATE-INDUCED CARDIAC CELL DEATH VIA ERK1/2-DEPENDENT P38 MAPK/AMPK SIGNALING PATHWAY.

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Fibroblast growth factor (FGF) 21 plays important role in lipid metabolism. However, whether FGF21 protects effect on lipid-induced cardiac damage was unknown. The present study examined whether FGF21 expresses in the heart of diabetic mice; whether FGF21 protects cardiac (H9C2) cells from palmitate-induced cell death. For animal study, diabetes was induced by multiple low dose of streptozocin. In these diabetic mice cardiac lipid accumulation was evidenced by increased lipid drops and triglyceride levels from 2 weeks after diabetes onset. Cardiac FGF21 mRNA expression significantly increased at 2 months (about 40 folds) and remained high at 4 (about 3 folds) and 6 (1.5 fold) months compared to controls, suggesting FGF21 up-regulation at the early stage may be a protective response. To test this assumption, H9C2 cells were exposed to palmitate at 62.5 nM for 15 h, which caused a significant apoptotic effect, examined by DNA fragmentation and cleaved caspase-3. Pre-incubation of palmitate-treated cells with different doses of FGF21 significantly reduced palmitate apoptotic effect within a dose range of 25-250 nM with a maximal protection at 50 to 100 nM. Mechanistic study showed that palmitate down-regulated and FGF21 up-regulated phosphorylated Erk1/2, p38 MAPK and AMPK. Pre-inhibition of either Erk1/2, p38 MAPK or AMPK with its specific inhibitor can significantly abolish the preventive effect of FGF21 on palmitate-induced apoptosis. Furthermore, inhibition of Erk1/2 blocked the activation of p38 and AMPK. Neither p38 MAPK nor AMPK inhibitor affected Erk1/2 phosphorylation in response to FGF21, but inhibition of p38 MAPK blocked AMPK activation. FGF21 also found to prevent palmitate-induced PTEN activation and activated Akt in H9C2 cells. These results suggest that palmitate induces cardiac apoptosis through activation of PTEN and inactivation of Akt. FGF21 prevents the cardiac apoptotic effect of palmitate via up-regulation of Erk1/2-dependent p38 MAPK/AMPK signaling pathway.

2052 EMBELIN INDUCES APOPTOSIS THROUGH DOWNREGULATION OF AKT-DEPENDENT PATHWAY IN PC-3 HUMAN PROSTATE CANCER CELLS.


Embelin is known as a potent small-molecule inhibitor of X-linked inhibitor of apoptosis (XIAP) that abrogates binding of XIAP to procaspase-9. Although embelin induces apoptosis in human cancer cells, the detailed mechanisms is still unknown. In this study, the inhibitory effects of embelin on cellular proliferation of PC-3 human prostate cancer cells were determined. Treatment with embelin showed a strong inhibition of cell growth in a concentration-dependent manner with IC50 values of 12.6 μM. Western blot analysis showed that the level of proapoptotic protein Bax was up-regulated and anti-apoptotic proteins such as Bcl-2 and Bcl-xL were down-regulated in embelin-treated mitochondria. Embelin also increased the release of mitochondrial proapoptotic factors such as AIF leading to activation of caspases. Interestingly, prosurvival factor Mcl-1 was significantly suppressed by embelin. We found that embelin-dependent Mcl-1 downregulation is mediated by suppressing Akt phospho-tylation and cyclooxygenase-2 expression. Activation of Akt, induction of COX-2 and Mcl-1 by tumor necrosis factor (TNF-α) were also blocked by embelin. Taken together, these findings suggest that embelin results in human prostate cancer cells apoptosis through downregulation of Akt-dependent pathway.

2053 DEFECTIVE EIF2α PHOSPHORYLATION CORRELATES WITH ENHANCED PROTEIN AGGREGATION AND CELL DEATH IN RESPONSE TO PROTEASOME INHIBITION.

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Protein synthesis in mammalian cells is tightly regulated in response to a variety of stresses and plays an essential role in proteostasis. The hub of this translational control network is the eukaryotic initiation factor 2 (eIF2), upon which phosphorylation of the α-subunit at the S51 residue effectively attenuates cap-dependent protein synthesis. Proteasome inhibitors (PI's), a class of anti-neoplastic agents, are known to cause eIF2α phosphorylation, but conflicting studies report this phosphorylation as being both pro-death and pro-survival. We have preliminary evidence from pancreatic cancer cells indicating that there appears to be heterogeneity in both basal and PI-induced eIF2α phosphorylation, with PI-induced phosphorylation being both pro-death and pro-survival. Further investigations into more cell types and other proteotoxic stress-inducing agents (thapsigargin, tunicamycin, arsenic triox-ide) will be needed to determine the extent and prevalence of this defect in cancer
2054 CHRONIC EXPOSURE TO CADMIUM INDUCES APOPTOSIS THROUGH THE ACCUMULATION OF P53 IN KIDNEY OF MICE. 
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We have found that overaccumulation of p53 by cadmium (Cd) may relate to induction of apoptosis and may be due to the suppression of p53 degradation through the inhibition of expression of ubiquitin-conjugating enzyme family, Ubc2d family in rat proximal tubule cells (NRK-52E cells). In this study, we examined the effect of chronic exposure to Cd on the expression of Ubc2d family genes and accumulation of p53 in the mouse kidney. Five weeks old female C57BL/6J mice were fed diet containing 300 ppm Cd without restraint for 12 months. Cd slightly elevated blood plasma urea nitrogen value but did not increase creatinine value in serum. Some of mice exposed Cd was detected histopathological change (e.g., urinary casts and cell swelling) in the renal tubules. Thus, Cd-induced renal toxicity was weakly developed. Ubc2d family (Ubc2d1, Ubc2d2, and Ubc2d3) mRNA levels significantly increased and p53 accumulated in the kidney of Cd group. Moreover, apoptotic cells were predominantly detected in both tubules and were absent in glomeruli of all Cd-exposed mice. These results suggest, therefore, that Cd causes p53-dependent apoptosis in not only NRK-52E cells but also the renal tubules of mice, and that Cd-induced accumulation of p53 may be due to down-regulation of Ubc2d family genes in the kidney of mice.

PS

2055 APPLICATION OF IN VITRO CYTOLETHALITY AND PROLIFERATION ASSAYS TO IMPROVE KINASE INHIBITOR SELECTIVITY PROFILE AND REDUCE PRECLINICAL HEMATOXICITY. 
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Hematotoxicity, leading to myelosuppression and neutropenia, is the most common clinical dose-limiting toxicity (DLT) encountered during development targeted kinase inhibitor compounds. Hematotoxicity may be driven by multiple biological, chemical and physicochemical (pChem) properties. In these studies, rat primary hepatocytes were utilized to assess pChem toxicity, and bone marrow mononuclear cells were employed to evaluate biologic (on- and off-target) activity. Rat primary hepatocytes were isolated and assessed for cytotoxicity after treatment with different concentrations of compound for 24 hours. Bone marrow mononuclear cells, KG-1 meyloblasts, or CD34+ bone marrow cells were incubated with different concentrations of compound for either short term (4 – 24 hrs) or long-term exposure (10 – 14 days). Inhibition of proliferation (IC50, IC90) or cytotoxicity (LC50) values were calculated and used with pChem and kinase selectivity data to categorize compound risk. Greater viability was observed with compounds exhibiting better overall pChem properties and, therefore, was used as a prefilter to select compounds ahead of the proliferation assays. Kinase inhibitor compounds with improved selectivity, specifically against inhibition of CDK9, exhibited less inhibition of proliferation in the models utilized to assess hematotoxicity risk. In summary, we have applied a strategy that utilizes multiple in vitro tools to guide kinase inhibitor compounds toward optimal pChem properties and kinase selectivity, resulting in overall lower toxicity. Furthermore, the in vitro assay output translated to reduced hematotoxicity and increased margin of safety to dose-limiting myelo-suppression prior to clinical development. This strategy has also produced a higher throughput surrogate assay to assess hematotoxicity risk that can be applied during preclinical drug development.

PS

2056 ROLE OF OXYGENATED PHOSPHATIDYLSERINE AND ITS METABOLITES—LYSO-PHOSPHATIDYLSERINES PRODUCED AFTER OXIDATION AND HYDROLYSIS BY PLASMA LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A2 IN CLEARANCE OF APOTOTIC CELLS BY MACROPHAGES: LC-ESI-MS STUDY. 
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Peroxidized phospholipids and their metabolites are known modulators of inflammatory responses. We suggested that during apoptosis, oxidative modification of externalized on the cell surface phosphatidylserine (PS) and its subsequent hydrolysis by LpPLA2VIIA would yield a pattern of diversified molecular products with different affinity and recognition by macrophage (PS) receptors. To make HL60 cells susceptible to oxidation we treated them with linoleic acid (LA) (100 nmol/106 cells). We found that phagocytosis of LA-enriched apoptotic HL60 cells (100 μM H2O2) by RAW 264.7 macrophages was significantly higher compared to naive apoptotic HL60 cells and was suppressed by annexin V. Using oxidative lipidomics approach we were able to detect the presence of oxidized PS species containing LA with two and three oxygen in LA-enriched apoptotic HL60 cells. Further, we determined whether treatment of LA-enriched apoptotic HL60 cells with LpPLA2VIIA will affect their phagocytosis. We found that the ability of macrophages to engulf apoptotic cells was significantly reduced after treatment of cells with LpPLA2VIIA. In addition, MS analysis revealed the presence of lyso-PS and oxygenated LA accompanied by a decrease amounts of oxidized PS (but not non-oxidized PS). Further, we oxidized C18/0/C18:2-PS (c1y H2O2) and integrated it into naive HL-60 cells. We found that phagocytosis of HL60 cells containing oxygenated PS on cell surface was ~two time higher compared to cells with incorporated non-oxidized PS. We suggest that oxidatively modified externalized PS and its hydrolysis products are important regulators of phagocytosis and inflammatory responses. Supported by a contract with GlaxoSmithKline, OH008282, U19AI068021, HL70755, HL094488, ES020693.

PS

2057 INTRACELLULAR ACCUMULATION OF SULFIDES AND INDUCTION OF APOPTOSIS DEPEND ON PH IN NAHS-EXPOSED JURKAT CELLS. 
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Hydrogen sulfide (H2S) is a toxic gaseous substance and has an odor of rotten eggs. Accidental exposure to high concentrations of H2S has been reported to be lethal in human. Inhaled and absorbed H2S may be partially ionized in blood and cause toxic effects on lymphocytes (pK1 of H2S is 6.8 at 37 °C). However, the mechanism for toxicity of H2S has not been well documented. In this study we studied cellular uptake and cytotoxicity of sulfides in human lymphoma cells (Jurkat) following in vitro exposure to NaHS at different pHs. The cells were exposed to 0-5 mM NaHS in HBSS in a screw-capped plastic conical tube for 1 hr at 37 °C with gentle shaking. The pH of HBSS was adjusted to pH 6.0, 7.0 or 8.0 and the air was removed by filling the tube with HBSS to avoid a loss of dissolved H2S gas from HBSS. The cells were collected by centrifugation and cultured in RPMI1640 culture medium for 6-24 hrs in a culture dish. The cytotoxicity of NaHS increased with the decrease of pH in HBSS. The cell viability was not changed by the pH in the absence of NaHS. The activity of caspase-3/7 in NaHS-exposed cells was measured by colorimetric method and was found to be increased with the decrease of pH in HBSS. Western blotting using anti-PARP and anti-caspase-3 also revealed that exposure to 5 mM NaHS at pH 6.0 induced apoptosis. Z-VAD-fmk, a pan-caspase inhibitor, reduced the NaHS-induced activation of caspase-3, indicating that pH-dependent cell death caused by NaHS was due to activation of caspases. The concentration of sulfides in the cells was measured by an HPLC with a fluorescent detector. The cellular sulfide concentration in NaHS-exposed cells was significantly higher compared to cells without NaHS. The air was removed from the culture tube to keep the sulfide concentration constant. The extracellular sulfide concentration increased with the decrease of pH, and the intracellular sulfide concentration also decreased with the decrease of pH. At pH 6.0 the ratio of the intracellular sulfide concentration to the extracellular sulfide concentration was 1:8. Therefore the larger number of cells underwent apoptosis in the acidic condition.

PS

2058 EFFECT OF SURFACE-MODIFIED TITANIUM BY CHEMICAL TREATMENT ON THE GENE EXPRESSION PROFILE IN OSTEOGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS. 
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Titanium is the material widely used for orthopedic and dental applications. Surface properties of material play a major role in cell-material interaction. Adult human mesenchymal stem cells (hMSCs) have the pluripotency to differentiate into cells of mesodermal origin, e.g., bone, cartilage, adipose, and muscle cells. In the present study, we evaluated the molecular responses of hMSCs to three modified titanium surfaces. Experimental titanium disks were treated with NaOH, NaOH+CaCl2, and NaOH+Ca(OH)2. Untreated titanium disks served as control. Then, hMSCs were cultured on each surface-modified titanium disk for 7 days. Comparative gene expression in hMSCs were analyzed by the DNA microarray. The gene expressions in hMSCs cultured on three kinds of chemically treated surfaces were compared with that on untreated titanium. The expressions of

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the osteogenic promoter WNT and OPN (osteoprogenitor) were up-regulated by NaOH treatment of titanium surface. In addition, NaOH+CaCl2 treatment increased the expressions of IGF-1, BMP-2, and OPN. Moreover NaOH+Ca(OH)2 treatment increased the RANKL (receptor activator of nuclear factor kappa-B ligand) and osteocalcin expressions. The highest expression level of NFAT (nuclear factor of activated T-cells) was NaOH+Ca(OH)2, followed in order by NaOH+CaCl2 and NaOH. Furthermore, the amount of Ca2+ ions incorporated into the titanium surface may increase osteogenic differentiation. These results suggest that modified titanium surfaces may promote osteogenic differentiation of hMSCs, and that Ca2+ ions incorporated into the titanium surface may increase osteogenic responses in hMSCs.

2059 METHYL PARATHION-INDUCED EXPRESSION CHANGES OF PLURIPOTENCY MAINTENANCE GENES IN HUMAN EMBRYONIC STEM CELLS.


Given their wide spread use, ease of procurement, and potential for serious health consequences if deployed by terrorists, toxic industrial chemicals (TICs) represent a real threat to soldiers and civilians at home and abroad. Unfortunately, for a vast number of TICs, including the widely-used organophosphate insecticide, methyl parathion (MP) there is incomplete knowledge regarding the basic molecular toxicological consequences of exposure in humans. Although the literature suggests diverse toxicological consequences for MP exposure, it is all based on human epidemiological studies, in vivo animal studies, or in vitro studies using immortal cell lines. Therefore, there is no definitive connection to the molecular events that occur during MP exposure in "normal" human cells. Furthermore, chemicals that have certain known effects in adults can have dramatically different toxic effects during embryonic and prenatal development. Thus, an important part of any complete toxicological evaluation must include examination of the compound's effect on human embryonic development. A promising, relatively new development in the field of toxicology is the use of human embryonic stem (hES) cells to measure human cellular and molecular toxicological endpoints. Since undifferentiated hES cells maintain the ability to differentiate into any cell type in the body, they provide a unique window into the influence of toxicants on the entire early human development. We are studying the effects of MP on undifferentiated WA09 hES cells using an RT-PCR array (Qiagen) designed to reveal changes in expression of 84 genes known to be important in the maintenance of pluriptotency and differentiation of embryonic stem cells. These data suggest that exposure to MP alters the expression of a number of key genes involved in pluripotency and differentiation pathways. The results of this study have opened a new avenue toward a better understanding of how exposure to TICs, such as MP, may interfere with early human embryonic growth and development.

2060 COMPARISON OF PROTEIN EXPRESSION PROFILES IN HUMAN MENSECHYMAL STEM CELLS CULTURED ON SURFACE-MODIFIED TITANIUM WITH CHEMICAL TREATMENTS.


Titanium (Ti) is the most widespread materials for various types of biomedical applications, such as orthopedic and dental implants, because of its mechanical properties and good biocompatibility with bone tissue. Some studies showed that NaOH-treated Ti could form the apatite layer on its surface, which improved bone formation. However, what kind of change has occurred within the cells on surface-modified Ti are still poorly understood. On the other hand, human mesenchymal stem cells (hMSCs) are pluripotent adult stem cells that can differentiate into various cell types, including osteoblasts. Therefore, hMSCs are a promising candidate applied in regenerative medicine. In this study, we analyzed the protein expressions of hMSCs cultured on four different Ti surfaces: untreated Ti, NaOH-treated Ti, NaOH-CaCl2-treated Ti, NaOH-Ca(OH)2-treated Ti. Comparing the protein expression profiles of hMSCs cultured on untreated Ti and the others using LC-MS/MS shotgun analysis and Mascot/Swiss-prot database search followed by pathway analysis showed that above 2.5 fold upregulated proteins were categorized to mainly protein synthesis, molecular transport and protein trafficking. From the viewpoint of osteogenic differentiation, Msx1, fibronectin, collagen and MAFK which related to bone growth and development were upregulated. Additionally, PAI-1, Annexin A1, pyruvate kinase, superoxide dismutase, cathepsin D, enolase 1, versican core protein and tenascin which increased upon osteogenic differentiation that reported in some proteomics analysis of hMSCs, were also upregulated. These findings suggest that those chemical-treated Ti surfaces make hMSCs to enhance osteogenic differentiation.

2061 EFFECTS OF HIGH-FAT DIET AND PARTICULATE MATTER (PM2.5) EXPOSURE ON CIRCULATING AND BONE MARROW ENDOTHELIAL PROGENITOR CELLS IN MICE.

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Inhaled fine particulate matter (PM2.5) induces endothelial dysfunction and increases the risk of cardiometabolic disease. Endothelial progenitor cells (EPCs) contribute to endothelial repair and angiogenesis, and decreased EPC levels associate with increases in endothelial dysfunction and cardiovascular disease (CVD) risk. Acute exposure to either elevated ambient PM2.5 or to concentrated ambient PM2.5 (CAPs) decreases circulating EPC levels in humans or mice, respectively. Because diabetes and air pollution are worldwide health problems, we examined whether high-fat diet (HFD) enhanced CAPs-induced EPC suppression. Mice (male; C57BL/6) maintained on low fat or HFD (600 kcal fat) were exposed to HEPA-filtered air or urban Louisville CAPs (80-100 μg/m3) for 9-30 consecutive days (6h/d). Exposure to CAPs (30d) or to HFD (8 weeks) alone significantly decreased levels of circulating Flk-1/Sc-a1 EPCs by -54.7±6.7% or -30.1±6.9%, respectively. In contrast, bone marrow (BM) EPC numbers were significantly increased by CAPs (+41.3±11.7%) as well as HFD (+35.7±12.7%) alone, yet BM-EPCs were decreased by combined HFD+CAPs (-38.5±7.9%) treatment. Combined HFD+CAPs exposure decreased circulating EPC level (-51.0±7.5%) equivalent to the level observed with HFD or CAPs alone, indicating that HFD and CAPs may share a common mechanism. To test if CAPs inhibited EPC mobilization, EPCs were mobilized by VEGF (100ng/kg/d/4d) and AMD3100 (5mg/kg) treatment in mice exposed to either air or CAPs. VEGF+AMD3100 treatment doubled the number of circulating EPCs in air-exposed mice but not in CAPs-exposed mice (-48±14% control). Similarly, CAPs exposure prevented VEGF-induced Akt and eNOS phosphorylation in aorta. Stem cell factor (SCF, 200μg/kg/d) and AMD3100 co-treatment, however, mobilized EPCs equally well in air- or CAPs-exposed mice. Collectively, these results show that HFD or CAPs blocked VEGF-mediated EPC mobilization, which may diminish endothelium repair, induce endothelial dysfunction and increase CVD risk.

2062 CYTOTOXICITY AND INHIBITORY EFFECTS OF LOW-DOSE TRICLOSAN ON ADIPOGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELLS.

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Humans of all ages are continually exposed to triclosan (TCS), a widely used antimicrobial agent that can be found in many daily hygiene products, such as toothpaste and shampoos; however, the toxicological and biological effects of TCS in humans after long-term and low-dose exposure are far from being well understood. In the current study, we investigated the effects of TCS on the differentiation of human mesenchymal stem cells (hMSCs) by measuring the cytotoxicity, morphological changes, lipid accumulation, and the expression alterations of adipocyte differentiation biomarkers during a 21-day adipogenic differentiation process. Significant cytotoxicity was observed in hMSCs treated with high-dose TCS (~5.0 μM), but not at low-dose (~2.5 μM), treatments. TCS inhibited adipocyte differentiation of hMSCs in a dose-dependent manner from 0.156 to 2.5 μM, as indicated by changes in Oil Red O staining. The inhibitory effect was confirmed by a decrease in gene expression of specific adipocyte differentiation biomarkers, including ap2, LPL, and adiponectin at the mRNA level. Our study demonstrates that TCS inhibits adipocyte differentiation of hMSCs under non-cytotoxic dose conditions. (Supported by Interagency Agreement between NCTR/FDA IAG #224-07-007 and NIH/NTI JAG #Y1E51027.)

2063 KEY METABOLIC PATHWAY CHANGES IN HUMAN EMBRYONIC STEM CELLS EXPOSED TO METHYL PARATHION AND METHYL PARAOXON.

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Toxic industrial chemicals (TICs) represent a threat to soldiers, first responders and other civilians. One class of toxic industrial chemicals, pesticides, is particularly accessible and used widely in crop, industrial, and home applications. For many pes-
Potency Ranking of Monophthalates Studied by Transcriptomics in the Mouse Embryonic Stem Cell Test.

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The murine Embryonic Stem cell Test (EST) is widely studied as an animal-free screening method for potential developmental toxicants. The inhibition of contracting cell foci formation assessed after 10 days is taken as a parameter for developmental toxicity. Our research aims at introducing transcriptomics to decrease the duration of the test method and to increase the sensitivity and informative value of the readout of the test. Here we compared contracting cell foci formation and differential gene expression after exposure to four phthalates in a concentration-dependent manner. Phthalates are used as plasticizers in consumer products and are known to affect embryonic development and differentiation of embryonic stem cells towards cardiomyocytes. Three embryotoxic phthalates, monobutyl phthalate (MBuP), monononyl phthalate (MnP), and mono-(2-ethylhexyl) phthalate (MEHP) and the non-embryotoxic monomethyl phthalate (MMP) were tested. We observed compound specific concentration response effects on gene expression after 24 hours of exposure and on contracting cell foci formation 6 days later, when exposure was initiated at day 3 of culture. The concentrations of embryotoxic phthalates resulting in 50% inhibition of differentiation (ID50), based on the classical readout of the EST, were between 0.4 and 1.4 μM. The potency ranking of phthalates resulting in 50% inhibition of differentiation (ID50) was similar to that of known in vivo embryotoxicity. Concentration-response analysis of gene expression showed the same potency ranking. Moreover, gene expression appeared orders of magnitude more sensitive than the untreated cells with 13 human metabolic pathways exhibiting statistically significant enrichment in the treated cells. These data suggest that MP and MPO exposure may significantly impact the metabolism of undifferentiated hES cells.

Specification of Cardiomyocytes Using Small Molecules During the Differentiation of Human Pluripotent Stem Cells.


Pluripotent stem cells derived from human cells are a useful tool for drug discovery and development because they can be used to model many cell types important for toxicology studies, can be made from disease-specific patient populations, and represent relevant human biological pathways. An example of an important toxicology model is the use of cardiomyocytes differentiated from human embryonic (hES) and induced pluripotent stem cells (hiPS). In order to use these models for drug discovery, a reproducible and scalable method for differentiation needs to be developed. In addition, characterizing the specification of the resulting cells into atrial, ventricular, and nodal cell populations is also important for developing downstream assays. A focused library of small molecule kinase inhibitors that were capable of inhibiting +100 kinase targets was used to screen across the cells. These compounds were added to the hES and hiPS cells during the last week of the differentiation, and the gene expression of these cells was examined via immunofluorescence of the cardiac progenitor marker NKX2.5 and the cardiomyocyte marker Tropomin T after 12 days. Twenty-four compounds were found to significantly increase the expression of at least one of these markers indicating that these compounds were increasing the efficiency of the differentiation. In addition, 75% of the initial “hits” were confirmed in secondary screens. Overall, these data suggest that addition of small molecules during the differentiation of human pluripotent stem cells can lead to more efficient production of cardiomyocytes that can be used in toxicology assays for drug discovery.

Development of a High-Throughput, In Vitro Human Pluripotent Stem Cell Test (hPST) for the Identification of Potentially Teratogenic Compounds.


Teratogens are compounds that disturb the development of the embryo or fetus, and result in birth defects, while remain relatively nontoxic to the maternal body. Currently, drugs are tested for teratogenicity in pregnant animal models such as rats or rabbits. However, using animal tests to detect potential human teratogens is problematic because of inter-species differences in teratogenesis mechanisms as tragically demonstrated in thalidomide case that resulted in more than 10,000 cases of birth defects. In addition a continuous effort to reduce the number of animals used during drug development is important for ethical reasons. However, creating robust in vitro screens for teratogenicity has been challenging due to the dynamic cellular changes that occur during embryonic development. Here, we describe a human Embryonic Stem cell Test (hEST) for potential teratogens. This methods allowed us to successfully classify 55 out of 59 tested compounds (93% accuracy) with three false positives and one false negative. This suggests that the human pluripotent stem cell model may be used as a convenient yet sensitive time point to test potential teratogens. Compared to the other existing in vitro teratogenicity assays such as whole embryo culture (WEC), MicroMass test (MM), and the mouse Embryonic Stem cell Test (mEST), the hPST assay is faster (1 week), requires less compound (<1mg), is amenable to high-throughput screening, and can detect teratogenic compounds undetected in animal models such as teratogenic thalidomide.

Derivation of Cyromolgus Macaque-Induced Pluripotent Stem Cells (iPSCs) for In Vitro Toxicology Assay Development.


Induced pluripotent stem cells (iPSCs) are a useful tool for drug discovery and development because they can be used to model many cell types important for in vitro toxicology studies, can be made from disease-specific patient populations, and can be used when embryonic stem cells are not available for a particular species. Generating iPSCs from pre-clinical toxicity species, such as the cyromolgus macaque, would aid in developing mechanistic toxicology assays to further investigate in vivo study findings. iPSCs have been generated using many viral—mainly lentivirus and retrovirus—and nonviral reprogramming methods—including mRNA transfection, plasmid transfection, and recombinant proteins. Our work has focused on finding a reprogramming method that is efficient, suitable for species including the cyromolgus macaque and other preclinical species, and produces fully reprogrammed cells. Specifically, we used primary cells cultured from in-house kidney tissue samples and Sendai virus constructs for the delivery of the reprogramming transcription factors. In comparison to other methods, the Sendai virus infections were easier to perform, infect cells from multiple species, and produce fully reprogrammed cells quickly. To date, we have produced many cyromolgus macaque iPSC cell lines that exhibit the morphology, growth characteristics, and gene expression associated with iPSC and embryonic stem cells. These cell lines can be used in the future to develop in vitro mechanistic toxicology assays for early drug development.
MECHANISM OF ACTION OF THE HERBICIDE PARAOXON ON ERYTHROID DIFFERENTIATION PATHWAY IN BONE MARROW OF MICE.

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Paraquat (1,1'-dimethyl-4,4'-bipyrindinum dichloride), the most widely used herbicides in the world is toxic to human beings. Exposure to Paraquat causes anemia in humans but the mechanism of its action on the differentiation of erythroid cells in bone marrow and erythrocyte turnover in blood circulation is not known. In the present study, the effect of Paraquat administration on erythroid differentiation in mouse bone marrow was studied. Stages of erythroid differentiation were enumerated by double staining of bone marrow cells with Ter 119 (Glycoporphin) and CD 71 (Transferrin) markers (Marinkovic et al. J Clin Invest. 117, 2133, 2007). This methodology allows identification and enumeration of four different precursors of erythroid population, i.e. early pro-erythroblast (Termed CD71high), basophilic erythroblast (Terhigh CD71high), late basophilic, polychromatophilic erythroblast and orthochromatophilic erythroblast (Terhigh CD71med), and orthochromatophilic erythroblast with mature erythrocytes (Terhigh CD71low). C57Bl/6 mice were administered 10 mg/kg of Paraquat i.p. on alternate days and precursor cells representing different stages of erythrocytes pathway enumerated on day 7, 14 and 21. Proportion of cells of erythroid lineage decreased significantly in response to Paraquat; the decline being 46%, 50.4% and 47.1% respectively on day 7, 14 and 21 of Paraquat treatment. Pro-erythroblast progenitors were significantly reduced (40-50% reduction) whereas the decline in erythroblast population was more severe (70-80% reduction). Further analysis indicated that the decline in bone marrow erythropoiesis resulted from enhanced apoptosis as well as a decline in mitotic activity of the erythroid precursor cells of bone marrow. The changes induced by Paraquat on the mouse erythroid cells were transient since the mice regained normal levels of blood erythrocytes 28 days after the Paraquat treatment was stopped.

CELLULAR, METABOLIC, AND HISTOLOGICAL EVIDENCE FOR ARSENIC-INDUCED MYOPATHY.

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Stem and precursor cells are susceptible to the deleterious effects of arsenic. Previous studies have demonstrated that muscle precursor cell (MPC) exposure to arsenic in vivo inhibits MPC differentiation and gene activation of the myogenic program. Given the importance of stem cells for both developmental processes and tissue regeneration, this prevalent toxicant therefore presents a major public health concern. We investigated the hypothesis that in vivo arsenic exposure (100ppm) for 5 weeks induces epigenetic and metabolic changes in adult mouse MPCs. We further hypothesized these cellular changes to be associated with aberrant skeletal muscle tissue integrity. Indeed, we observed accelerated growth kinetics when MPCs were isolated after arsenic exposure through drinking water, as compared to controls. Transmission electron imaging of arsenic-exposed muscles revealed mitochondrial alterations. Among the highest ranking putative redox disrupting chemicals (pRDC) were mitochondrial disruptors such as rotenone, azoxystrobin, pyraclostrobin, fluoxastrobin and trifloxystrobin. For these 5 chemicals, the ToxCast™ ranking was 50% change in stem cell differentiation (AC50) were grouped with more potent pRDCs whereas chemicals that altered cell number by 50% were evenly distributed throughout the redox ToxCast™ ranking (Wilcoxon rank sum, p=0.03). To test the putative redox disrupting activity of pRDCs, 2,7'-dichlorodihydrofluorescein diacetate was used to measure ROS in exposed mESCs. Preliminary data indicated H2O2 at concentrations ~300uM produced a 50% decrease in ROS. Further analysis indicated that 50% decrease in ROS was 30% lower on Day 3 than Day 7, indicating a decline in antioxidant capacity with mESC differentiation. Thus, more differentiated mESCs may have decreased antioxidant capabilities. This finding indicates the importance of temporal considerations in the mESC differentiation assay when interpreting the pRDC ToxCast™ ranking of ToxCast chemicals on oxidative stress signaling/alterred redox pathways. In sum, altered redox potential may be an adverse outcome pathway linked to altered differentiation in mESCs and developmental toxicity in vivo. Identification of pRDCs may be useful in prioritizing chemicals as potential developmental toxicants. This abstract does not necessarily reflect US EPA policy.

A NEW STRATEGY FOR DRUG DISCOVERY AND DEVELOPMENT BY ANALYZING THE BEHAVIOR OF ES CELLS CULTURED ON TOSHI (TISSUE/ORGAN SECTIONS FOR HISTOPATHOLOGY)-SUBSTRATA.


It is reported that mouse embryonic stem (ES) cells injected into the tail veins of carbon tetrachloride (CCL4) liver-injured mouse were differentiated into hepatocyte-like cells in the host liver [Hepatology 37: 983-993, 2003]. Therefore, we investigated whether the ES cells could also be differentiated into hepatocyte-like cells when they were cultured on the TOSHI-substrata prepared from livers in various stages after CCL4 administration into mice. Consequently, it was found that the substrata derived from regenerating livers enhanced cell attachment, supported growth as clusters, and induced differentiation into cells expressing albumin, although the substrata from injured livers did not. In particular, the cells cultured on the most proliferative regenerating liver-derived substratum reconstructed the hepatic cord-like structures with bile canaliculus-like aspects in which some binucleated cells were involved, secreted albumin, and expressed cytochrome P450A1 activity within a few days [Tissue Eng. Part A 14: 267–274, 2008]. Here, I propose a novel strategy for drug discovery and development from the above data. The data suggest two advantages based on the behavior of ES cells in a culture system utilizing TOSHI-substrata: one is that TOSHI-substrata derived from regenerating livers with high proliferative potential efficiently induced the differentiation of ES cells toward hepatic lineage and another is that ES cells functions as a sensor recognizing liver toxicity in TOSHI-substrata derived from injured livers after CCL4 administration into mice. Therefore, it is considered that the former would be available for the novel approach of drug discovery to find bioactive factors and the latter provide a new alternative method of animal experiments in toxicology to support drug development [Methodological Advances in the Culture, Manipulation and Utilization of Embryonic Stem Cells for Basic and Practical Applications, Craig Atwood, (ed.), InTech, Croatia, pp. 473-488, 2011].

REDOX DISRUPTING POTENTIAL OF TOXCAST CHEMICALS RANKED BY ACTIVITY IN MOUSE EMBRYONIC STEM CELLS.


To gain insight regarding the adverse outcome pathways leading to developmental toxicity following exposure to chemicals, we evaluated ToxCast™ Phase I chemicals in an adherent mouse embryonic stem cell (mESC) assay and identified a redox sensitive pathway that correlated with altered myocardial differentiation. Here, we developed a weight-of-evidence ToxPi ranking for 309 chemicals across 19 ToxCast assays selected for cell-based and biochemical features that can be tied to cellular redox balance. Among the highest ranking putative redox disrupting chemicals (pRDC) were mitochondrial disruptors such as rotenone, azoxystrobin, pyraclostrobin, fluoxastrobin and trifloxystrobin. For these 5 chemicals, the ToxPi ranking followed their rank order potency in developmental toxicity (ToxRedDB). For the entire chemical library, those that produced a 50% change in stem cell differentiation (AC50) were grouped with more potent pRDCs whereas chemicals that altered cell number by 50% were evenly distributed throughout the redox ToxCast™ ranking (Wilcoxon rank sum, p=0.03). To test the putative redox disrupting activity of pRDCs, 2,7'-dichlorodihydrofluorescein diacetate was used to measure ROS in exposed mESCs. Preliminary data indicated H2O2 at concentrations ~300uM produced a 50% decrease in ROS. Further analysis indicated that 50% decrease in ROS was 30% lower on Day 3 than Day 7, indicating a decline in antioxidant capacity with mESC differentiation. Thus, more differentiated mESCs may have decreased antioxidant capabilities. This finding indicates the importance of temporal considerations in the mESC differentiation assay when interpreting the pRDC ToxCast™ ranking of ToxCast chemicals on oxidative stress signaling/alterred redox pathways. In sum, altered redox potential may be an adverse outcome pathway linked to altered differentiation in mESCs and developmental toxicity in vivo. Identification of pRDCs may be useful in prioritizing chemicals as potential developmental toxicants. This abstract does not necessarily reflect US EPA policy.

SMALL-MOLECULE KINASE INHIBITION TO EXPEDITE THE MATURATION OF HUMAN IPS-DERIVED CARDIOMYOCYTES.

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Human pluripotent stem cell-derived models are becoming increasingly important in drug discovery and development, because of a number of key attributes. In particular, they are human, euploid, genetically defined, can be derived from relevant patient populations, and are scalable. However, tissues differentiated from pluripotent stem cells are more similar to fetal tissues than adult, making their extrapolation to the adult, in vivo situation difficult. Our recent work studying long-term culture of stem cell-derived cardiomyocytes (hiPS-CMs) revealed a time-dependent activation of genes associated with the adult heart, suggesting these cells may be capable of maturing into a more adult-like phenotype. However, long-term culture (>4 months) is not feasible for examining toxicities associated with SAR of novel mo-
2073 EVALUATION OF CADMIUM BIPHASIC CYTOTOXICITY ON HUMAN EMBRYONAL CARCINOMA AND MOUSE EMBRYONIC STEM CELLS.

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Cadmium (Cd) is a complex metal that is classified as a nongenotoxic human carcinogen. Environmental and occupational exposure, including cigarette smoke and contaminated food and water, are among the possible sources of Cd. Although the exact mechanism of Cd carcinogenicity is debatable, current information suggests that exposure to low concentrations of Cd results in alterations in gene expression and mitochondrial dysfunction. Undifferentiated mouse embryonic (EC) and mouse embryonic stem (mES) cells are both undifferentiated pluripotent cell lines that are capable of unlimited proliferation and programmed differentiation in vitro. Based on the principle that altered cell proliferation leads to abnormal embryogenesis, we evaluated the effect of low concentrations of Cd in the stem cells by measuring cell viability (MTT assay), cell proliferation ([3H]-thymidine [3H-TdR] incorporation for DNA synthesis) and total protein (sulforhodamine B [SRB] assay). Cells were treated with various concentrations of Cd for 1-h (+ 23 hour recovery period), or 24-h. Cell viability significantly decreased at higher Cd concentrations (400-800 μM) following 1-h exposures, but recovery was evident within 23-h. Inhibitory concentration 50% (IC50) values for EC and mES cells were estimated at 64 and 107 μM, respectively, after 24-h exposures. Although there was a consistent, statistically noticeable, increase in cell proliferation following 24-h to low dose Cd (80 nM - 5 μM), this biphasic effect was not statistically significant. The study demonstrates that a toxicologically important hormesis effect from trace metals is distinguishable in cultured embryonic stem cells and depends on the indicator and exposure period. The ability to detect this in vitro phenomenon implies that similar processes occurring in vivo may be responsible for development, induction, or enhancement of the carcinogenic process.

2074 EXPRESSION OF THE ARYL HYDROCARBON RECEPTOR DURING MOUSE EMBRYONIC STEM CELL DIFFERENTIATION IS HIGHER IN MESODERMAL LINEAGES.

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The aryl hydrocarbon receptor (AhR) is a ligand activated transcription factor. Many of its ligands, including dioxin (TCDD) and the dioxin-like compounds, are known environmental toxicants with extensive biological effects. Upon TCDD binding, the AhR translocates into the nucleus, heterodimerizes with the Ah receptor nuclear translocator (ARNT) and binds to AhR response elements in the chromatin of target genes. As a consequence of AHR-ARNT complex binding, many target genes are up-regulated while others are down-regulated, or even silenced. AhR is one of the critical mediators of gene-environment interactions. Besides the canonical AhR signaling pathway, alternative AhR functions, resulting from cross-talk with other signaling pathways, have also been reported. A growing body of evidence indicates that AhR plays an important role in regulating cell differentiation and cycling, hormonal and nutritional homeostasis, immune responses, aging and cancer promotion. Recently, evidence in AHR knockout mice has also shown a functional role for the AhR in hematopoiesis and heart development. To characterize the role that the AhR plays in development and the consequences of dioxin exposure during embryonic differentiation, we have studied the effect of TCDD exposure on the differentiation of mouse embryonic stem cells (ESC) in culture and on the expression of marker genes of different cellular lineages. Stable ESC lines transfected by a Cyp1a1 promoter-driven puromycin-IRES-eGFP plasmid were allowed to differentiate in the presence of TCDD followed by puromycin treatment. Real-time PCR analyses of diagnostic ectoderm, endoderm, and mesoderm marker gene expression revealed that many mesoderm marker genes were up-regulated by TCDD treatment, while both endoderm and ectoderm markers were down-regulated. Since up-regulation of the transgene is AHR-dependent, these data indicate that during early differentiation, AhR activation might be committed to mesodermal lineages, suggesting a potential role for AHR in the regulation of their morphogenesis during embryonic development. Supported by NIH grant R01ES06273.

2075 ARSENIC INHIBITS DIRECTED DIFFERENTIATION OF MOUSE PLURIPOTENT STEM CELLS TO EPIDERMAL KERATINOCYTES.

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Skin is a primary target for chronic arsenic toxicity. Hyperkeratosis, hyperplasia and non-malignant skin cancer (NMSC) are well-established hallmarks of arsenicism. Keratoses develop slowly over many years with a wide range of severity and have been recognized as a pre-malignant lesion since the nineteenth century. Despite being strongly linked to these non-malignant skin diseases in humans, there is no mechanism that satisfactorily explains arsenic’s cutaneous effects. A growing body of literature suggests that arsenic disrupts epigenetic programming, thereby disrupting the expression of genes that regulate cell cycle and differentiation. The goal of this study is to test the possibility that arsenic disrupts stem cell differentiation, and in doing so, identify the stage(s) when disruption occurs. In order to get the greatest possible perspective, directed epithelial differentiation of mouse embryonic stem cells was employed. Mouse embryonic stem cells were differentiated by treating with all-trans retinoic acid (ATRA; 1 μM) BMP4 (25 μM) and 300 μM ascorbic acid for 7 days, then with keratinocyte media containing growth supplement for an additional day. Differentiation was preformed in the presence or absence of 0.5 μM arsenic throughout the experiment. Differentiation from embryonic stem cell to keratinocyte was followed using qRT-PCR to measure expression levels of key differentiation markers. Here we show that arsenic does not influence the differentiation of embryonic stem cells prior to acquiring an epithelial phenotype. After this point, however, markers of terminal differentiation are repressed by arsenic. We conclude that arsenic does not target early-stage differentiation of embryonic stem cells; rather, arsenic disrupts differentiation of more terminally differentiated keratinocytes and inhibits expression of early markers of stratified epithelium. This observation is consistent with the effects of arsenic on HaCaT cells (human keratinocytes) and human keratinocytes infected with HPV 16.

2076 IMPROVED HIPSCS-DERIVED CARDIOMYOCYTES (REPROCARDIO 2) FOR USE IN HIGH-THROUGHPUT ELECTROPHYSIOLOGICAL PLATFORMS AND HIGH-CONTENT IMAGING SYSTEMS.


Human induced pluripotent stem cells (hiPSCs) possess self-renewing potency and pluripotency, which are known to provide a promising source of human cardiomyocyte cell types. However, current cardiomyocytes differentiated from hiPSCs have limitations for use, due to their instability of their cardiac characteristics and functionality, which has given rise to problems when using electrophysiological assay systems. To overcome these limitations, ReproCELL has developed the ReproCardio2 cardiomyocytes, derived from hiPSCs that demonstrate stable cardiac characteristics and functionality and can therefore be used on electrophysiological assay systems. Furthermore, cardiomyocytes from the ReproCardio2 can produce beating clumps, thin layers or single beating cells and therefore have the versatility for use in high-throughput screening (HTS) platforms or high-content imaging systems. Whether as a single cell, clump or thin layer, ReproCardio2 cardiomyocytes stained positive for the representative cardiac markers, αMHC, cTnT, MLC-2a, MLC-2v, Cx43. Using the improved ReproCardio2 cardiomyocytes we have further developed our QTTempo® assay (beating clumps on the MEA platform) to better predict the drug induced QT interval prolongation (DIQITOP) of a wide spectrum of compounds including Sotalol and Verapamil. We are also developing a high-content imaging assay, utilizing the ReproCardio2 cardiomyocytes ability to form beating thin layers. Fluorescent microscopy clearly showed synchronized electrical potential using a Ca2+ transient assay (fluor8) and converting this to
and M. P. Waalkes

idly than with the total population.

target human skin SCs for carcinogenic transformation and this happens more rap-

malignant transformation to occur. Thus, it appears arsenic exposure can directly

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the evaluation of chemicals for epigenetic toxicity.

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toxicology test model. We suggest that the developed model could be a useful tool for

evaluation of chemicals for epigenetic toxicity.

Because of small portion of PGC in the embryo, large scale of screening is imposi-
to overcome these obstacles, we establish in vitro PGC differentiation model from

mouse embryonic stem cells (ESC) and evaluated the methylation patterns by

vinzclozolin in differentiated PGCs. To differentiate mouse ESC to PGC, hanging

drop method was used. SSEA-1 antibodies were used to isolate the primordial germ

cell. To verify the PGC, isolated PGC were cultured for 7 days on mouse embry-
one fibroblasts (3T3) in 2 mM retinoic acid supplemented medium and 2nd separa-
tions of PGC were done using SSEA-1 antibody. A well-known endocrine dis-
ruptor,vinzclozolin, was treated to ESC during differentiation and proliferation period

of PGC at 0.18mM, 0.27mM and 0.36mM concentration. To determine the methylation pattern in H19 gene, Biotinylated PCR products were purified with streptavidine- sepharose. 4 Ptg sites in H19 gene between 1594-1730 were analysed using P55G6MA system. Average methylation rate of H19 at Cpg sites was 84.3% in ESC, and it was decreased to 43.8% in 1st isolated PGC at day 5, and 33.4% in 2nd isolated PGC after further 7 day culture. The erase of H19 methylation was significantly delayed (70.5%) by vinzclozolin treatment to ESC during the differentiation to PGC for 5 days. But, it was delayed in PGC isolated after 7 day culture (2nd isolated PGC) at all vinzclozolin groups. The methylation of H19 gene was more significantly erased by 7 day culture of PGC after isolation of PGC differentiated for 5 days from ESC. The vinzclozolin showed significant delay the erase of methylation at H19 gene Cpg site by the present In vitro epigenetic toxicity test model. We suggest that the developed model could be a useful tool for the evaluation of chemicals for epigenetic toxicity.

ARSENIC-INDUCED MALIGNANT TRANSFORMATION OF HUMAN SKIN KERATINOCYTE STEM CELLS.

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Arsenic is a human skin carcinogen. Cancer is probably a disease driven by stem cells (SCs) and SCs are likely a key target during arsenic carcinogenesis. Accumulating evidence indicates that cancer SCs (CSCs) may arise from SCs. Our prior work showed arsenic-induced malignant phenotype was particularly pro-

ounced in the SCs subpopulation during transformation of human skin ker-
atinocytes or prostate epithelial cells and this occurred concurrently with an over-

production of CSCs. Further, arsenic can directly and rapidly transform a prostate

epithelial SC line into CSCs. Here we determine if arsenic can induce oncogenic

transformation in SCs isolated from a human skin keratinocyte line (HaCaT cells). SCs first were enriched from HaCaT cells by using a positive magnetic bead isola-
tion system for CD34 positive cells. CD34 is a robust cell surface marker for human skin SCs. Isolated SCs were then continuously exposed to sodium arsenite (100 nM) in collagen-coated dishes and compared to unexposed SCs. Potential ma-

lignant phenotype and SC/CSC characteristics were assessed. After 13 weeks con-
tinuous arsenic exposure, transcript level of K13, a biomarker for dermal cancer progression, markedly increased in arsenic exposed (As-E) SCs compared to control SCs. The expression of PTEN, a tumor suppressor gene often inactivated in tu-
mors, was markedly reduced in As-E SCs compared to control. Furthermore, com-
pared to control, As-E SCs showed markedly elevated secreted activity of MMP-9, an invasive ability, and colony formation in soft agar, all indicators of cancer phen-

type. The expression of SC markers CD34, Oct-4, K19, K5 and K15 markedly in-

creased in As-E SCs compared to control SCs. In prior work using total population

HaCaT cells, similar oncogenic changes were not observed in phenotype or expres-
sion as early as 13 weeks of exposure to 100 nM arsenic, and it took ~30 weeks for malignant transformation to occur. Thus, it appears arsenic exposure can directly target human skin SCs for carcinogenic transformation and this happens more rap-

idly than with the total population.

REVIEWAL OF THE US NATIONAL TOXICITY PROGRAMS (NTP) MULTILYMPHOMA FORWARD MUTATION ASSAY (MLA) DATA USING CURRENT STANDARDS REVEALS LIMITATIONS OF USING THE PROGRAM'S SUMMARY CALLS.

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The NTP developed an extensive database on the mutagenicity of chemicals in the MLA during the 1980s. These data are frequently used to evaluate the performance of this assay to predict in vivo mutagenicity and carcinogenicity. Since the MLA has undergone significant procedural enhancements in recent years, a project was un-
taken to reevaluate the NTP data according to the current standards (IWGT) to asess the assay performance capabilities. Data from more than 1900 experiments representing 342 chemicals were examined against acceptance criteria for back-
ground mutant frequency, cloning efficiency, positive control values, and appropri-
ate dose selection. In this reanalysis, only 17% of the experiments and 40% of the “positive” calls met the current acceptance standards. Approximately 20% of the test chemicals required >1000 µg/ml to satisfy the criteria for the selection of the top concentration. When the concentration is expressed in molarity, approximately 58, 32, and 10% of the chemicals required ≤1 mM, ≤1 ≤10 mM, and >10 mM, respectively, to meet the criteria for the top concentration. More than 60% of the chemicals were judged as having insufficient data to classify them as positive, nega-
tive, or equivocal. Of the 265 chemicals from this list evaluated by Kirkland et al. (2005, Mutat Res., 584, 1), there was agreement between Kirkland calls and our calls for 32% of the chemicals. A detailed listing of the chemicals along with their analysis calls will be presented to highlight the limitations of using the NTP’s summary calls. This evaluation also revealed the need for expert review of primary data to assess specific chemical results and to develop structure-activity and read-

across relationships.

ARISTOLOCHIC ACID IS A MORE POTENT GENE MUTAGEN THAN CLASTOGEN IN F344 RATS.


The herbal drug aristolochic acid (AA) is a mixture of two structurally related ni-

trogenated compounds with the potential to induce DNA damage is an important step in

assessing the assay performance capabilities. Data from more than 1900 experiments

representing 342 chemicals were examined against acceptance criteria for back-
ground mutant frequency, cloning efficiency, positive control values, and appropri-
ate dose selection. In this reanalysis, only 17% of the experiments and 40% of the “positive” calls met the current acceptance standards. Approximately 20% of the test chemicals required >1000 µg/ml to satisfy the criteria for the selection of the top concentra-
tion. When the concentration is expressed in molarity, approximately 58, 32, and 10% of the chemicals required ≤1 mM, ≤1 ≤10 mM, and >10 mM, respectively, to meet the criteria for the top concentration. More than 60% of the chemicals were judged as having insufficient data to classify them as positive, nega-
tive, or equivocal. Of the 265 chemicals from this list evaluated by Kirkland et al. (2005, Mutat Res., 584, 1), there was agreement between Kirkland calls and our calls for 32% of the chemicals. A detailed listing of the chemicals along with their analysis calls will be presented to highlight the limitations of using the NTP’s summary calls. This evaluation also revealed the need for expert review of primary data to assess specific chemical results and to develop structure-activity and read-

across relationships.

DEVELOPMENT OF A HIGH-CONTENT HIGH-

THROUGHPUT SCREENING PLATFORM FOR GENOTOXICITY ASSESSMENT.

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DNA damage causes chromosome instability and gives rise to impaired cell func-
tion, apoptosis, or carcinogenesis. Identification of environmental and pharmaceu-

tical compounds with the potential to induce DNA damage is an important step in

a high-content system will provide a more robust and predictive set of tox-assays for drug discovery.
investigating chemical safety. Micronuclei formation is a key characteristic of geno- toxicity, and can be assessed using the in vitro micronucleus (MN) assay. This assay detects aneugenic and clastogenic compounds in cells that have divided during treatment. A high-content high-throughput MN assay was developed in CHO-k1 cells in 384-well format, as part of the U.S. Tox21 program, following OECD guidelines. 1000 cells/well were imaged on the ImageXpress® system, and MetaXpress was used to identify and quantitate micronuclei after compound exposure. To further classify active chemcials as aneugens or clastogens, a 1536-well high-content screen was developed for detection of pH2AX, a phosphorylated histone protein that accumulates in foci at the sites of DNA double strand breaks. Detection of pH2AX was achieved using a FITC-conjugated anti-pH2AX antibody, and analysis was completed using MetaXpress. To complement the pH2AX results, a Rad51-GFP redistribution assay was developed in 1536-well format. Rad51 is a component of homologous recombinination DNA repair and is recruited to nuclear foci to coordinate repair. Using this battery of high-throughput assays, we successfully characterized the clastogenic and aneugenic activity of over 20 known genotoxic compounds (e.g., mitomycin C, etoposide, teniposide, bis-D-arabinofuranoside). The results obtained support the use of this battery in Tox21 Phase II, where a 10K compound library will be screened for genotoxicity. Supported by NIEHS Interagency Agreement Y3-ES-7020-01.

2082 OPTIMAL DOSE SETTINGS TO EVALUATE DNA DAMAGE IN DIFFERENT ORGANS IN A RAT COMET ASSAY.


The comet assay is a promising technique to evaluate DNA damage in vivo. However, evaluation techniques have not been optimized; there is no agreement on a method to evaluate DNA damage in organs where the cytotoxicity was observed. As part of the JaCVAM international validation study to develop the testing guideline, we examined DNA damage on the liver and stomach cells in the comet assay. Male SD rats were treated three times orally at 3, 24 and 48 hours before sacrifice with N-methyl-N-nitrosourea (MNU, coded by JaCVAM when tested) at 50, 100 and 200 mg/kg. The maximum tolerated dose, 200 mg/kg, was set based on the systemic toxicity in the dose-finding test. MNU significantly increased the % tail DNA both in the liver and stomach. Histopathological analysis showed no cytotoxic effect on the liver, indicating clearly that MNU has a DNA damaging potential to the liver. In the stomach, however, the extremely severe cytotoxic effects such as degeneration/necrosis in mucosa were observed at any doses including systemically non-toxic dose of 50 and 100 mg/kg. The increase in the % tail DNA was considered to be caused by a secondary effect of severe cell damage. The additional study was conducted with lower doses of 6.25, 12.5 and 25 mg/kg and the increase in the % tail DNA at no cytotoxic dose of 6.25 mg/kg was observed. Therefore, it was concluded that MNU has the DNA damaging potential in the stomach. Above findings indicate that an optimal dose for detecting DNA damage may vary among organs and that it is not adequate to select a top dose for the comet assay solely based on the systemic toxicity like mortality and clinical observations. We concluded that when the increase in the % tail DNA was observed, the criteria for a positive response in the comet assay should include the confirmation of no cytotoxic effect, judged by histopathological examination to ensure well-designed dose settings.

2083 IDENTIFICATION OF GENOMIC INSERTION SITES OF LEEP10 DNA IN GPT DELTA TRANSGENIC MICE AND RATS BY HIGH-THROUGHPUT DNA SEQUENCING.


Transgenic gpt delta mice and rats are developed for in vivo mutation assays. The assays allow detection of gene mutations in any organs of mice and rats, and therefore frequently used for identification of mutations in target organs of carcinogenicity. The transgenic rodents have been established by microinjection of LEEl0 phage DNA into the fertilized eggs of C57BL/6J mice and Sprague Dawley (SD) rats. Recently, Fischer 344 gpt delta rats have been established by backcross from the SD gpt delta transgenic rats. LEEl0 DNA carries reporter genes for point mutations in the gpt gene and the mouse chromosome contains 70 base-pair (bp) duplicated sequences, two-bp insertion at one end and five bp sharing between LEEl0 and the mouse chromosome at the other end. In a similar manner, transgenic mice from male F344 gpt delta rats were applied to high-throughput DNA sequencing analysis. The result indicated that multi-copy of LEEl0 was inserted at a single position in the rat chromosome 4, where 72 kb genomic DNA was deleted. The junction contained a 14 bp-insertion sequence, among which 10 bp was identical to the end of inserted LEEl0. Based on the DNA sequence information, PCR primers that could distinguish between homo and hetero status of the transgene were designed for mice and rats. High-throughput DNA sequencing is a powerful tool to analyze complex genome rearrangements in detail such as identification of insertion sites of the transgene in rodents for in vivo mutation assays.

2084 EVALUATION OF IN VITRO MICRONUCLEUS TEST IN TK6 LYMPHBLASTOID CELLS: COMPARISON WITH CHL CELLS AND HUMAN PERIPHERAL LYMPHOCYTES USING PHARMACEUTICAL DRUG CANDIDATES.


In vitro micronucleus test (MN) using mammalian cells is widely used to assess the genotoxic potential of compounds. Human TK6 lymphoblastoid cell, one of the cell lines used for genotoxicity studies, has the great advantage of p53 proficiency, which is expected to decrease false positive results caused by underestimation of cytotoxicity. However, TK6 cell has not been well validated in MNT so far. In this study, to evaluate the sensitivity of TK6 cells in MNT, we tested our internal pharmaceutical drug candidates with in vivo positive and negative genotoxic effects and compared the results with those in CHL cells and human peripheral lymphocytes (HLs). TK6 cells were treated with 11 compounds for 3 hours with or without S9 followed by the recovery period for 21 hours or continuously treated for 24 hours without S9. The frequency of micronucleated cells in 1,000 cells was analyzed for each treatment. Chromosomal aberration tests in CHL cells and HLs and in vivo MNT in rats were also conducted with those compounds.

Even for a shorter treatment period, most of the compounds produced a more severe cytotoxicity in TK6 cells than in CHL cells. All in vivo MNT-positive compounds showed a statistically significant, clearly positive response in TK6 cells. In vivo MNT- and HL-negative, CHL-positive compounds also produced a statistically significant increase in the incidence of micronuclei in TK6 cells, but those compounds showed a weaker response compared to the in vivo MNT-positive compounds. Hence, in vivo MNT-positive and negative compounds were able to be discriminated with the introduction of a cut-off value for the incidence of micronuclei. These results suggested that actual in vivo genotoxic potential of a compound reflected the TK6 response.

From these results, it is considered that TK6 cells are useful and sensitive enough for in vitro MNT if the threshold for micronucleus induction is incorporated into the evaluation criteria together with a statistical method.

2085 COMBINATION OF MUTATION AND CHROMOSOMAL DAMAGE ENDPOINTS USING PIG-A GENE AND MICRONUCLEUS ASSAY IN PERIPHERAL BLOOD IN RATS.

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Two endpoints of genetic toxicity, mutation at the x-linked Pig-a gene and chromosomal damage in the form of micronucleated reticulocytes were evaluated in blood samples of rats. Wistar Han rats were treated with three prototypical mutagens: N-ethyl-N-nitrosourea (ENU), 2-acetylaminofluorene (2-AAF) and cyclophosphamide (CPA). Animals were treated on three consecutive days (days 1-3) via oral gavage and blood specimens were obtained on days: -1, 4, 15, 30 and 45. A second endpoint of genotoxicity the frequency of peripheral blood micronucleated reticulocytes was measured on day 4. Each chemical induced micronuclei and the GPl-anchor-deficient phenotype. Increased mutant cell fractions were applied to CPA on day 15. Mutant reticulocyte frequencies remained relatively stable for some chemicals CPA, but the other two chemicals peaked and the dropped significantly. The differences in kinetics observed are presumably related to the degree to which mutation occurs in hematopoietic stem cells versus more committed cells with limited self-renewal capacity. The results indicate that determination of the frequency of GPl anchor-deficient erythrocytes is an efficient test system of evaluating the in vivo mutagenic potential of chemicals. Integration into routine toxicology studies is feasible.
2086 GENOTOXICITY OF FURAN DERIVATIVES AS ASSESSED BY THE COMET ASSAY.
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Furan derivatives are produced during food processing such as cooking and frying, and some are added to food as flavourings. When ingested, these compounds are substrates for sulfotransferases (SULTs) which transform the substrate to a water soluble compound to ease excretion. However, in some cases this transformation can lead to the formation of DNA reactive metabolites. Most in vitro genotoxicity tests do not include SULT activation systems, and thus compounds that are acti-
vated by SULTs may give negative results in conventional genotoxicity tests sys-
tems. The aim of this study is to identify the genotoxic potential of selected furan derivatives with SULT activation both in vitro and in vivo. Chinese hamster V79 cell lines expressing combinations of a human cytochrome P450 (CYP) and human SULT1A1, and transgenic mice expressing human SULT1A1 and 1A2 (SULT mice) were used in the study. DNA damage was assessed using a modified version of the single cell gel electrophoresis assay (alkaline Comet assay). This method de-
tects DNA strand breaks and alkali labile sites. Enzyme treatment was used to de-
tect oxidative damage by specifically inducing gaps at these lesions. A concentra-
tion-dependent increase in genotoxicity was shown for furfuryl alcohol in vitro, but it appeared independent of SULT activation. Furfuryl alcohol did not cause detectable DNA damage in SULT mice. A dose-dependent increase in DNA damage in the liver of 5-hydroxymethylfurfural (HMF) treated SULT mice was found when specifically testing for oxidative damage. The project continues studying the geno-
toxicology of alkyl-substituted furans. In conclusion, the genotoxicity of furan deriva-
tives seems to be dependent on the side chains. It remains to be determined whether the difference in genotoxicity is a consequence of SULT activation.

2087 FLOW CYTOMETRY-BASED IN VITRO MICRONUCLEUS ASSAY: MITIGATING THE INFLUENCE OF APOPTOTIC ACTIVITY.
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Overt toxicity, including apoptotic activity, is known to generate misleading re-
sults in mammalian cell cytogenetic assays. This laboratory has previously described a flow cytometric approach for scoring in vitro micronuclei (MN). An important component of this method is the application of the dye ethidium monoazide (EMA) to help differentially label the chromatin associated with dead and dying cells from actual MN. The current work with human TK6 cells was initiated to criti-
cally investigate the ability of this methodology to discriminate apoptosis-inducing compounds that are thought to possess little or no direct genotoxic activity from genotoxicants with appreciable apoptotic activity. The genotoxicants were etopos-
ide, colchicine, taxol, camptothecin, aphidicolin, and 5-fluorouracil, and the non-
genotoxic apoptosis inducers were anti-FAS, tunicamycin, dexamethasone, car-
bonyl cyanide m-chlorophenylhydrazine, and tributylin. Chemicals were studied over a range of concentrations in quadruplicate, and each compound was evaluated in two independent experiments. Treatment occurred for 24-30 continuous hours in 96-well plates, and staining, plasma membrane lysis and flow cytometric analysis occurred in these same plates according to In Vitro MicroFlow® Kit instructions. Flow cytometric MN scoring was accomplished based on the acquisition of approx-
imately 5,000 nuclei per replicate well. Cytotoxicity was evaluated via relative sur-

2088 GENOTOXICITY ASSESSMENT OF MOLINDONE.

Antipsychotic drug, molindone, has been on the market since 1970s. It is currently being developed for new indications for the CNS-related diseases. As part of an ef-
fort to fill the nonclinical information gap for this drug, a complete genotoxicity as-
essment was conducted. Studies were conducted in compliance with GLP regula-
tions and included tests recommended by ICH for new pharmaceutical products using OECD developed protocols. Genotoxicity test battery included: assessment of gene mutations in bacteria, chromosome aberrations in human lymphocytes and micronuclei in rats. Results showed that molindone was negative in the chromo-
some aberration assay with and without S9 up to appropriate cytotoxicity levels and in rat micronucleus assay up to limits of toxicity. In the bacterial Ames Salmonella and E. coli assay, molindone was negative, except for a weak positive response (2.5-
3-fold) at high doses in a single strain TA100. This finding was further investigated using human S9 to evaluate the background genotoxic risk. With the human S9, molindone demonstrated a weaker activity in TA100 achieving a 2-fold increase in revertants/plate only at a dose of 5000 μg/plate. But, the mutagenic response was abolished in the presence of human S9 and phase II enzyme system (uridine-5-
diphosphoglucuronic acid and glutathione), with a demonstration of conjugates, suggesting a detoxification mechanism. Further, an additional in vivo rat comet assay was performed as a follow up study. Standard procedures were used in the conduct of this assay. The comet assay results were negative. Based on these data, it was concluded that the genotoxicity risk of molindone, if any, is negligible to hu-
man.

2089 ASSESSMENT OF AN INTEGRATED IN VITRO GENOTOXICITY SCREENING TEST FOR AN EARLY AND MORE ACCURATE DETECTION OF GENOTOXIC COMPOUNDS.
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The integration of innovative genotoxicity screening testing into the early discovery phase of drug development processes is required for several reasons, including the potential to improve predictivity of mammalian cell test systems and to optimize learning cycles with regard to a sensible selection of high quality candidates for fur-
ther development. The recently developed GADE45x GreenScreen HC geno-
toxicity assay (GreenScreen assay) (Hastwell et al. 2006) offers this potential because of its higher specificity compared to other in vitro mammalian cell assays. This study aimed to assess the accuracy of the GreenScreen assay by testing four later stage development drug candidates along with four reference compounds (in-
cluding known genotoxicants and non-genotoxicants), which were compared to re-
sults obtained from other genotoxicity test systems. In addition, a set of in-house early drug discovery compounds with compound purities between 90% and 100% were tested. The overall concordance of the GreenScreen assay in this study with expected re-
sults of drug candidates and reference compounds was found to be very high, which confirmed findings reported in the literature. The assessment of the in-house drug discovery compounds revealed a lower genotoxicity rate with the GreenScreen assay when compared with the in vitro Micronucleus test indicating a reduced false posi-
tive rate. Importantly, our results clearly showed that low-level impurities at 1% or higher concentrations can have a serious impact on the GreenScreen assay result.

Takings together our results provide additional evidence to support the use of the GreenScreen assay as an effective mammalian cell test system in the genotoxicity screening battery to improve predictivity power of detecting genotoxic com-

2090 CYTOTOXIC AND MUTAGENIC EFFECTS OF HALOQUINONE DRINKING WATER DISINFECTION BYPRODUCTS.
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The haloquinone (HQ) class of disinfection byproducts was recently identified. Little is known about HQ toxicity, but they are predicted to be potent carcino-

gens. This study examined cytotoxicity and mutagenicity of 5 HQ DBPs: 2,6-
dichloro-1,4-benzoquinone (DCBQ); 2,6-dichloro-3-methyl-1,4-benzoquinone (DCMBQ); 2,3,6-trichloro-1,4-benzoquinone (TCBQ); 2,5-dibromo-1,4-benzo-
quinone (2,5DBQ); and 2,6-dibromo-1,4-benzoquinone (2,6DBQB). Impairments based on real-time cell electronic sensation (RT-CES) was used to examine cytotoxicity. Dye- and label-free RT-CES measures cell proliferation, viability, and morphology as cell index (CI). Increasing CI indicates cell proliferation, growth, and/or attachment; decreasing CI indicates cell death, detachment, and/or a cyto-

static state. The normal human fibroblast BJ cell line (CRL-2522) was used in the RT-CEs system. Cells were exposed to 5-50 μM of individual HQ and monitored for 24 h. No change in CI was observed in BJ cells exposed to DCBQ, TCBQ, or 2,5DBQ; however, decreased CI was observed in BJ cells exposed to 12.5-50 μM DCMBQ and 50 μM 2,5DBB. A bacterial reverse mutation (Ames) test was used }
to examine mutagenicity of 3.1-50 μM HQ DBPs +/- rat S9 fraction. Salmonella typhimurium strains TA98 and TA100 were used to primarly detect frameshift and substitution mutations, respectively; Escherichia coli WP2 uvrA was used to detect mutagens acting at AT base pairs. Significant mutations compared to negative control group (Student’s t-test, p<0.05 + two-fold increase from negative control) were observed in TA100 exposed to ≥1.25 μM 2,6DBBQ and ≥1.5 μM DCMBQ without S9. TA98 exposed to ≥5 μM 2,6DBBQ was found to have significant mutations compared to negative control group ≥25 μM in all HQs except 2,6DBBQ. Few mutations were observed ≥/S9 in TA98 or E. coli strains. In summary, 2,5DDBQ and DCMBQ are cytotoxic to BJ cells under these experimental conditions. Some HQs appear to be mutagenic in the Ames test, primarily producing substitution mutations; addition of S9 increases mutagenicity of some HQs but decreases mutagenicity of others.

This study was conducted to further optimize smoke exposure procedures, measure the mutagenicity of mainstream cigarette whole smoke (WS) and the contribution of the gas vapor phase (GVP) and wet total particulate matter (WTPM) impart to the WS activity, as determined by the Salmonella Reverse Mutation (Ames) Assay. WS, GVP and WTMP were prepared from Kentucky Reference 3RF4 cigarettes smoked under ISO puff profile (35 mL volume, 2 second puff duration and 1 minute puff interval) on a VITROCCELL® VC10 smoking robot. TA98 and TA100, in the presence of increased (S9)- in metabolic activation, were exposed to WS or GVP from three (3) 3RF4 cigarettes via the VITROCCELL® Dilution / Distribution System with dilution air flow rates set at 1, 2, 4 and 8 L / minute, allowing the delivery of four doses of WS or GVP to the Ames exposure modules during each exposure. For GVP experiments, a Cambridge filter pad was placed in-line prior to the puffing syringe to remove the smoke particulate fraction, which was subsequently extracted in dimethylsulfoxide (DMSO) for use in WTMP exposures. Quantification of several carbonyls verified the delivery of GVP to the bacteria. WTMP exposures (S9+/S9-) utilized a 30 minute preincubation with DMSO limited to 2.5% v/v final concentration. WS mutagenicity was detected in both strains (S9+/S9-); however, TA100 S9- WS activity was approximately 30% of TA100 S9- WS activity while TA98 S9- WS activity was considerably lower at 5% of measured TA98 S9+ WS activity. No GVP mutagenicity was detected in both strains (S9+/S9-). Lack of GVP activity was not due to cytotoxicity since no significant decrease in cell viability was observed over the delivered GVP dose range. WTMP activity was detected in both strains (S9+ only) at approximately 67% and 94% of the WS activity measured in TA100 and TA98, respectively. Under the exposure conditions used in this study, the majority of the WS mutagenic activity was found to reside in the particulate fraction, with no apparent contribution to WS activity coming from GVP.

**2093 RELATIONSHIP BETWEEN GENETIC DAMAGE AND DERMAL LESION OF ARSENICOISIS PATIENTS CAUSED BY BURNING COAL.**

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Arsenicosis caused by burning coal has become a public health problem in Guizhou province, China. Skin carcinoma and hepatoma are the main cancers of it. But till now, the carcinogenic mechanism is as yet unknown. In view of mutation closely related to carcinogenicity, we selected 95 cases exposed to arsenic caused by burning coal as research objects. 41 villagers living 1km away were chosen as control group, sharing a similar lifestyle but not using high arsenic coal. The objects were divided into groups in accordance with their clinic symptoms (Chinese National Arsenicosis Diagnosis Criteria, WS/T211-2001) and dermal histo-pathological results. Genetic damage situation of them were determined. And the relationship between genetic damage and dermal lesion were analyzed meanwhile, to discuss arsenicosis carcinogenic mechanism and provide evidences for dynamic monitoring and prevention. Results 1. Compared to the normal control, chromosome aberration and sister chromatid exchange ratios were significantly higher in exposed groups. While micro nucleoli only significantly increased in carcinoma group. 2. DNA single strand breaks increased significantly in groups exposed to arsenic. DNA-protein crosslinks were remarkable higher in hyperkeratosis, pre-carcinoma and carcinoma group. 3. The genetic damage rates of every index increased coincide with the development of dermal lesion. An obvious positive correlation relationship existed between them(with r=0.9955, p<0.01). Conclusions 1. Arsenic exposure caused by burning coal can result in human body significant genetic damage. 2. The genetic damage of arsenicosis patients is closely related to dermal lesion. 3. In the process of arsenicosis dermal carcinoma, genetic damage might play a role.

**2094 ERYTHROSINE, A FOOD DYE, SHOWED MUTAGENIC POTENTIAL IN HEPG2 CELLS BY CYTOKINESIS-BLOCK MICRONUCLEUS CYTOME ASSAY.**

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Introduction: Erythrosine is a colorant widely used on foods, drugs and cosmetics. This study aimed at determining the frequency of binucleated cells with micronuclei (MNs), nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs) which provides a measure of genome damage and/or chromosomal instability. Methods: Mutagenicity and damage DNA were evaluated using the cytokinesis-block micronucleus cytose assay (CBMN-cyt). HepG2 cells, a human-derived hepatoma cell line that have retained the activities of several phase I and phase II drug metabolizing enzymes, were treated with different concentrations of erythrosine dye (25, 50, 100, 300 and 500 μg/mL) for 24 h. To obtain binucleated cells, cytokinesis-B was added to the culture after exposure to the dye. The incubation time of the HepG2 cultures was 68 h at 37 °C. At the end of the culture period the cells were fixed. Positive (0.05 μM doxorubicin) and negative (1% w/w dimethyl sulfoxide) controls were included. In addition, nuclear division index (NDI) was calculated. Results: CBMN-cyt was used as an endpoint for in vitro DNA damage and showed mutagenic effect at all five erythrosine concentrations evaluated in HepG2 cells. On the other hand, the concentration at 100 μg/mL showed the highest number of MNs and the lowest NDI, indicating reduction in cell proliferation. Furthermore, NPBs and NBUDs scored were similar to negative control. Conclusions: Considering that erythrosine is an approved food dye and even the lowest concentration induces the MNs formation in HepG2 cells, its intake can be considered a human health risk. Therefore, this colorant should be better evaluated and used carefully. This study was supported by FAPESP.

**2095 SYSTEMATIC EVALUATION OF EXPERIMENTAL VARIABILITY IN THE COMET ASSAY IN VIVO.**

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Over the last years the comet assay in vivo has increasingly been used for regulatory genotoxicity testing, for the evaluation of DNA damage in a number of organs of rats and mice. In the pharmaceutical industry the assay is mainly carried out to follow the guidelines (1) all in vivo mutagenicity results, primarily in liver, (2) unexpected proliferative findings in chronic toxicity studies, to elucidate the contribution of organ-specific genotoxicity or (3) local genotoxicity, mainly in the GI tract.

**2092 COMET ASSAY IN RAT SKIN—ESTABLISHMENT OF A RELIABLE AND ROBUST CELL ISOLATION METHOD.**

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The in vivo COMET assay is increasingly being used to evaluate the genotoxic potential of industrial chemicals, agrochemicals, and pharmaceuticals and it provides more detailed information when a positive test results is obtained in the in vitro genotoxicity tests. This assay makes it possible to perform genotoxicity testing at the organ tissue level across a range of organ types. The skin is playing a major role in the occupational exposure to chemicals and agrochemicals and is regarded as one of the main target organs. In order to investigate a potential DNA damage to skin, the COMET assay in rat keratinocytes was established together with a reliable and robust cell isolation method, avoiding artefacts of DNA damage. For this different enzyme cocktails for skin digestion were investigated and also different mechanical techniques were used for isolation of the keratinocytes. For the performance of the COMET assay in rat keratinocytes was established together with a reliable and robust cell isolation method, avoiding artefacts of DNA damage. For this different enzyme cocktails for skin digestion were investigated and also different mechanical techniques were used for isolation of the keratinocytes. For the performance of the COMET assay in rat keratinocytes was established together with a reliable and robust cell isolation method, avoiding artefacts of DNA damage. The incubation time of the HepG2 cultures was 68 h at 37 °C. At the end of the culture period the cells were fixed. Positive (0.05 μM doxorubicin) and negative (1% w/w dimethyl sulfoxide) controls were included. In addition, nuclear division index (NDI) was calculated. Results: CBMN-cyt was used as an endpoint for in vitro DNA damage and showed mutagenic effect at all five erythrosine concentrations evaluated in HepG2 cells. On the other hand, the concentration at 100 μg/mL showed the highest number of MNs and the lowest NDI, indicating reduction in cell proliferation. Furthermore, NPBs and NBUDs scored were similar to negative control. Conclusions: Considering that erythrosine is an approved food dye and even the lowest concentration induces the MNs formation in HepG2 cells, its intake can be considered a human health risk. Therefore, this colorant should be better evaluated and used carefully. This study was supported by FAPESP.
Recommenensions for conducting the in vivo comet assay have been published by expert panels and currently an international validation study coordinated by the JaCVAM is ongoing. It is hoped that these activities will result in a standardized protocol which is a key element to limit the known experimental variability of the comet assay. It has been demonstrated that several technical parameters, such as electrophoresis and scoring, have an impact on the assay variability. In contrast, lit- tle is known regarding the influence of the initial steps, i.e. from tissue sampling to slide preparation, on assay variability. Here we present a systematic evaluation of the experimental variability brought by different key steps of organ sampling, tissue storage and slide preparation. We closely examined the influence of (1) the time be- tween euthanization of the animal and tissue sampling; (2) the size of the sampled tis- sue; (3) the composition, temperature and volume of the sampling buffer; and (4) the time of the tissue or single cell suspension in the sampling buffer etc. These data will help to further clarify and define a common standardized protocol for the Comet assay in vivo.

2096 EVALUATION OF THE NUCLEOTIDE ANALOGS, 5-FLUOROURACIL AND CYTOSINE ARABINOSIDE IN THE IN VITRO MICRONUCLEUS ASSAY: COMPARISON OF FLOW CYTOMETRY AND SLIDE ANALYSIS.


With the adoption of OECD Guideline 487, the in vitro micronucleus (MNvit) assay has become a prevalent genetic toxicology test. This laboratory has conducted the MNvit assay with TK6 cells comparing flow cytometric methods and slide analysis using various measures of cytotoxicity. Compounds used were cystosine ara- binoside (CA), 5-fluorouracil (5-FU) and, as a positive control, mitomycin C (MMC). Cultures were incubated for 24 hours and either harvested (24-4) or al- lowed to recover for 24 hours prior to harvest (24-24). Cytotoxicity was determined via calculations of relative population doubling (RPD), relative cell count (RCC), and relative increase in cell count (RICC) using a Coulter Counter; and relative survival (RS) using a flow cytometer. A cytotoxicity target of ~55% relative to the vehicle control was used. Appropriate cytotoxicity was observed at 0.02 g/mL (RS), 0.5 μg/mL (RPD and RCC), and 0.9 μg/mL (RPD with CA), at 0.3 μg/mL (RS), 0.5 μg/mL (RCC and RS), and 0.05 μg/mL (RPD with CA), at 0.3 μg/mL (RS), 0.5 μg/mL (RCC and RRP, and 0.05 μg/mL (RPD) with CA, at 0.5 μg/mL (RS), 0.5 μg/mL (RCC and RRP, and 0.05 μg/mL (RPD with 5-FU, and at 0.01 μg/mL (RS), 0.02 μg/mL (RCC and RCC), and 0.03 μg/mL (RPD with MMC. Statistically significant responses in micronuclei (p <0.05) were observed with CA using both flow cytometry and slide analysis (5-fold increases) and MMC (5-fold increases) at ~55% cytotoxicity using all measures of cytotoxicity, and with 5-FU with flow cytometry (7-fold increases) using RPD, RCC, and RICC as measures of cytotoxicity. Negative results were observed with 5-FU flow cytometric data (<2-fold increase) using RS as a measure of cytotoxicity and in slide analysis (<2-fold increases) using all measures of cytotoxicity. These data indicate that for CA flow cytometry analysis is comparable to slide analysis, but for 5-FU flow cytometry appears more sensitive with respect to cytotoxicity measurements versus Coulter Counter analysis and to micronucleus evaluation versus slide analy- sis.

2097 THE DYE REACTIVE BLACK 5 INDUCES DNA DAMAGE IN HEPG2 CELLS.

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Introduction: The fabrics dyeing began thousands of years ago and the commercial availability of dyes is enormous. In Brazil textiles production is a major economic activity. Unlimited and uncontrolled use of such dyes can lead to serious conse- quences in terms of human health and ecological balance. Therefore, the cytotoxic- ity, genotoxicity and mutagenicity testing of textile dyes is a crucial tool for accu- rately predicting health risks for consumers exposed to dyes and impact of them to the environment. The dye Reactive Black 5 (RB5), widely used during the fibers dyeing process, has the group dichlorotriazine as reactive and the group azo as chromophore. Our objective is to evaluate the hepatotoxicity, genotoxicity and mutagenicity of the RB5 dye. Methodology: Cultures of the HepG2 cells were exposed to RB5 dye at concentrations ranging from 0.1 to 50.0 μg/mL. Cytotoxicity was evaluated after 24, 48 and 72 hours using MTT assay. Comet and cytokinesis-block micronucleus tests were carried out according to Tice et al. (2000) and Natarajan and Darroudi (1991), respectively, with slight modifications. Results: No citotoxic ef- fect was observed after the exposure of HepG2 cells to RB5 dye. However, the dye studied induced DNA damage and the formation of micronucleus in HepG2 cells in a dose-dependent manner. Discussion: Both comet and micronucleus assays de- tected chromosomal damage leading the formation of micronuclei in binucleated HepG2 cells. Conclusion: Based on our data, we can say that the RB5 dye is a mutagenic substance and that these bioassays can thus be used as an initial screening test to analyze various dyes and dye containing effluents avoiding damages on hu- mans and to the aquatic environment.

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2098 DEVELOPMENT OF A NOVEL GLP 3D EPIDERM™ RECONSTRUCTED HUMAN SKIN MICRONUCLEUS ASSAY.


The 3D reconstructed human skin micronucleus assay (RSMN) in EpiDerm™ is a promising new in vitro assay for the assessment of genotoxicity. Analysis of mi- cronuclei in a 3D primary skin model offers a more biologically relevant in vitro ap- proach to assess genotoxicity of many types of dermal exposures including drugs, chemicals and cosmetics, compared to standard in vitro genotoxicity assays. EpiDerm™ provides a functional stratum corneum that takes into account perme- ability, appears to have normal dermal metabolic capability, normal DNA repair and cell-cycle control. The RSMN assay provides a new animal alternative for follow- ing up chemicals that are positive in current in vitro genotoxicity assays and is especially useful for cosmetics that can no longer be tested in vivo assays accord- ing to the 7th Amendment to the EU Cosmetics Directive. To meet the increasing interest in this assay, we have developed a GLP RSMN assay. Results for model chemicals including mitomycin C and vinblastine sulphate show dose-dependent increases of MN and demonstrated good reproducibility and comparability to pre- viously published results. Studies are ongoing with genotoxins and nongenotoxins with various modes of action. Results to date demonstrate the transferability of this novel assay into a robust GLP setting.

2099 EVALUATION OF THE MUTAGENICITY OF REACTIVE DYES.

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Introduction: In Brazil, textile industry is considered as one of the main economic activities in the country. This fact is relevant, since textile dyes are discharged into the aquatic ecosystem via industrial effluents and potentially expose humans and biota to adverse effects. The class of reactive dyes includes colored compounds highly water soluble, little absorbed by biomass and not degraded by conventional methods used in wastewater treatment plants. The dye Reactive Blue 4 (RB4) is widely used in the textile industry and it has the group dichlorotriazine as reactive and the group anthraquinoine as chromophore. The dye Reactive Blue 15 (RB15) is also an important textile commercial dye based on copper-phthalylocyanine as chro- mophore and a monochlorotriazine group as reactive site. Objective: Evaluate the mutagenicity of the dyes RB4 and RB15 using the Salmonella mutagenicity with the strains TA98, TA100 and YG1041. Methodology: Salmonella mutagenicity assay was carried out according to Maron and Ames (1983), with or without S9 metabolic activation. Different concentrations of the dyes ranging from 0.25 to 5000 μg/plate were examined. Results: RB4 dye showed positive mutagenic re- sponse for TA100 and YG1041 strains in presence of S9 (1.44 and 1.56 revertants/μg, respectively), as well as for YG1041 without S9 (0.15 revertants/μg). RB15 dye showed no mutagenic activity under the conditions tested. Discussion: RB4 dye induces damages to DNA by both base-pair substitution and frame-shift mutations. Possibly products generated after metabolic activation have been given a higher interaction with DNA. Conclusion: These results can provide relevant infor- mation for the textile sector, contributing to the production of dyes safer, protect- ing the environment and human health.

2100 CYTOTOXIC AND GENOTOXIC PROPERTIES OF THE MIXED HERBAL TEAS.

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The quality of herbal tea is influenced by many toxic contaminants originating from industries, agriculture and private households including mutagenic and car- cinogenic effects. Therefore, the increased consumption of herbal teas is a crucial problem in Turkey as well as in other countries and makes necessary determining the possible carcinogenic and mutagenic ingredients or contaminants of them. Generally, identification of all compounds present in herbal teas is a difficult task. Moreover, chemical analysis does not evaluate the cyto- or genotoxic effects of

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chemicals nor their possible additive, synergistic or antagonistic interactions in complex samples. The toxicity of mixture could be different from the sum of the effects of the components. Therefore, the present study investigated the cytogenic and genotoxic activities of 6 mixed herbal teas. Herbal teas were collected from points around Istanbul, Turkey. Water, methanol and chloroform extracts of all the herbal preparations were assayed by LDH (lactate dehydrogenase activity for cell membrane integrity) and XTT test (mitochondrial succinate dehydrogenase activity) for cytotoxic activities, Ames (bacterial reverse mutation assay by Salmonella typhimurium TA98 and TA100) and umu-test (short-term bacterial assay by Salmonella typhimurium TA1535/pSK1002 strain) for genotoxic activities. In some extracts, it was found genotoxic and cytotoxic effects. It is believed the results to be obtained from the study is useful to determine their toxicological effects and to take precautions about consumption of herbal teas in our country.

2101 MICRONUCLEI INDUCTION BY ETHYL-4-BROMOPHENYL-CARBAMATE AND ETHYL-4-CLOROPHENYL-CARBAMATE AFTER ACUTE AND SUBCHRONIC RAT EXPOSURE USING PERIPHERAL BLOOD ERYTHROCYTES.

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Ethyl-4-bromophenyl-carbamate (LQM 919) and ethyl-4-chlorophenyl-carbamate (LQM 996) are newly synthesized chemicals with acarcidical properties. Previous data about toxicological capacity are unknown. Genotoxicity capacity was assessed using peripheral blood smears from treated rats. Micronucleus technique as genotoxicity testing was conducted at acute and subchronic exposure. For acute exposure, oral single doses from 50 and 300 mg/kg for both LQM 919 and LQM 996 were used and for subchronic exposure male and female Wistar rats were treated with 12.5, 25 and 50 mg/kg during 90 days for both compounds. The micronuclei frequency data was analyzed using normochromatic erythrocytes (MN-NCE) and polychromatic erythrocytes (MN-PCE). In order to determine cytotoxicity, the ratio of polychromatic erythrocytes to normochromatic erythrocytes was calculated.

In the acute exposure, LQM 919 and LQM 996 induced a significant increase (p<0.05) in the frequency of MN-PCE in rats treated with both doses 50 and 300 mg/kg. After subchronic exposure both compounds induced a significant increase (p<0.05) in the frequency of MN-PCE and MN-NCE in the rats exposed in all the range of doses. Both, LQM919 and LQM996 had not cytotoxic capacity at least in this testing. This study shows that both carbamates derivatives significantly increased the frequency of micronuclei in polychromatic erythrocytes showing genotoxic capacity.

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2102 FINE PARTICLE EMISSION FROM TOBACCO SMOKE IN ENCLOSED ENVIRONMENTS.

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Fine particle are effective vehicles to transport toxicants into the lung; depending on the size particles may reach the bronchiolar or alveolar space. In recent years particular matter (PM) has been correlated with both pulmonary and cardiovascular diseases. Smoking machines used to generate main and side stream tobacco smoke have been designed for animal experiments; although they mimick smoking cycles their geometry bears little similarity to human environments (e.g. car, office). In order to better characterize PM emission and distribution from cigarette smoke (SRA 4 Kentucky standard cigarette) we have developed an ETS emitter to simulate human smoking emission and measured fine particle concentration in a telephone booth (1.75 m3 volume) as a substitute for similar spaced environments like cars, in order to imitate realistic situations. Fine particulate matter was measured using an aerosol spectrometer with 6 sec time resolution; laser scatter allowed the resolution from 0.25 µm to 32 µm to determine PM10, PM2.5 and PM1. Cumulative fine particulate matter was measured as AUC values (µg/m3/sec), maximum exposure as cmax values in µg/m3. Cigarette smoke produced particulate AUC values of 60 000 +/- 15 000 µg/m3/sec PM10 with maximum concentrations of ~ 1000 µg/m3. When opening the door air circulation reduced the AUC for PM10 by 90%, whereas maximum concentrations were reduced by 80%. For PM2.5 and closed door conditions the maximum concentrations were reduced by 90%(1.900 000 +/- 15 000 µg/m3) and cmax values were obtained (1035 +/- 230 µg/m3). Again, air circulation by opening the door reduced the AUC by 90%, and the cmax to 185 +/- 130 µg/m3. This method mimicked human smoking behaviour in confined spaces like cars and confirmed high fine and ultrafine particle concentrations and cumulative particle load in small compartments. This method may be adapted to measurements of other microenvironments which are polluted but are not amenable to direct measurements.

2103 EFFECT OF COMMERCIALLY USED AIR FILTERS ON ENVIRONMENTAL TOBACCO SMOKE CONSTITUENTS AND CYTOKINE-MEDIATED INFLAMMATORY RESPONSE.

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Lung inflammation, chronic obstructive pulmonary disease and asthma are linked to ETS and these can be exacerbated as a result of lung infections. The claim that air filtration systems eliminate the risk from ETS exposure has no scientific basis. In this study air filters commonly used in commercial buildings, MERV 4 (fiberglass), MERV 8 (charcoal) and MERV 8 (pleated), were tested for filtration efficiency of ETS constituents and ETS induced cytokine-mediated inflammatory response using a murine model. ETS was generated and passed through MERV 4, 6 & 8 air filters. Total suspended particulate (TSP) levels and carbon monoxide (CO) levels were measured with and without filtration through these filters and C57BL/6 mice were exposed with and without the air filters. Bronchoalveolar lavage fluid (BALF) was extracted and the alveolar macrophages (AM) were cultured and stimulated with lipopolysachcaride (LPS). Levels of TNF-a, IL-1b, IL-6 and GM-CSF secreted by the LPS stimulated and unstimulated AM's of control, unfiltered exposed and filtered smoke exposed mice were determined. TSP's were reduced less than 45% and CO was reduced less than 5% by the MERV 8 filters. TNF-a levels were significantly attenuated in the unfiltered, MERV 4 and MERV 8 exposed groups, while MERV 8 (charcoal) caused attenuation close to significance. Attenuation of IL-1b was observed in unfiltered and MERV 8 filtered ETS exposed mice. Both IL-6 and GM-CSF cytokines were attenuated by MERV 8 (charcoal) filtered ETS. Based on our results air filters do not eliminate ETS constituents or recover the attenuation of the cytokine response. This study was supported by a grant from the Flight Attendant Medical Research Institute and by the Nevada Agricultural Experiment Station.

2104 EFFECT OF COMMERCIALLY USED AIR FILTERS ON ENVIRONMENTAL TOBACCO SMOKE CONSTITUENTS AND OXIDATIVE STRESS.

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Environmental tobacco smoke (ETS) contains an estimated 1016 radicals per cigarette. Reactive oxygen species formed as a result of exposure to ETS can oxidize cell membrane phospholipids causing lipid peroxidation that can further damage the cells and tissues. Products of lipid peroxidation include malondialdehyde (MDA) and 4-hydroxyalkenals (HAE). Gluthathione (GSH) is an important antioxidant in maintaining the cellular redox status. In this study, the claim that ventilation systems eliminate the risk of exposure to ETS is investigated. Air filters commonly used in commercial buildings, MERV 4 (fiberglass), MERV 8 (charcoal) and MERV 8 (pleated), were tested for filtration efficiency of ETS constituents and ETS induced oxidative response using a murine model. ETS was generated and passed through MERV 4, 6 & 8 air filters. Total suspended particulate (TSP) and carbon monoxide (CO) levels were measured with and without filtration through these filters and C57BL/6 mice were exposed with and without the air filters. MERV 8 filters reduced TSP levels by less than 45% and CO was reduced less than 5% by the MERV-8 filters. Control, unfiltered ETS exposed and filtered ETS exposed lung homogenates were assayed for total glutathione levels and MDA+HAE. Unfiltered exposure led to highly significant elevated levels of GSH and MDA+HAE compared to the unexposed levels. Filtered ETS still led to elevated GSH and MDA+HAE compared to the unexposed levels. These results suggest that constituents of ETS or the oxidative damage caused by exposure to ETS smoke was not completely eliminated by using the air filters. This study was supported by a grant from the Flight Attendant Medical Research Institute and by the Nevada Agricultural Experiment Station.
2105 COMPARISON OF MOUSE STRAINS AND EXPOSURE CONDITIONS IN 2-WEEK CIGARETTE SMOKE INHALATION STUDIES.

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Cigarette smoke inhalation studies in mice have been conducted under various exposure regimens using different strains as animal models of smoke-related diseases. We exposed cigarette smoke to two strains of mice (C57BL/6J (C57) and AKR/J (AKR)) under two different exposure regimens (1 or 4hr/day) at equivalent daily exposure amounts (concentration x time). After 2 weeks exposure, mice were evaluated for exposure markers and biological responses. Smoke exposure suppressed respiratory functions dependent on exposure concentration. The 1 hr regimen groups generally showed a greater degree of respiratory suppression and relatively lower exposure markers of urinary nicotine metabolites than the corresponding 4-hr regimen groups. Tidal volume was more suppressed in AKR compared to C57, while respiratory rate was more suppressed in C57. Changes in those parameters suggested that C57 inhaled relatively more volume of smoke than AKR. Changes in bronchoalveolar lavage fluid (BALF) cytokine and enzyme parameters were most noticeable in the 1 hr AKR groups. For BALF cytokines, TARC concentration in C57 was higher than AKR, while KC and MCP-1 in C57 were lower than AKR. Relative lung/body weight ratio in smoke-exposed C57 was generally higher, as well as the incidence and severity of lesions in respiratory organs compared to AKR. In summary, C57 appeared to inhale relatively more smoke and displayed greater inflammatory changes in respiratory tract than AKR. Comparison of exposure regimens suggests that longer exposure durations at lower WTTPM concentration will deliver a larger dose of smoke than shorter exposure durations at higher WTTPM concentration.

2106 AN ANALYSIS OF HISTORICAL EXPOSURES OF PRESSMEN TO AIRBORNE BENZENE (1930s TO 2006).

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Benzene is an aromatic hydrocarbon and human carcinogen that was extensively used in the printing industry from the 1930s to the 1970s. Benzene is volatile and an effective solvent which explains its use in the easily vaporized low viscosity inks, ink solvents, and cleaning agents in the printing industry. Frequent benzene use explained by its use in the easily vaporized low viscosity inks, ink solvents, and cleaning agents in the printing industry. Frequency of benzene use was limited by biological factors such as species and age, test material and dosing conditions, testing conditions, animal model, and the evidence on experimental and organismic determinants of pyrethroid neurotoxicity in small rodents. A comprehensive analysis of these studies was conducted focusing on test material and dosing conditions, testing conditions, animal model, and other determinants such as test species. Variations in the severity of the neurotoxicity under laboratory-controlled conditions was found to be mostly explained by the influence of biological factors such as species and age, test material features such as chemical structure, stereochemistry and vehicle, and the potency of pyrethroids in small rodents. According to current guidelines, the estimation of absolute and relative potencies is a critical step in chemical risk assessment. In order to identify the variables influencing neurobehavioral findings across pyrethroid studies, we have reviewed the evidence on experimental and organismic determinants of pyrethroid neurotoxicity in small rodents. A comprehensive analysis of these studies was conducted focusing on test material and dosing conditions, testing conditions, animal model, and other determinants such as test species. Variations in the severity of the neurotoxicity under laboratory-controlled conditions was found to be mostly explained by the influence of biological factors such as species and age, test material features such as chemical structure, stereochemistry and vehicle, and dosing conditions such as route of exposure and dose-volume. Theoretically, interplay between all effective factors might result in up to -1,000-fold (o higher) variation in potency estimates. This critical analysis of the pyrethroid case examines the scope of toxicological data required to assure the safety of pesticide products, and the factors and on the towels and estimates of transfer for towel-to-hand, hand-to-mouth, and towel-to-lip. We estimated exposure to metals on the LSTs assuming either typical or high-end use of towels, represented by the mean (12 towels) and the 95% upper confidence limit on the mean (UCLM) (26 towels), respectively, as estimates of daily use per employee. We compared intake estimates to agency criteria and doses estimated at maximum contaminant levels (MCLs). For typical use of 12 towels a day, exceedances of Proposition 65 limits and toxicity criteria of the US Environmental Protection Agency (EPA) and the Agency for Toxic Substances and Disease Registry (ATSDR) may occur for seven metals. Assuming maximum daily intake concentrations, calculated intakes were up to 3,600-fold higher than their respective toxicity criterion. Intake of nine metals may also exceed that associated with the MCL. If the number of towels increases to 26 a day, additional exceedances of USEPA and ATSDR toxicity criteria may occur. Intakes were up to 7,700-fold higher (lead) than their respective criterion. Also, intake for 10 metals could be greater than that associated with the MCL. A sensitivity analysis indicated that alternate plausible assumptions could increase or decrease the magnitude of exceedances but was unlikely to eliminate certain exceedances, particularly for lead.

2109 REVISITING THE IMPACT OF BIOLOGICAL AND EXPERIMENTAL CONDITIONS OF LABORATORY ANIMAL STUDIES ON ESTIMATIONS OF RISK OF NEUROTOXICITY BY EXPOSURE TO PESTICIDES IN HUMANS: THE PYRETHROID CASE.

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Pyrethroids are potent insecticides that alter nervous system function in target and nontarget species by disrupting neuronal excitability. While the accumulated evidence consistently shows that this neurophysiologic action is followed by alterations in motor, sensorimotor, neuromuscular and thermoregulatory responses, there are some equivocal results regarding the potency of pyrethroids in small rodents. According to current guidelines, the estimation of absolute and relative potencies is a critical step in chemical risk assessment. In order to identify the variables influencing neurobehavioral findings across pyrethroid studies, we have reviewed the evidence on experimental and organismic determinants of pyrethroid neurotoxicity in small rodents. A comprehensive analysis of these studies was conducted focusing on test material and dosing conditions, testing conditions, animal model, and other determinants such as test species. Variations in the severity of the neurotoxicity under laboratory-controlled conditions was found to be mostly explained by the influence of biological factors such as species and age, test material features such as chemical structure, stereochemistry and vehicle, and dosing conditions such as route of exposure and dose-volume. Theoretically, interplay between all effective factors might result in up to -1,000-fold (o higher) variation in potency estimates. This critical analysis of the pyrethroid case examines the scope of toxicological data required to assure the safety of pesticide products, and the factors and
covariates which must be controlled for in order to ensure that predictive power and precaution are balanced in a risk assessment process within a reasonable time frame.

2110 OUTDOOR AIR RISKS FROM SUBSURFACE CONTAMINATION BY VOLATILE CHEMICALS.
Exposure to contaminants in outdoor air, resulting from migration of volatile chemicals from soil matrix, soil gas or groundwater, can present a significant human health risk. Vapor intrusion into indoor air usually results in greater exposure and risks than migration into outdoor air. This is because wind disperses contaminants into a large volume of outdoor air, plus advective forces of HVAC systems create negative pressure and draw subsurface air into buildings. At contaminated sites with no existing or potential future buildings, exposure to volatile chemicals in outdoor air should be considered. In the absence of existing State or Federal guidance for estimating outdoor air inhalation risks using soil gas data, we present methodology for a screening-level health risk assessment. The effective diffusion through the unsaturated vadose zone is assumed to be the sole mode of emission. Dispersion is based on Q/C values derived from the Industrial Source Complex model. Standard risk assessment methods are used to estimate inhalation exposure and associated risks. Sensitivity analyses were performed, comparing DTSC default soil assumptions to sand, source areas of 0.5 acres and 30 acres, and dispersion models (Q/C versus box model). Soil gas to outdoor air attenuation factors ranged from 10³ to 10⁶ for tetrachloroethylene, trichloroethylene, and benzene. Case studies demonstrate that potential outdoor air exposures may drive the risk assessment when buildings with indoor air exposures do not exist or are prohibited. If screening-level risks exceed acceptable levels, collecting indoor air samples and/or conducting site-specific refined estimates of outdoor air concentrations and inhalation risks is recommended.

2111 COMPARISON OF HUMAN EXPOSURE PATHWAYS FOR CONTAMINATED SOIL IN AN ARTIC URBAN BROWNFIELD.
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Risk assessments of contaminated soil often overlook the risk associated with soil inhalation as soil ingestion is typically the dominant exposure pathway. Iqaluit, Nunavut is one of many sites in northern Canada with a history of soil contamination and conditions in northern or rural centres favour the re-suspension of soil particles, making soil inhalation a relevant risk pathway. Here we determine and compare human exposure to metals and polycyclic aromatic hydrocarbons from soil ingestion and inhalation and analyze the carcinogenic and non-carcinogenic risk before and after roads were paved in Iqaluit. To determine the inhalation exposure, three size fractions of airborne particulate matter were collected (TSP, PM10 and PM2.5) before and after roads were paved. Paving roads reduced the concentration of many airborne contaminants by 25-75%, thus reducing risk. For example, before paving the carcinogenic risk associated with the inhalation of chromium was 3.4 in 100,000 whereas after paving this risk was reduced to 1.6 in 100,000. Paving roads reduced the concentrations of TSP (p < 0.1) and PM10 (p < 0.05) but not PM2.5. We conclude that re-suspended soil is likely an important source of risk for many northern communities and that paving roads is an effective method of reducing risk from the inhalation of soil particles.

2112 EXPOSURE AND TOXICITY OF CHEMICALS RESULTING FROM NATURAL GAS EXTRACTION AND HYDRAULIC FRACTURING.
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The number of sites extracting natural gas from shale formations has greatly increased following improved technologies allowing horizontal drilling within the shale. Hydraulic fracturing (hydrofracking) uses a combination of water, sand, and chemicals injected into the ground under high pressure to release natural gas or oil.

Along with economic benefits, hydraulic fracturing has led to public concerns over potential human health impacts. A recent DOE study (2011) has identified 4 major areas of concern: possible water contamination; air pollution; community disruption during production; and cumulative adverse impacts on communities and ecosystems from intensive shale production. However, much of the perceived concern is not based on measurements of chemicals at and surrounding operations. This study examines the first two of the DOE concerns, water contamination and air pollution, by surveying studies that report measured concentrations of potential pollutants associated with hydraulic fracturing in the ambient environment. The most abundant chemicals measured on- and off-site include many VOC (methane, ethane, propane, n-butane, isobutane, isopentane, and n-pentane), with lower amounts of aromatic hydrocarbons (benzene, toluene, and xylene) and other chemicals. Data from state monitoring sites, as well as independent research studies are compiled and analyzed to give an overall picture of potential exposure patterns resulting from hydraulic fracturing operations. The evaluation of off-site measurements allows us to better understand the temporal and spatial patterns of these chemicals in the ambient environment. Such information is necessary to be able to evaluate the potential impact of the drilling process on air and water burden in the surrounding communities. The evolving Federal and State regulatory climate regarding hydrofracking is reviewed to evaluate how different regions are approaching the regulation of this expanding industry.

2113 FORMALDEHYDE EXPOSURE ASSOCIATED WITH USE OF PROFESSIONAL KERATIN HAIR-SMOOTHING PRODUCTS.
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Formaldehyde (CH2O) is a ubiquitous air contaminant. Numerous indoor sources include glues, paper, coatings, particle board and household products. Occupational exposures occur in many industries including health care, pharmaceutical, paint and coating production. Recently, occupational exposure to CH2O via keratin hair-smoothing treatment has become a health concern. Keratin hair-smoothing products contain methylene glycol (MG) which exists in dynamic equilibrium with CH2O gas that can be released during treatment. The purpose of this study was to quantify potential occupational CH2O exposures in professional salons performing keratin hair-smoothing treatments. METHODS: Four specific products with [MG] at 7,582 to 22,200 ppm and estimated free [CH2O] at <11 ppm based on quantitative 13-C NMR spectroscopy were evaluated at 6 salons. Integrated treatment and task-specific levels were measured during pre-application preparation, application, blow-drying, and ironing. Air CH2O was analyzed per NIOSH Method 16676. Personal breathing zone (PBZ, n=35) and area samples (n=28) including pretreatment levels were collected during 9 treatments. Amount of product used and salon characteristics including ventilation parameters were recorded. RESULTS: PBZ [CH2O] during treatments lasting 63-93 min ranged from 0.11-0.33 ppm. Pre-treatment background [CH2O] ranged from 0.0068-0.032 ppm, ave 0.017 ppm. Area [CH2O] samples in the first 30 min after treatment ranged from <0.0058-0.055 ppm, ave 0.022 ppm. In the subsequent 50 min, [CH2O] ranged from <0.0050-0.03 ppm, ave 0.013 ppm. CONCLUSIONS: All PBZ (CH2O) were well below the OSHA PEL (0.75 ppm, 8 hr TWA) and STEL (2 ppm over 15 min). In 6 of the 9 applications, the ACGIH Ceiling of 0.3 ppm was exceeded for a limited period. Contributing factors to potential CH2O exposures include volume of product used, application methods, and proximity of stylist to hair being treated. These integral and task-based CH2O exposure data serve to characterize exposure potential opportunities and health risks in stylists performing these treatments.

2114 DERIVATION OF LOAEL AND NOAEL FOR TREMOLITE ASBESTOS.
Tremolite is a noncommercial form of amphibole mineral that is present in some chrysotile, talc, and vermiculite deposits. Some in the scientific and medical communities believe that exposure to tremolite asbestos, including tremolite, caused or contributed to an increased incidence of mesothelioma (MM) in occupational and non-occupational settings where individuals handle or otherwise come into contact with chrysotile, talc and vermiculite and/or end-products manufactured with these materials. However, very little is known about the magnitude of tremolite asbestos exposure that occurred from these scenarios, nor the exposure-response relationship for tremolite asbestos. In order to evaluate the exposure-response relationship for tremolite asbestos, a literature search was conducted to identify all tremolite asbestos-exposed occupational cohorts for which cumulative exposure
2115 TREMOLITE ASBESTOS EXPOSURES ASSOCIATED WITH THE USE OF COMMERCIAL PRODUCTS.


Tremolite is a member of the amphibole group of minerals and may be present in some chrysotile, talc, and vermiculite deposits. Increased incidence of malignant mesothelioma has been suspected to be caused by exposure to fibrous or asbestos-form tremolite in certain occupational (Thetford Mines, Canada and Libby, Montana) and non-occupational settings. However, very little is known regarding the magnitude of exposure to tremolite asbestos that occurred in these occupational environments, and even less is known about airborne tremolite exposure experienced by consumers handling or otherwise coming into contact with chrysotile, talc, and vermiculite-containing products. The purpose of this analysis was to develop estimates of cumulative tremolite asbestos exposure in various consumer product use scenarios and compare these values to a lowest-observed-adverse-effect level (LOAEL). Using measured and estimated airborne tremolite asbestos concentrations for simulated and actual product use, we conservatively estimated the following cumulative tremolite asbestos exposure: career auto mechanic: 0.028 f/cc-year; non-occupational use of joint compound: 0.0006 f/cc-year; non-occupational use of vermiculite-containing gardening products: 0.034 f/cc-year; home owner removal of Zonolite insulation: 0.0002 f/cc-year. In a separate analysis of the tremolite asbestos exposure–response relationship observed for the Thetford chrysotile mines and the Libby vermiculite workers, a LOAEL for mesothelioma of 35-73 f/cc-year was derived. Consequently, the estimated cumulative tremolite asbestos exposures experienced from handling chrysotile, talc and vermiculite-containing consumer products are well below the derived LOAEL for asbestiform tremolite.

2116 EVALUATION OF ASBESTOS EXPOSURE ASSOCIATED WITH ASBESTOS-CONTAINING GAUZE PADS.

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Gauze pads have been used for decades in chemical laboratories when heating flasks and other glassware using gas burners. These pads are typically composed of woven wire with a plaster center and in the past, many such gauze pads contained asbestos fibers within the plaster. In an effort to determine the potential for asbestos fiber exposure to laboratory personnel and others, we have undertaken testing involving normal use of asbestos-containing pads over a variety of different time periods from ½ to 2-hours. Industrial hygiene personal and area air samples were collected during all testing following NIOSH 7400/7402 methodology. Analysis of personal air samples (n=10) using phase contrast microscopy (PCM), showed airborne fiber concentrations ranging from less than 0.014 to 0.048 fibers per milliliter (f/ml) for sampling durations 30 to 120 minutes. Further analysis using transmission electron microscopy showed no asbestos fibers present in any of the personal samples. Analysis of area air samples (n=12) using (PCM) showed airborne concentrations ranging from less than 0.014 to 0.067 f/ml. Without the use of TEM, all but one of these area samples showed no asbestos present. An asbestos adjusted PCM concentration of 0.029 f/ml was indicated for the single area sample. These results indicate that use of asbestos–containing gauze pads produces a low and insignificant potential for asbestos fiber exposure.

2117 DO WOODWORKING OPERATIONS GENERATE POLYCYCLIC AROMATIC HYDROCARBONS?

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Occupational exposure to wood dust has been associated with an elevated risk of sino nasal cancer. Wood dust is recognized as a human carcinogen but the specific cancer causal agent remains unknown. One possible explanation is that co-exposure to wood dust and polycyclic aromatic hydrocarbons (PAHs) are known carcino- gens. PAHs may be generated from wood during the use of power tools due to heat created from friction. To determine if PAHs are emitted from wood materials during common woodworking operations, we measured PAHs levels in wood dust from various wood materials under controlled conditions in an exposure chamber. Firstly, wood dust was generated from fir, Medium Density Fiberboard, beech, sipo, oak and wood melamine by means of a vibrating sander. Secondly, three woodworking operations were performed using vibration sander, belt sander and saw to generate dust from fir, oak and wood melamine. During these operations, wood dust samples were collected and analysed for PAHs. This study shows that PAHs are emitted from wood materials during woodworking operations. We found that PAH level differed according to wood materials and wood processing operations. Our results suggest that PAHs emitted from wood play a role in wood dust hazards by inhalation of wood dust particles with PAHs.

2118 POLYFLUOROALKYL COMPOUNDS (PFCs) IN TEXAS—CHILDREN FROM BIRTH THROUGH 12 YEARS OF AGE.

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The Centers for Disease Control and Prevention (CDC) reports serum concentrations of polyfluoralkyl compounds (PFCs), including perfluorooctanoic acid (PFOA), perfluorooctanesulfonic acid (PFOS), perfluorohexane sulfonic acid (PFHxS), and perfluorohexane sulfonic acid (PFHxS), for the US general population ages 12 and older as part of the National Health and Nutrition Examination Survey (NHANES) but does not include PFCs data for children younger than 12. To address this gap, we analyzed serum collected in 2009 for 8 PFCs in 300 infants and children 0 – 12 years of age from Dallas, Texas. The PFCs serum concentrations were measured using on-line solid phase extraction–high performance liquid chromatography–isoctane dilution–tandem mass spectrometry (LC-ID/MS/MS). PFOS, PFHxS, PFOA, and PFNA were each detected in ≤93% of the samples; the other four PFCs were detected less frequently. Median concentrations of PFOS (4.1 ng/mL) were higher than for PFHxS (2.9 ng/mL), PFOA (1.2 ng/mL), and PFHxS (1.2 ng/mL). We found no significant differences by sex, but observed a positive association between age and PFOS and PFOA concentrations. This is one of the few reports of PFC serum concentrations in US infants and young children, and suggests ongoing exposure to these potentially toxic compounds years after changes in some PFCs production. (This abstract does not represent NIH or CDC policy.)

2119 DIETARY EXPOSURE TO ACRYLAMIDE IN ADOLESCENTS FROM A CANADIAN URBAN CENTER.

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Acrylamide (AA), a probable carcinogen in humans, is formed during high-temperature cooking and is detectable in common foods such as french fries and potato chips. In this study, dietary exposure to AA was investigated in adolescents from the Island of Montreal, Quebec, Canada. A total of 196 non-smoking adolescents of both genders, aged between 10 and 17 years old were randomly recruited in the general population. Consumption of specific foods containing AA was documented using a daily self-administrated questionnaire during two consecutive days. Based on the dietary questionnaire, a total of 195 duplicates of different combinations of consumed foods (french fries, potato chips, corn chips, pretzels, roasted almonds, crackers, breakfast cereals, toasted bread, black olives and coffee) were selected and analyzed for AA concentration by LC-MS/MS. Results obtained for AA levels in food ranged from 1 to 2390 ng/g with a mean of 288 ng/g. Using these data we estimated the total daily dietary intake of AA (both days) at 0.59 μg/kg bw/d with a 97.5th percentile of 2.85 μg/kg bw/d. No significant difference in AA intake was observed between boys and girls. With an average level of AA of 1053 ng/g, deep-fried french fries was identified as the major contributor to AA exposure in these adolescents. In this study, dietary exposure to AA was investigated in adolescents from the Island of Montreal, Quebec, Canada. A total of 196 non-smoking adolescents of both genders, aged between 10 and 17 years old were randomly recruited in the general population. Consumption of specific foods containing AA was documented using a daily self-administrated questionnaire during two consecutive days. Based on the dietary questionnaire, a total of 195 duplicates of different combinations of consumed foods (french fries, potato chips, corn chips, pretzels, roasted almonds, crackers, breakfast cereals, toasted bread, black olives and coffee) were selected and analyzed for AA concentration by LC-MS/MS. Results obtained for AA levels in food ranged from 1 to 2390 ng/g with a mean of 288 ng/g. Using these data we estimated the total daily dietary intake of AA (both days) at 0.59 μg/kg bw/d with a 97.5th percentile of 2.85 μg/kg bw/d. No significant difference in AA intake was observed between boys and girls. With an average level of AA of 1053 ng/g, deep-fried french fries was identified as the major contributor to AA exposure in these adolescents.

2120 CONSUMER USE OF POLYFLUOROALKYL COMPOUNDS AMONG THE US POPULATION.


Consumer products are well below the derived LOAEL for asbestiform tremolite. In an effort to determine the potential for asbestos fibers within the plaster. In an effort to determine the potential for asbestos fiber exposure to laboratory personnel and others, we have undertaken testing involving normal use of asbestos-containing pads over a variety of different time periods from ½ to 2-hours. Industrial hygiene personal and area air samples were collected during all testing following NIOSH 7400/7402 methodology. Analysis of personal air samples (n=10) using phase contrast microscopy (PCM), showed airborne fiber concentrations ranging from less than 0.014 to 0.048 fibers per milliliter (f/ml) for sampling durations 30 to 120 minutes. Further analysis using transmission electron microscopy showed no asbestos fibers present in any of the personal samples. Analysis of area air samples (n=12) using (PCM) showed airborne concentrations ranging from less than 0.014 to 0.067 f/ml. Without the use of TEM, all but one of these area samples showed no asbestos present. An asbestos adjusted PCM concentration of 0.029 f/ml was indicated for the single area sample. These results indicate that use of asbestos–containing gauze pads produces a low and insignificant potential for asbestos fiber exposure.
Quantification of Benzene, Toluene, and UFP During Surgery with Electrocautery

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Introduction: Electrocautery is frequently used in surgery. It produces less bleeding and is effective in tumor removal. However, the electrocautery smoke generated can be irritating to the respiratory tract and contains carcinogetic substances. We have investigated the occurrence and concentrations of benzene and other volatile organic compounds (VOC) in electrocautery smoke during peritonectomy with cytoreductive surgery. We have also measured the concentration of ultrafine particles (UFP) and the correlation with benzene levels. IARC has classified benzene as carcinogetic to humans. The aim was to quantify benzene, toluene and ultrafine particles in electrocautery smoke and assess of possible health effects risk for operating room personnel. Materials and methods: Patients with peritoneal carcinoma were treated with cytoreductive surgery using high voltage electrocautery. Personal sampling was performed with Anasorb tubes connected to a portable air-sampling pump. Anasorb tubes were desorbed with carbon disulfide and analysed with GC-MS. Also UFP in electrocautery smoke was measured and air quality parameters: carbon dioxide, temperature and relative air humidity was measured. Results: The average exposure for benzene in electrocautery smoke was 1.78 microg/m3, range 1.09-2.44 and average toluene concentrations were 5.70, 5.31-12.49 microg/m3, the mean concentration of UFP was 9.150 ptc/c (110-4372000). Aldehydes, such as nonenal and decenal, could be detected. No correlation was shown between benzene or toluene concentration and UFP. Conclusion: The concentrations of benzene and toluene were below the Swedish occupational exposure limit value. Peak exposure of UFP was high which may cause irritating symptoms in the respiratory tract. Further the mixed exposure situation during electrocautery may give rise to synergistic or additive effect of VOC and UFP that could result in e.g. cardiovascular effects.

Derivation of Route-Specific Maximum Allowable Dose Levels (Safe Harbor Levels) for Lead for the Assessment of Risks from Consumer Products

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The existing Proposition 65 MADL for lead that is based on the OSHA PEL of 50 microg/m3 is 0.5 microg/day. Route-specific MADLs, which are more suitable for the assessment of exposure from consumer products, have not been derived by OEHHA although hand-to-mouth transfer of lead has been raised as a concern. In this work, route-specific MADLs for lead and worst case estimated exposures due to hand-to-mouth transfer of lead (using data from lead sheeting) were determined. The latter results were used to estimate theoretical transfer from a product containing 600 ppm lead. Adjusting for differences in bioavailability, oral MADLs for children and adults of 0.95 and 4.75 microg/day, respectively, were derived. Although blood lead levels are commonly used as a reliable indicator of the potential for adverse effects, MADLs are in units of microg/day. Using the relationship determined for dietary lead, 1 microg lead/day resulting in blood lead increases of 0.16 microg/day. Route-specific MADLs are in units of microg/day. Using the relationship determined for dietary lead, 1 microg lead/day resulting in blood lead increases of 0.16 microg/day. The correlation with benzene levels. IARC has classified benzene as carcinogetic to humans. The aim was to quantify benzene, toluene and ultrafine particles in electrocautery smoke and assess of possible health effects risk for operating room personnel. Materials and methods: Patients with peritoneal carcinoma were treated with cytoreductive surgery using high voltage electrocautery. Personal sampling was performed with Anasorb tubes connected to a portable air-sampling pump. Anasorb tubes were desorbed with carbon disulfide and analysed with GC-MS. Also UFP in electrocautery smoke was measured and air quality parameters: carbon dioxide, temperature and relative air humidity was measured. Results: The average exposure for benzene in electrocautery smoke was 1.78 microg/m3, range 1.09-2.44 and average toluene concentrations were 5.70, 5.31-12.49 microg/m3, the mean concentration of UFP was 9.150 ptc/c (110-4372000). Aldehydes, such as nonenal and decenal, could be detected. No correlation was shown between benzene or toluene concentration and UFP. Conclusion: The concentrations of benzene and toluene were below the Swedish occupational exposure limit value. Peak exposure of UFP was high which may cause irritating symptoms in the respiratory tract. Further the mixed exposure situation during electrocautery may give rise to synergistic or additive effect of VOC and UFP that could result in e.g. cardiovascular effects.

High Mercury Levels in the Air of Dental Centers in Cartagena, Colombia

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Mercury vapor generated by the placement and removal of dental amalgams is a contaminant in dental centers. The kinetic energy given to perform these activities increases the degree of exposure through the generation of mercury vapors. The primary objective of this study was to determine mercury levels in the air of dental centers in Cartagena, Colombia. For this purpose, air mercury concentrations were measured by atomic absorption spectroscopy at sixty four locations, using the mercury analyzer RA-915+. Air mercury concentrations measured in areas without the presence of patients were 1390±232 ng/m3 for the workplace, 2686±452 ng/m3 at cuspidors, and 1262±209 ng/m3 on the dental tray. Moreover, when clinical work was performed on amalgams, air mercury concentrations reached 9510±1950 ng/m3 in the workplace, 23983±2670 ng/m3 at cuspidors, and 39983±2058 ng/m3 near the patient mouth, values up to 133 times greater than the reference concentration established by the U.S. Environmental Protection Agency-EPA (300 ng/m3). It is clear that the high levels of mercury recorded in the air present at these dental centers increase the risk of exposure to dentists and patients, with potential serious health consequences, especially in the case of dentists and assistants, as they spend greater time in these working places. These results pointed out the necessity to eradicate the use of mercury in dental fillings in Colombia, as it is a prominent exposure source of this toxic metal to dental care providers.
are provided along with the role of the Community. Lead isotope analyses can pro-
vide significant benefits to regulatory agencies, interested parties, and the commu-
nity where the signature is able to be characterised with a high degree of certainty.

2125 SIMULATION OF HEAVY METAL CONTAMINATION OF FRESH-WATER BODIES: TOXIC EFFECTS IN THE CATFISH AND ITS AMELIORATION WITH COCONTAMINATION WITH GLYPHOSATE.

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The toxic implications of fresh water contamination with zinc in the catfish, Clarias albopunctatus (Lamont and Nicole, 1927), and the effect of a co-contamination with a sub-lethal dose of glyphosate (Roundup) was studied using the static bioassay model. Thirty six fish were divided into 3 equal groups. Fish in Group 1 were placed in normal tap water, and served as the control group, while fish in Groups 2 and 3 were placed in water contaminated with ZnSO4 and ZnSO4 + glyphosate, respectively. The study lasted for 96 hours (though sampling was done at the 48th hour). Biochemical markers of toxicity were measured and the fish liver and gill histology were studied using standard protocols. The results show that ZnSO4 was sig-
nificantly toxic to the fish only after 96 hours. Co-contamination of the water with both toxicants was found to ameliorate the toxic effects of ZnSO4 significantly. The metal chelating property of glyphosate may be responsible for the observed attenuation of toxicity in the fish.

2126 MANAGING HUMAN HEALTH RISK THROUGH A COMPREHENSIVE AIR-MONITORING PLAN AT A FORMER MGP SITE.

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Monitoring for potential emissions from a Manufactured Gas Plant (MGP) reme-
diation site is implemented to reduce or prevent a potential inhalation pathway for VOCs such as benzene, toluene, ethylbenzene, and xylenes; and contaminated par-
ticulates acting as a conduit for PAhS and heavy metals. This risk management case study presents a USEPA-approved air monitoring program implemented to manage human health risks at a former MGP site located in the southeast U.S. Risk-based Acceptable Air Concentrations (AACs) were developed and a sampling regime esti-
mated to monitor potential emissions to maintain contaminant concentrations below the AACs. The AAC for benzene was based on carcinogenic effects using the current IUR from the USEPA's IRIS database. The AACs for toluene, ethyl benz-
ene, and xylenes were based on non-carcinogenic effects using the current RfC from the IRIS database. The AACs for the carcinogenic PAhS were based on car-
icinogenic effects using the current IUR from California EPA. The AAC for res-
pirable particulate matter (PM10) was the National Ambient Air Quality Standard (NAAQS) for PM10 and was used as a surrogate for both the PAhS and heavy met-
als. Site-specific AACs were calculated using a target cancer risk (TR) value of 1X10-6 for carcinogens and a target hazard quotient (THQ) of 1 for non-carcino-
gens. The exposure duration used was based on a twelve-month project duration and an exposure time of 24-hours per day; equations, toxicity values and sources were based on USEPA's Regional Screening Levels website (2009). A total 535 twenty-four hour time weighted samples (269 VOC samples and 266 PAH sam-
ple) were collected over the project duration. Only minor levels of VOCs and PAhS were detected and no results were above the AACs. These time-weighted av-
erages demonstrate that the real-time air monitoring and control measures imple-
mented at the Site effectively maintained concentrations below the AACs and were protective of human health.


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NAD(P)H:quinone oxidoreductase (NQO1) catalyzes the obligatory two electron reduction of quinones and quinoline compounds bypassing redox cycling and pro-
duction of reactive oxygen radicals and protecting cells from toxicity. Induction of NQO1 is considered as a model for analyzing transcriptional regulation of many cytoprotective enzymes and proteins. The aryl hydrocarbon receptor (AhR) medi-
ates the induction of NQO1 by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and benzo[a]pyrene (BaP); whereas the antioxidant activator/repressor/ transcription factor Nrf2 is critical for induction by antioxidants such as tert-butylhydroquinone (tBHQ). Induction of the genes by AhR agonists or antioxidants requires “dioxin response element” (DRE) or “antioxidant response element” (ARE), respectively. We previously reported a cross-interaction between AhR and Nrf2 signal transduc-
tion is required for induction of NQO1 by TCDD (Ma et al, Biochem J 377, 205-
213, 2004). In this study, we analyzed the interaction between AhR and Nrf2 at the promoter of NQO1. Chromatin immunoprecipitation analyses revealed that treat-
ment with TCDD recruits both AhR and Nrf2 to the promoter region where a DRE and an ARE locate; the finding is in agreement with the result from genetic studies in which induction by TCDD or BaP was shown to require both AhR and Nrf2. TCDD-induced binding of AhR and Nrf2 to DNA is time-dependent. Consistent with the activation of Nrf2, TCDD treatment inhibits Keap1-dependent ubiquitination and proteosomal degradation of Nrf2 resulting in the stabiliza-
tion and nuclear accumulation of Nrf2. Co-immunoprecipitation experiments re-
vealed that AhR directly interacts with Nrf2 in the presence of TCDD. Our findings demonstrate that AhR interacts with Nrf2 to control induction of NQO1 by AhR ligands.

2128 ESTROGEN RECEPTOR ALPHA AND ARYL HYDROCARBON RECEPTOR DIFFERENTIALLY MODULATE THE TRANSCRIPTIONAL ACTIVITY OF NUCLEAR FACTOR ERYTHROID-2-RELATED FACTOR 2 (NRF2).

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Nuclear Factor-Erythroid 2 Related Factor 2 (NRF2) regulates the expression of a battery of Phase II detoxifying enzymes and provides cellular protection against electrophiles and oxidative stress. Sulforaphane (SFN) is a naturally occurring NRF2 activator in cruciferous vegetables and has been undergoing clinical trials for the prevention of breast cancer due to its pro-apoptotic and anti-mitotic effects in MCF7 breast cancer cells. Modulation of the NRF2 signalling pathway by estrogen receptor alpha (ERα) and the aryl hydrocarbon receptor (AHR) was investigated in this study. SFN induced NADPH-dependent oxidoreductase 1 (NQO1) and heme oxygenase 1 (HMOX1) mRNA expression, which was significantly diminished when MCF-7 ERα cells were co-treated with various estrogenic compounds with the exception of diethylstilbestrol (DES). DIM + SFN induced NQO1 and HMOX1 mRNA to levels greater than SFN alone. RNAi-mediated knockdown of AHR abrogated the supra-induction effect of DIM + SFN on NQO1 and HMOX1 expression, whereas knockdown of ERα abrogated the inhibitory effect of resvera-
trol (RES). In cells treated with SFN +17beta-estradiol (E2), ChIP assays revealed significant decrease in p300 recruitment which coincided with 1) decreased local Histone H3 Lysine 9 acetylation and 2) time-dependent increase in ERα recruit-
ment at the NQO1 and HMOX1 enhancer regions. Taken together, our findings suggest that both ERα and AHR modulate the activity of NRF2. E2-mediated re-
pression of HMOX1 and NQO1 might involve the recruitment of ERα, resulting in a decrease in p300 recruitment and an associated decrease in local H3K9Ac/H3. This study is funded by the Canadian Breast Cancer Foundation (CBCF) Doctoral Fellowship, the CBCF Grant Program and Canadian Institutes of Health Research Operating Grant.

2129 NUCLEAR FACTOR E2-RELATED FACTOR-2 (NRF2) REGULATES P-GLYCOPEPTIDE EXPRESSION AT THE BLOOD-BRAIN BARRIER (BBB) BY ACTING THROUGH P38 MAP KINASE.

X. Wang and D. Miller. Laboratory of Pharmacology, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

At the BBB, the ATP-driven efflux pump, P-glycoprotein (Pgp) is a major impedi-
ment to CNS pharmacotherapy. Signals that modulate Pgp function are currently sought. In this study, when MCF-7 breast cancer cells transfected with Pgp are upregulated by xenobiotics acting through nuclear receptors. Here we show that ligands for Nrf2 increase Pgp-mediated transport and transporter protein expression in rat brain capillaries. Nrf2 senses oxidative stress and induces multiple cytoprotective pro-
teins, including antioxidant and glutathione generating enzymes, but its ability to modulate transport proteins is largely unexplored. We used freshly isolated rat brain capillaries, a fluorescent Pgp substrate and confocal microscopy to monitor changes in Pgp transport activity. Exposure capillaries to the Nrf2 ligand, sulforaphane (SFN, 0.1-10 μM), a naturally occurring compound present in cruciferous vegeta-

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bles, increased Pgp activity in a concentration-dependent manner. Inhibiting trans-
scription with actinomycin D or inhibiting translation with cycloheximide abol-
ished SFN-induced upregulation of transport. Another Nr2 activity, tert-butyl-
hydroquinone (tBHQ), widely used in food preservatives, also significantly
increased Pgp transport activity. Exposing rat brain capillaries to SFN (1-10 μM)
caus a concentration-dependent increase of Pgp protein expression assayed by
Western blot. Electrophoretic Mobility Shift Assay (EMSA) detected binding of
Nr2 to ARE in nuclei from rat brain capillaries exposed to SFN. Pretreatment with
SB203580, a p38 mitogen-activated protein kinase inhibitor, abolished SFN- and
tBHQ-induced upregulation of Pgp transport, while inhibitors of MEK and PI3
kinase were without effect. These results implicate p38 signaling in Nr2 induction of
Pgp activity. Thus, the BBB is tightened selectively by dietary constituents that are
Nr2 ligands, providing increased neuroprotection but at the expense of reduced
penetration of certain therapeutic drugs.

2130 THE ROLE OF SIRTUIN-1 ON TBHQ INDUCTION OF
NRF2 AND PROTOTYPICAL NRF2 TARGET GENE
EXPRESSION.

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Multidrug Resistance-associated Proteins (Mrcp, Abcc) are key transporters in the excretion of chemicals out to bile and blood from hepatocytes. Tertiary butylhydro-
quinone (tBHQ) is a phenolic antioxidant that stabilizes the protein of NF-Ε2-related factor 2 (Nr2), enhances antioxidant response element-mediated gene tran-
scription. Recent studies demonstrated that the histone deacetylase, Sirtuin-1
(Sirt1), is necessary for nuclear receptor signaling via peroxisome proliferator activat-
ed-receptor α (Ppar-α) in vivo. The current study was to determine whether tBHQ activation of the NRF2 target gene expression occurs via Sirtuin-1-dependent
mechanisms in vitro and in vivo. Primary hepatocytes were isolated from male wild-type or hepatocyte-specific Sirt1 knockout mice (Sirt1-KO). The cells were treated with tBHQ (100 μM) for 24 hours, and then total RNA as well as proteins were isolated. T:BHQ increased Ppar-α, glutamate-cystine ligase catalytic subunit
(Gclc), Naf(p)H:quinone oxidoreductase (Nqo1), Tmp2, and Merp4 mRNA expres-
sion in wild-type hepatocytes, but this induction was attenuated in Sirt1-KO hepa-
tocytes. T:BHQ induced Nr2, Gclc, Nqo1, Mrp2, and Merp4 protein expression in
wild-type hepatocytes, but this induction was attenuated in Sirt1-KO hepatocytes.
T:BHQ increased Nr2 mRNA and protein expression in HEK293 cells, but not HEK293 cells after Sirt-1 knockdown. In contrast, BHA treatment increased Gclc
and Nqo1 protein expression in liver of wild-type mice and Sirt1-KO mice to a
similar degree. T:BHQ and BHA treatment increased Sirt1 expression in hepatocytes
and liver, respectively. Overall, tBHQ induction of NR2 targets was attenuated
in Sirt-1 KO hepatocytes and Sirt1-Knockdown cells, but not liver from
BHA-treated mice. Together, the data demonstrate Sirt1-dependent modulation of
Nr2 expression and target gene expression in hepatocytes and cells. This work was supported by NIH 3R01ES016042.

2131 XBP1 AND NRF2, AT THE CROSSROADS OF
ENDOPLASMIC RETICULUM STRESS AND
OXIDATIVE STRESS.


X-box binding protein 1 (XBP1) is one of the key players of the unfolded protein
response (UPR) and is critical for cell fate determination in response to endoplas-
mic reticulum (ER) stress. During UPR, active inositol-requiring enzyme 1
(IRE1) splices XBP1 mRNA into XBP1s. XBP1s then binds to ER stress response
elements (ERSE) and activates transcription of a variety of UPR target genes in-
cluding ER chaperones and components of the ER-associated degradation (ERAD)
pathway. The bZIP Cap ’n’ Collar transcription factor nuclear respiratory factor 2
(NR2) is another master regulator of cellular redox homeostasis. As an adap-
tive response to oxidative stress, Nr2 activates transcription of genes encoding for
antioxidants, detoxification enzymes, and xenobiotic transporters, by binding the
cis-antioxidant responsive element (ARE) in the promoter regions of the genes. In
our study, we found that XBP1-/- mouse embryonic fibroblast (MEF) cells have a
lower Nr2 expression compared to XBP1+/+ MEFs. Ectopic XBP1s expression in
HEK293, MDA-MB-231 and A549 cells decreased Nr2 protein expression over
control, suggesting that the observed effect is not cell type specific. In addition, we
saw a reduction in Nr2 transcriptional activity as well as Nr2 downstream gene

expression by reporter gene assay and qPCR, indicating XBP1s may be responsible for
modulating anti-oxidant response by suppressing expression of Nr2. Treating
hepatocytes with proteasome inhibitor (MG132) or lysosome inhibitor (Chloroquine)
in the presence of XBP1s cannot restore Nr2 protein expression to control level, lead-
ing us to think that XBP1s may modulate Nr2 though some mechanism other than
protein degradation. In summary, our data revealed a possible crosstalk be-
tween ER stress pathway and oxidative responses via UPR and Nr2 signaling path-
way in order to protect cells against environmental stress. Further investigation of
the mechanisms of this coordinate regulation will not only shed light on principles
of stress response but may also lead to new approaches to the treatment of stress re-
lated diseases.

2132 REGULATORY ROLE OF NRF2 AND KEAP1 IN PPAR-
GAMMA EXPRESSION AND CHEMORESISTANCE IN
HUMAN NON-SMALL CELL LUNG CARCINOMA
CELLS.


The Nuclear factor-E2-related factor 2 (NR2) serves as a master regulator in cell-
ular defense against oxidative/electrophilic stress and in chemical detoxification. How-
ever, persistent activation of NR2 resulting from gain-of-function mutation of
NR2 and/or loss-of-function mutation of its suppressor Kelch-like ECH-asso-
ciated protein 1 (KEAP1) are associated with tumorigenicity and chemoresistance of
non-small-cell lung cancer cells (NSCLC). Thus, inhibition of mediated antioxidative response in NSCLCs is widely considered as a promising strat-
egy to prevent tumor growth and reverse chemoresistance. Unexpectedly, stable
knockdown of KEAP1 by lentiviral shRNA sensitized three independent NSCLC
cells line (A549, HTB-178 and HTB-182) to multiple chemotherapeutic agents, including arsenic trioxide (A2O3), etoposide and doxorubicin, despite increased
NR2 activity attained by KEAP1 silencing. In lung adenocarcinoma epithelial cell
line A549, the silencing of KEAP1 augmented the expression of peroxisome prolifer-
ator-activated receptor γ (PPARγ), which was accompanied by enhanced expres-
sion of cell differentiation-related genes, including E-CADHERIN and GEL-
SOLIN. In addition, KEAP1-KD A549 cells displayed attenuated expression of CYCLIN
D1 proto-oncogene and markers for cancer stem cells (CSCa) and reduced
non-adherent sphere formation. Moreover, deficiency of KEAP1 led to ele-
vated induction of PPARα in response to A2O3 treatment. Pretreatment of A549
cells with PPARγ agonists augmented the effects of A2O3 on activation of PPARα
and cytotoxicity in a NR2-dependent fashion. Collectively, the current study demonstrates that suppression of KEAP1 expression in human NSCLC cells results in
sensitization to chemotherapeutic agents, which may be attributed to activation of
PPARα and subsequent alterations in cell differentiation and CSC abundance.

2133 UPREGULATION OF QUINONE OXIDOREDUCTASE 1
ENZYME BY THE STRONGLY BASIC ALKALOIDAL
FRACTION OF RHAYZA STRICTA THROUGH NRF2-
DEPENDENT MECHANISM.

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NAD(P)H:quinone oxidoreductase 1 (NQO1) is an important detoxifying enzyme that prevents the formation of highly reactive oxygen radicals. NQO1 is regulated by the aryl hydrocarbon receptor (AhR) and nuclear factor erythroid 2-related fact-
or 2 (Nr2) transcription factors. Rhazya stricta is a common traditional anti-
tumor medicinal plant in the Arabian Peninsula, Pakistan and India. However, its
expression in NQO1 was not studied before. The current study aims to address the ef-
ficacy of the plant extract strong basic alkaloidal fraction (AF) on the regulation of
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tumor medicinal plant in the Arabian Peninsula, Pakistan and India. However, its
effect on NQO1 was not studied before. The current study aims to address the ef-
ficacy of the plant extract strong basic alkaloidal fraction (AF) on the regulation of
levels suggesting the role of Nrf2 in its regulation. This was confirmed by increased
the nuclear translocation of Nrf2 by AF. Finally, the AF induced human NQO1 at
mRNA and catalytic activity levels in a concentration-dependent manner in
HepG2 cells. We concluded that the strongly basic alkaloidal fraction of Rhazya
stricta upregulates NQO1 through Nrf2-dependent mechanism. These data may
represent novel mechanisms by which the strongly basic alkaloidal fraction of
Rhazya stricta confers chemoprevention. Acknowledgements: This work was
supported by NSERC of Canada grant 250139-07 to AOSE. ME is the recipient of
the Dissertation Fellowship, University of Alberta.

2134 NRF2 ACTIVATION PREVENTS ETHANOL-INDUCED HEPATIC ALTERATIONS.
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Oxidative stress and lipid accumulation play important roles in alcohol-induced liver injury. Genes involved in antioxidant defense are induced, whereas genes in-
volved in lipid biosynthesis are suppressed in livers of nuclear factor erythroid 2-re-
lated factor 2 (Nrf2) activated mice. To investigate the role of Nrf2 in alcohol-
induced hepatic alterations, Nrf2-null mice, wild-type mice, kelch-like ECH-associated protein 1-knockdown (Keap1-KD) mice with enhanced Nrf2, and
Keap1-hepatocyte knockout (Keap1-HKO) mice with maximum Nrf2 activation were treated with ethanol (6 g/kg, po). Blood and liver samples were collected 6 h
thereafter. Ethanol increased the enzyme activities of serum alanine aminotrans-
ferase and lactate dehydrogenase as well as the amount of serum triglycerides and
acid reactive substances (TBARS) in Nrf2-null and wild-type mice, but not in Nrf2-
enhanced mice. After ethanol treatment, mitochondrial glutathione concentrations decreased markedly in Nrf2-null mice, moderately in wild-type mice, but not in Nrf2-enhanced mice. H2DCFDA staining of primary hepatocytes isolated from the four genotypes of mice indicated that oxidative stress was higher in Nrf2-null cells, and lower in Nrf2-enhanced cells than wild-type cells. In addition, ethanol in-
creased serum triglycerides and free fatty acids in livers of Nrf2-null mice, and these increases were blunted in Nrf2-enhanced mice. To further investigate the role of Nrf2 in preventing hepatic lipid accumulation, mRNA and nuclear protein of sterol regulatory element-binding protein 1(Srebp-1), the master regulator of lipid biosynthesis, were quantified. The basal levels of Srebp-1 mRNA and nuclear pro-
tein were decreased with graded Nrf2 activation. Ethanol treatment further in-
duced Srebp-1 mRNA in Nrf2-null mice but not in Nrf2-enhanced mice. In con-
clusion, Nrf2 activation prevented alcohol-induced oxidative stress and accumula-
tion of free fatty acids in liver by increasing genes involved in antioxidant defense and decreasing genes involved in lipogenesis. (Supported by NIH grants
DK-081461, ES-019487, and ES-009649).

2135 NRF2 ACTIVATOR BARDOXOLONE METHYL TRANSCRIPTIONALLY REGULATES TRANSMANISASES AND INCREASES GLUTATHIONE IN MULTIPLE TISSUES.
S. A. Reimsain1, G. A. Miller1, R. Bumeister1, B. Probst1, C. D. Klaassen2, S.
Ruiz1, C. J. Meyer1, W. Wigney1 and D. A. Ferguson1, 1Beata Pharmaceuticals,
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First introduced into the clinic almost 60 years ago, serum alanine and aspartate transaminases have become standard biomarkers for detection of liver injury. However, concomitant with being expressed both intra- and extra-hepatically and critical for amino acid metabolism, increases in serum transaminases are not always associated with liver injury. Bardoxolone methyl, the lead molecule from the Antioxidant Inflammation Modulator (AIM) class, binds to Keap1 and potently in-
duces Nrf2, a transcriptional regulator of many cytoprotective genes, including glu-
tathione (GSH) synthetic enzymes. In clinical studies with bardoxolone methyl, al-
most all patients have increased serum transaminase activity above baseline upon
initiation of treatment. These elevations follow a consistent pattern and are not as-
sociated with elevations in bilirubin. Such elevations are typically mild, resolve
within two to four weeks after peaking without discontinuation of study drug, and
do not recur. Because transaminases catalyze reactions which produce glutamate, a necessary amino acid for the synthesis of GSH, it was hypothesized that the role of Nrf2 in GSH production might extend beyond regulation of GSH synthetic en-
zyme expression. In cells derived from liver, muscle, kidney, and macrophages, bar-
doxolone methyl and other AIMs, dose- and time-dependently increased transami-
nase mRNA and catalytic activity levels, GSH concentrations, and mRNA levels of GSH synthetic enzymes. In vivo, serum transaminase activity, hepatic GSH content, he-
patic GSH synthetic enzyme mRNA levels, and renal and hepatic transaminase
mRNA levels were increased in Keap1-knockdown mice, but decreased in Nrf2-
null mice compared to wild-type mice. Collectively, these data suggest that genetic
activation of Nrf2 and AIM pharmacology lead to elevated transaminase activity in
response to increased demand for glutamate for the production of GSH.

2136 BACOPA MONNIERI EXTRACT ACTIVATES THE NRF2 PATHWAY AND PROTECTS AGAINST MPP+ AND PARAquat-INDUCED TOXICITY THROUGH THE MODULATION OF INTRACELLULAR OXIDATIVE STRESS AND MITOCHONDRIAL FUNCTIONS.
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of Medicine, Laval University, Québec, QC, Canada. Sponsor: T. Sanderson.

Parkinson’s disease (PD) is one of the most common age related neurodegenerative
diseases and affects millions of people worldwide. Environmental factors especially,
pesticides represent one of the primary classes of neurotoxic agents associated with
PD. Strong evidence supports the role of oxidative stress, mitochondrial and pro-
teasomal dysfunctions underlying neuronal death in PD. Activation of the nuclear
efactor E2-related factor 2 (Nrf2), a master regulator of cellular antioxidant response
has shown neuroprotection in the PD models. The objective of our study was to in-
vestigate the neuroprotective effects of the standardized extracts of Bacopa monniera
(BM) against paraquat (PQ) and MPP+ induced toxicity and to elucidate the
mechanisms underlying this protection. Our results show that a treatment with BM
extract activated the Nrf2 pathway by modulating the expression of Keap1, Akt and
GSK3beta thereby up-regulating the endogenous GSH synthesis and phase two an-
tioxidant enzymes. We further demonstrate that a pre-treatment with the BM ex-
tact from 0.5 to 1.0 mg/ml protected the dopaminergic SK-N-SH cell line against MPP+
and PQ-induced toxicity in various cell survival assays, prevented the depletion of
GSH, preserved the mitochondrial membrane potential and maintained the mito-
ochondrial complex I activity. BM pre-treatment from 10.0 μg/ml also prevented the
PQ mediated generation of intracellular reactive oxygen species (ROS) and de-
creased the mitochondrial superoxide level. We further validated the involvement
of triterpenoid saponins Bacosides in the neuroprotection. By preserving the cel-
ular redox homeostasis and promoting cell survival under oxidative stress BM extract
may have therapeutic uses in various age related neurodegenerative diseases such as
PD.

2137 NRF2 DEFICIENCY PREVENTS GLUCOSE INTOLERANCE INDUCED BY A HIGH-FAT DIET IN MICE.
Y. I. Zhang, K. C. Wu, J. Liu and C. D. Klaassen, University of Kansas Medical
Center, Kansas City, KS.

Both proteomic and microarray studies have indicated that Nrf2 is involved in fatty
acid metabolism in the liver. The current study examined the effect of Nrf2 activa-
tion on high-fat diet-induced obesity. Nrf2-null mice, wild-type (WT) mice, and
Keap1-knockdown (Keap1-kd) mice with enhanced activation of Nrf2, were fed ei-
ther a high-fat Western diet (HFD) or a control diet for 12 weeks. The HFD-fed
mice gained approximately 11 g more weight than control mice, though no differ-
ces were observed between genotypes on either diet. However, Keap1-kd mice
did not gain weight as much as Nrf2-null mice, which indicated the activation
of the HFD or control diet. Both the nuclear accumulation of Nrf2 protein and
the mRNA expression of prototypical NRF2-target genes suggested that HFD did
not markedly activate Nrf2 in livers of any of the three genotypes of mice. These
data suggest that Nrf2 is not directly involved in regulating fatty acid metabolism.
Screening the mRNA expression of genes involved in hepatic gluconeogenesis and fatty
acid metabolism revealed that insulin-like growth factor binding protein 1 and 2 (Igfbp
1 and -2), as well as fibroblast growth factor 21 (Fgf21) were markedly elevated in
livers of Nrf2-null mice, regardless of diet. Moreover, Fgf21 protein expression in-
creased in Nrf2-null but decreased in Keap1-kd mouse livers. The mRNA of TNFα
and IL-1 was also higher in livers of Nrf2-null mice, which indicated the activation
of NF-κB. According to published data, increased expression of Fgf21, Igfbp-1, as
well as activation of NF-κB can all contribute to the lower blood glucose level ob-
served in Nrf2-null mice. In conclusion, Nrf2 activity affected the cellular environ-
ment (i.e., oxido-reductive balance) in liver, which led to altered expression of key
metabolic factors, including Fgf21 and Igfbp-1, as well as inflammatory factors.
These changes contribute to glucose in blood glucose levels in mice during various
levels of Nrf2 activity. (This research is supported by NIH funding:
DK-081461, ES-019487, and ES-009649).
2138 THERAPEUTIC POTENTIAL OF NRF2 ACTIVATORS IN STREPTOZOTOCIN-INDUCED DIABETIC MICE.

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Rationale—Diabetes is a disease with several secondary complications including nephropathy, skeletal muscle atrophy and cardiomyopathy, many of which are caused by oxidative stress in local tissues and throughout the vasculature. In the current study, the therapeutic potential to alleviate diabetic symptoms and complications of the antioxidant transcription factor, NRF2, was investigated. Methods—Diabetes was induced in Nrf2+/+ and Nrf2-/- mice by streptozotocin (STZ) injection. Nrf2 activators, sulloraphane (SF) or cinnamic aldehyde (CA) were administered 2 weeks after STZ injection and markers of diabetes including blood glucose, insulin, polydipsia, polyuria, and weight loss were measured. Pathological alterations, protein expression changes and oxidative damage in the kidney were also determined. The molecular mechanisms of Nrf2 mediated protection were investigated in vitro using human renal mesangial cells (HRMCs). Results—SF or CA significantly attenuated common metabolic disorder symptoms associated with diabetes in Nrf2+/+ but not in Nrf2-/- mice, indicating SF and CA function through specific activation of the Nrf2 pathway. Furthermore, SF or CA improved renal performance and minimized pathological alterations in the glomerulus of STZ-Nrf2+/+ mice only. Nrf2 activation reduced oxidative damage and suppressed the expression of TGF-β1, extracellular matrix (ECM) proteins and p21 both in vivo and in HRMCs. In addition, Nrf2 activation reverted p21-mediated growth inhibition and hypertrophy of HRMCs under hyperglycemic conditions.

Conclusions—We provide experimental evidence indicating that dietary compounds targeting Nrf2 activation can be used therapeutically to improve metabolic disorder and relieve renal damage induced by diabetes.

2139 TOO MUCH OF A GOOD THING: ENHANCED NRF2 ACTIVITY INCREASES OBESITY, STEATOSIS, AND GLUCOSE INTOLERANCE.

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Nuclear factor-E2 related factor 2, Nrf2 is implicated in a variety of therapeutic applications because of its ability to combat oxidative stress. In addition to regulating the expression of Phase-II biotransformation enzymes and transporters, Nrf2 also regulates several transcription factors and enzymes involved in lipid biosynthesis. Mixed findings are reported regarding the role of Nrf2 in contributing to adiposity in liver and adipose tissue. The present study was conducted to determine whether enhanced Nrf2 activity confers protection or augments diet-induced obesity, steatosis, and insulin resistance. C57Bl/6 and Keap1-Knockdown (Keap1-KD) mice, which exhibit enhanced Nrf2 activity, were fed a high fat diet (HFD, 60% kcal) for 24 weeks from 3 weeks of age. Age-matched Keap1-KD mice had significantly higher body mass, white adipose tissue mass, and hepatic triglyceride content as compared to C57Bl/6 mice on HFD. Pathology findings indicated that the HFD increased hepatic steatosis to a greater extent in Keap1-KD mice compared to C57Bl/6 mice. In addition, the HFD resulted in liver and adipose tissue inflammation, which was not observed in C57Bl/6 mice. Messenger RNA expression of Pparg-γ, the master regulator of adipogenesis, was significantly increased in livers of Keap1-KD mice, along with increased Cd36, Fabp4, and Lpl expression, which shuttles TG deposition in liver, leading to steatosis. The HFD increased the protein expression of lipogenic enzymes, such as acetyl CoA carboxylase-1, Sterol CoA desaturase to a greater extent in Keap-KD mice compared to C57Bl/6 mice. Increased Tnf and Mcp-1 mRNA expression was also observed in livers from Keap-KD mice fed the HFD. The HFD decreased glucose tolerance to a greater degree in Keap-KD mice compared to C57Bl/6 mice, which was accompanied by down regulation of insulin receptor substrate 1 and Glut-4 mRNA expression in skeletal muscle, as well as Akt1 protein expression in liver. The data demonstrate that enhanced Nrf2 activity potentiates diet-induced obesity, fatty liver and diabetes.

2140 ANTIOXIDANT-ACTIVATED PI3K/AKT BLOCKS GSK3β FROM REGULATING NRF2 EXPORT AND DEGRADATION THAT ALLOWS UNIMPEDED NRF2 ACTIVATION OF CYTOPROTECTIVE GENE EXPRESSION.

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NF-E2-related factor 2 (Nrf2) is a transcription factor that regulates a battery of cytoprotective genes that are critical for the maintenance of cellular redox balance. In the presence of oxidative stress Nrf2 dissociates from the negative regulator Nrf2 (Keap1) and translocates into the nucleus where it coordinates induces the transcription of a battery of defensive genes that combat reactive oxygen species and electrophiles. Subsequently, the Src-A subfamily kinase members Fyn, Src, Yes, and Fgr phosphorylate Nrf2 at tyrosine 568, which triggers Nrf2 nuclear export and degradation during the “post induction” phase. We have previously reported that activation and nuclear accumulation of the Src-A subfamily are regulated by the upstream kinase, GSK3β. In this study, we investigated the upstream factors responsible for regulating stress-activated GSK3β in human hepatocellular carcinoma, HepG2, cells. Here we demonstrate that one of the “early induction phase” responses to the antioxidant tert-Butylhydroquinone (t-BHQ) is the activation of the PI3K/Akt pathway. Within 0.5-1 hr of t-BHQ treatment Akt was activated upon being phosphorylated at S473 and T308, which allowed it to phosphorylate its substrate GSK3β at S9, resulting in the inhibition of GSK3β. In this inactive, closed state GSK3β is unable to interact with and phosphorylate its substrate Fyn, thereby preventing Fyn nuclear accumulation. Treatment of cells with the PI3K inhibitors, Wortmannin or LY294002, or an Akt inhibitor blocked GSK3β phosphorylation, which correlated with altered levels of Fyn and disrupted Nrf2 nuclear accumulation and activation. Together, this study demonstrates that the upstream PI3K/Akt cascade inactivates GSK3β mediated Fyn localization, thereby enabling Nrf2 to enter the nucleus unimpeded, where it can up-regulate cytoprotective gene expression.

2141 ARSENIC INDUCES CHRONIC NRF2 ACTIVATION.

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The Nrf2-Keap1 signaling pathway is an endogenous protective mechanism promoting detoxification and cell survival against cellular stress and environmental insults. Previous research has demonstrated that the Nrf2-dependent defense response is imperative in protecting cells against arsenic toxicity. However, chronic activation of Nrf2 has been shown to be present in many types of cancers, contributing to chemoresistance. Here, we demonstrate that arsenic-mediated Nrf2 activation is dependent on p62, a specific substrate of autophagy, and can be attenuated when expression of p62 is silenced by siRNA. This non-canonical Nrf2 pathway results in a chronic, sustained increase of Nrf2 activation. Moreover, we demonstrate by live cell imaging that arsenic causes deregulation of autophagy, specifically resulting in accumulation of autophagosomes and leading to colocalization of Keap1, p62, and LC3 (a marker of autophagosomes). Utilizing a tandem RFP-GFP-LC3 construct, arsenic was shown to inhibit the later stages of autophagy, thereby affecting autophagic flux in vitro models. Interestingly, the aforementioned arsenic-induced effects on autophagy were reversible by sulforaphane, a well-characterized chemopreventive compound that specifically activates Nrf2 through the Keap1-mediated pathway. Collectively these findings provide experimental evidence that arsenic causes prolonged activation of Nrf2 through autophagy dysfunction, which may lead to a novel mechanism for arsenic carcinogenicity in humans.

2142 INCREASE IN NRF2 CONTRIBUTES TO REDUCED APOPTOSIS AND RESISTANCE TO AROMATASE INHIBITORS.

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Breast Cancer is the leading cause of deaths in women. Excellent chemotherapeutic drugs including aromatase inhibitors (AIs) are available to reduce or eliminate breast cancer. However, there is often the problem of drug resistance. Nfn2 (Keap1)-Nrf2 serve as sensors of drugs and radiation-induced oxidative/electrophilic stress. Nfn2 constitutively suppresses Nrf2 in the absence of stress by functioning as an adapter protein for Cul3/Rbx1 mediated ubiquitination/degradation of Nrf2. Upon exposure to stress, Nfn2 is dissociated from Nfn2, stabilized and translocate to the nucleus and coordinately induce 200+ cytoprotective gene
Inhibition of paraquat-induced oxidative stress, proinflammatory cytokine expression, and fibroblast-to-myofibroblast transformation by resveratrol! via the NRF2 pathway.

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Paraquat (PQ) is a most widely used herbicide in the world. PQ selectively accumulates in the lungs and induces lung injury and fibrosis in humans. Redox cycling has been linked with PQ pulmonary toxicity but no effective antidote is available. Resveratrol (Res) is a natural phytoalexin with multiple functions including antioxidant, anti-inflammatory in animals and humans. In this study, we found that Res at pharmacological doses effectively attenuated PQ-induced cell toxicity and fibrogenic response in human lung cells. PQ dose-dependently caused toxicity in normal human bronchial epithelial BEAS-2B cells including increased cell death, oxidative stress, and loss of mitochondrial inner membrane potential. Res at 10 to 20 uM markedly inhibited PQ toxicity. PQ at 10 uM induced transformation of normal human lung fibroblast WI-38 cells into myofibroblasts, as shown by the de novo synthesis of a-smooth muscle actin, and heightened production of inflammatory cytokines TNFα and IL-6 and growth factor TGFβ1. On the other hand, pre- or co-treatment with Res blocked the fibrogenic reactions to PQ. Mechanistic analyses revealed that Res activated the antioxidant/antioxidant-activated receptor Nrf2 to induce cytoprotective genes. Nrf2 was required for normal defense against PQ toxicity and fibrogenic reactions as loss of Nrf2 significantly increased PQ toxicity, myofibrob- last transformation, and cytokine expression. Finally, Nrf2 mediated the protective response to PQ by Res because protection was lost in Nrf2-deficient cells. The study demonstrated that Res prevents PQ-induced ROS production, inflammation, and fibrogenic reactions in cultured cells by activating Nrf2 signaling. The findings provide new insights into the understanding and chemoprotection of PQ lung toxicity and potential intervention through Nrf2-based mechanisms.

Nrf2 (KEAP1) and Nrf2 both control Bcl-2 and cellular apoptosis.

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Nrf2/Inrf2 complex serves as a sensor of chemical and radiation-induced oxidative stress. Under normal conditions, Nrf2/Cul3-Rbx1 ubiquitin ligase complex constantly ubiquitinates and degrades Nrf2. When cells encounter stressor, Nrf2 is dissociated from Nrf2/Cul3-Rbx1 complex, translocates in the nucleus and coordinately activate a battery of cytoprotective proteins that provide the critical protection against oxidative stress, cellular transformation and neoplasia. However, persistent exposure to stressors or mutational inactivation of Nrf2/Cul3-Rbx1 complex cause nuclear accumulation of Nrf2 that leads to enhanced cell survival and drug resistance. In this study, we demonstrated that antioxidant control of both Inrf2 and Nrf2 leads to increase in anti-apoptotic protein Bcl-2 that prevents apoptosis and promotes cell survival and drug resistance. Nrf2/Cul3/Rbx1 complex ubiquitinates and degrades Bcl-2 protein as observed with Nrf2. The DGR domain of Nrf2 interacts with the BH2 domain of Bcl-2 and facilitates Nrf2/Cul3-Rbx1-mediated ubiquitination of Bcl-2 by the conjugation of ubiquitin molecules to lysine17 of Bcl-2. Antioxidant t-BHQ antagonized Nrf2/Bcl-2 interaction, led to the release and stabilization of Bcl-2, increased Bcl-2:Bax heterodimers and reduced apoptosis. In addition, antioxidant-induced Nrf2 binds with an ARE located between nucleotides -3148 to -3140 on the reverse strand of Bcl-2 gene promoter and increased Bcl-2 gene transcription leading to elevated levels of Bcl-2. The antioxidant control of Inrf2 and Nrf2 led to increased Bcl-2 that decreased etoposide-mediated accumulation of Bax, release of cytochrome c from mitochondria and activated caspase-3/7. These alterations led to significantly reduced DNA fragmentation and apoptosis. Together, these results provide the first evidence of Nrf2 and Nrf2 control of anti-apoptotic protein Bcl-2 and apoptosis.

Nrf2 protects human and mouse alveolar epithelial cells against injury by cigarette smoke.


Oxidative stress caused by cigarette smoke (CS) directly causes lung injury and cell death. The epithelium is the barrier between inhaled air, which contains the toxic compounds in CS and the underlying tissue. Nuclear factor erythroid 2-related factor 2 (Nrf2) is the principle transcription factor that regulates expression of phase II detoxifying antioxidant enzymes. We studied injury by cigarette smoke extract (CSE) of human primary alveolar cells isolated from lung donors in vitro and Nrf2-/- and wild-type C57Bl/6 mouse alveolar cells and lung tissue in vivo. We found that CSE induces Nrf2 translocation to the nucleus in human primary alveolar type I-like (ATI-like) cells. Moreover, Nrf2 overexpression protected these cells against injury by CSE and Nrf2 knockdown sensitizes these cells to CSE as detected by propidium iodide and Hoechst 33342 double staining. We also found that nucrosin of ATI-like cells induced by CSE was prevented by the antioxidant compounds NAC and trolox. Furthermore, we also studied lung injury by CS in Nrf2-/- mice and wild-type C57Bl/6 mice in vivo. We found that activation and induction of Nrf2-deficient genes HO-1 and NQO1 by western blotting and real time-PCR in alveolar type II cells isolated from wild-type mice but not from Nrf2-/- mice. This suggests involvement of the Nrf2 pathway in protection against lung injury by CS. Moreover, oral administration of NAC or trolox decreased expression of Nrf2 and Nrf2-dependent genes in the lung tissue of wild-type mice but not in Nrf2-/- mice exposed to CS as detected by real time-PCR. Our results suggest Nrf2 protects against CS-induced injury in human alveolar cells in vitro and in mice in vivo. This work is supported by a Young Clinical Scientist Faculty Award to B. Kosmider from the Flight Attendant Medical Research Institute.

Nrf2 deficiency attenuates fat accumulation in white adipose tissue by inhibiting SREBP1C transcription in Lepob/ob mice.

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It is well described Nuclear factor erythroid 2-related factor 2 (Nrf2) regulates cellular electrophilic and oxidative stress in liver and kidney. While our recent findings along with others indicate Nrf2 pathway is inducible in adipose tissue. Nrf2 signaling is enhanced in obese and diabetic animal models, and the transcriptional levels of Nrf2, Nqo1 and Gclc was induced by fasting, suggesting Nrf2 signaling plays key roles in regulating glucose and lipid metabolism. However the exact function of Nrf2 on lipid metabolism is still ambiguous. CDDO-Imidazole induced Nrf2 activation prevented high fat diet (HFD)-induced obesity, decreased hepatic fat accumulation and lipogenic gene expression (Zhang. YK, 2010). In contrast, recent work from Pi et al. illustrated Nrf2-null mice displayed less fat mass and smaller adipocytes formation, protection against weight gain and obesity (Pi. et al., 2010). In the current study, we generated the double knockout mice of Srebp1c by obesity in white adipose tissue, deficiency of Nrf2 attenuated the induction of Srebp1c by obesity in white adipose tissue, and subsequently blocked the induction of FAS, ACC-1, and SCD-1. Double knockout mice deposited less fat (triglyceride) in livers, suggesting Nrf2 deficiency prevented hepatic steatosis and fatty liver process in Lepob/ob mice. However Nrf2 deficiency impaired glucose tolerance and more severe diabetic status, which displayed higher hyperinsulinemia and hyperglycemia in Lepob/ob mice. Overall, Nrf2 deficiency attenuated fat accumulation to adipose tissue; this attenuation may mediate by decreased Srebp1c transcriptional levels and the decreased lipid synthesis.
2147 MAP KINESES REGULATE NITROGEN MUSTARD-INDUCED ACTIVATION OF DAMAGE-ASSOCIATED MOLECULAR PATTERNS IN Murine Keratinocytes.

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Exposure to chemical vesicants results in inflammation and blistering in the skin. To investigate mechanisms underlying these responses, we evaluated the effects of the nitrogen mustard vesicant 2-chloro-N-(2-chloroethyl)-N-methylharnitaine (HN2) on cellular signaling pathways initiated by endogenous molecules released by dying cells, damage-associated molecular patterns (DAMP). Proteins active in DAMP signaling include members of the toll-like receptor (TLR), TLR adaptor protein, and NOD-like receptor (NLR) families. Using RT-qPCR, we found concentration- and time-related increases in mRNAs for TLR2, 3, 6, 7, and 8 following treatment of PAM 212 murine keratinocytes with 1-30 μg/mL HN2. Increases in TLR mRNAs ranged from 4-15 fold, reached maximal levels by 24 hr and persisted for 72 hr. HN2 also increased TLR adaptor protein mRNA for TRAF6 (10-fold) and MyD88 (4-fold), and NOD2 (13-fold), maximal responses were observed after 12 hr. HN2 also increased mRNAs downstream of DAMP including COX2, IL-1β, NF-KB and PPARgamma, maximal effects were evident 24-72 hrs after HN2 treatment. Taken together these data indicate that DAMP signaling is altered in response to HN2 in a process mediated by TLR and NOD.

In further studies, SB 203580, a p38 MAP kinase inhibitor, was found to suppress HN2-induced expression of MyD88. TRAF6 and NOD2 indicating that MAP kinases regulate DAMP responses to HN2. We speculate that DAMP signaling is important in vesicant-induced skin toxicity and that regulation of DAMP by MAP kinases may be important in modulating HN2-induced injury in the skin. (With support by AR057037)

2148 MULTIPLEXED HIGH-CONTENT SCREENING REVEALS THAT CIGARETTE SMOKE CONDENSATE-ALTERED CELL SIGNALING PATHWAYS ARE ACCENTUATED THROUGH FAK INHIBITION IN HUMAN BRONCHIAL CELLS.

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Bronchial cells are one of the primary cell types affected by inhaled particulates. Treatment of immortalized human bronchial epithelial cells (BEAS-2B) with cigarette smoke condensate (CSC) disrupted the F-actin cytoskeleton, and decreased cell spreading and motility (Carter and Hamm, 2009). The current study sought to further elucidate the mechanisms by which these changes occurred. We hypothesized that CSC activated the tyrosine kinase proteins focal adhesion kinase (FAK) and paxillin along with mitogen-activated protein kinases (MAPKs) in BEAS-2B cells. Analysis was done using multiplexed high-content screening and Western blots. When BEAS-2B cells were treated with 20-100 μg/mL CSC for 1 hour, FAK staining and phosphorylation increased until the 100 μg/mL CSC dose was reached. CSC induced an increase in F-actin disruption but FAK inhibition alone, using FAK inhibitor NSC 667249, a p38 MAP kinase inhibitor, was found to suppress HN2-induced expression of MyD88. TRAF6 and NOD2 indicating that MAP kinases regulate DAMP responses to HN2. We speculate that DAMP signaling is important in vesicant-induced skin toxicity and that regulation of DAMP by MAP kinases may be important in modulating HN2-induced injury in the skin. (With support by AR057037)

2149 EXPRESSION OF STEROID SULFATASE INDUCES WNT SIGNAL PATHWAY AND CELL MIGRATION IN HUMAN PROSTATE CANCER CELLS.


Steroid sulfatase (STS) is responsible for the conversion of estrone sulfate to estrone that can stimulate growth in endocrine-dependent tumors such as prostate cancer. Although STS is considered as a therapeutic target for the estrogen-dependent dis-

2150 IDENTIFICATION OF A NOVEL PATHWAY INVOLVED IN THE NEGATIVE REGULATION OF METALLOTHIONEIN TRANSCRIPTION.

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Analysis of transcriptome data from multiple species indicate that exposure to the carcinogenic metal cadmium alters the expression of hundreds of genes that are regulated by multiple signal transduction pathways, many of which remain to be defined. In response to cadmium, cells increase the expression of highly conserved, small, cysteine-rich metal-binding proteins known as metallothioneins (MTs), which function in metal detoxification. 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 mtl-1 regulatory region were constructed, mtl-1::GFP Transgenic strains constitutively express GFP in the pharynx and following cadmium exposure, express GFP in the intestine. In a reverse genetic screen, genes involved in various stress response pathways were tested for their potential role in controlling mtl-1 expression. Increased GFP expression was observed when either pdk-1 was knocked out or the simultaneous knock out of akt-1 and akt-2. AKT-1 is a serine/threonine kinase involved in the insulin signaling pathway which complexes with AKT-2 to regulate transcription of downstream factors and directly interacts with PDK-1. Interestingly, mtl-1 transcription was not affected when other insulin signaling pathway genes were knocked out suggesting that PDK-1 and the AKT-1/2 complex act independently of this pathway to control mtl-1 transcription. To identify other transcriptional regulators, genes involved in various MAPK pathways were tested. A deletion mutant of atf-7, a negative transcription factor involved in the JNK/p38 pathway, resulted in an increase in GFP expression. Additionally, the atf-7 mutant failed to complement an EMS mutant isolated from a forward genetic screen. PMK-1 regulates ATF-7 and the loss of pmk-1 results in a decrease in mtl-1 expression, as determined through qRT-PCR. Pathway analysis indicates that ATF-7 and PMK-1 regulate cadmium-inducible MT transcription downstream of PDK-1 and AKT-1/2.

2151 REGULATION OF MnSOD BY THE AHR AGONIST PCB126: THE ROLE OF DIETARY MANGANESE.

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PCB126 is the most potent aryl hydrocarbon receptor (AhR) agonist among all polychlorinated biphenyls, which are persistent organic pollutants. PCB126 has been shown to increase reactive oxygen species including superoxide radicals in vitro and in vivo. MnSOD is one of the most efficient antioxidant enzymes, converting superoxide to hydrogen peroxide. However, its activity was reduced by PCB126, thereby probably aggravating the oxidative stress. To elucidate the mechanisms of PCB126's modulation of MnSOD expression, we conducted dose- and time-response and a manganese dietary study of PCB126 effects in male Sprague Dawley rats. The expression and activity of hepatic MnSOD was determined. Several redox-sensitive transcription factors of MnSOD as well as post-translational modifications of the protein were also studied. The results show a dose-and time-dependent modulation of MnSOD mRNA and protein. The transcriptional induction of MnSOD is consistent with an observed increase of Nrf2 binding activity and the direct binding of AhR to the promoter region of MnSOD, which contains two core XRE sequences. However, MnSOD activity was reduced by PCB126, which could not be accounted for by acetylation, nitration or phosphorylation of the protein. Dietary Mn supplementation enhanced the increase in MnSOD mRNA but did not reverse the loss of its activities by PCB126, even though it significantly reduced the PCB126-driven liver hypertrophy. Mn supplementation also elevated the Mn concentration in the liver, which was nevertheless decreased by PCB126 both in the

cases, the cellular function of STS are still not clear. Here, we found that treatment with basic lipopolysaccharides (LPS)-induced STS expression in concentration- and time-dependent manners in human prostate cancer PC-3 cells. We found that LPS-induced STS mRNA expression was suppressed by NF-κB inhibitors such as Bay 11-7082 and Bortezomib. To determine whether IkBe is involved in NF-κB-dependent STS expression, the effect of IkBe siRNA was elucidated. Western blot and RT-PCR analyses followed by vector-mediated overexpression of STS cDNA showed that STS expression induced Wnt signal pathway through increased level of cyclin D1, b-catenin and MMP7. We determined that STS overexpression induced tumor cell migration by using wound healing assay. Tumor cell migration was suppressed by knockdown of STS and recovered by LPS. These data suggest that STS expression induces Wnt signal pathway and tumor cell migration in PC-3 cells.

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whole liver and in liver mitochondria. In conclusion, we discovered that the complex regulation of hepatic MnSOD expression by PCB126 occurred at both transcriptional and post-translational levels. Mn supplementation is protective, although far from complete, against PCB126-induced toxicities. (Supported by NIEHS P42 ES013661 and DAMD17-02-1-0241)

2152 EVALUATION OF PERFLUOROALKYL ACID ACTIVITY USING PRIMARY MOUSE AND HUMAN Hepatocytes.


While perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) have been studied at length, less is known about the biological activity of other environmental perfluoroalkyl acids (PFAAs). Using a transient transfection assay developed in COS-1 cells, our group has previously evaluated a variety of PFAAs for activity associated with activation of peroxisome proliferator-activated receptor alpha (PPARα). Here we use both mouse and human primary hepatocytes to further assess the biological activity of a similar group of PFAAs using custom designed Taqman Low Density Arrays. Commercially available primary mouse and human hepatocytes were cultured for 48 hours in the presence of varying concentrations of 12 different PFAAs or Wy14,643, a known activator of PPARα. Total RNA was collected and the expression of 48 mouse or human genes was evaluated. Gene selection was based on either in-house liver microarray data (mouse) or published data using primary hepatocytes (human). Gene expression in primary mouse hepatocytes was more restricted than expected. The expression of genes typically regulated in whole tissue by PPARα agonists, such as Acox1, Me1, Aca1a, Hmgcs1, and Sk27a1 was not altered in mouse cells. Cyp2b10, a gene regulated by the constitutive androstane receptor and a transcript normally up-regulated by in vivo exposure to PFAAs, was also unchanged in mouse cells. Cyp8a1, Ehahd1, Pdk4, Cpt1b, and Fabp1 were regulated as expected in mouse cells. Human primary hepatocytes, changes in gene expression were not robust making the determination of dose response across a wide group of genes and compounds difficult. This likely reflects weaker activation of PPARα in human versus rodent cells. Unlike mouse cells, CYP2B6 was up-regulated in human primary hepatocytes by certain PFAAs as well as PPARα. Ranking of biological activity was determined based on a limited list of responsive genes and contrasted to data collected from COS-1 cells. (This abstract does not reflect US EPA policy.)

2153 ARYL HYDROCARBON RECEPTOR KNOCKOUT RATS ARE INSENSITIVE TO THYMIC ATROPHY, CHANGES IN LIVER WEIGHT AND AHR-MEDIATED GENE INDUCTION FOLLOWING ACUTE TCDD EXPOSURE.

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) has been shown to produce both hepato- and cutaneous carcinomas and cholangiocarcinomas in 2-year cancer bioassays performed using the Sprague Dawley outbred rat model. Aryl-hydrocarbon (AhR) activation is a key event in the tumor promotion Mode-of-Action (MOA) for TCDD, although data gaps remain regarding how sustained AhR activation culminates in cellular and histopathological changes leading to liver tumors in rodents. To examine the contribution of AhR activation in liver carcinogenesis, we have developed an AhR-knockout rat model on the Sprague-Dawley outbred background. The knockout was generated by targeting exon 2 within the AhR gene using zinc-finger nuclease (ZFN) technology. The ZFN knockout produced two different rat lines containing either a 2-bp or 29-bp deletion in AhR exon 2. Breeding pairs heterozygous (Het) for either AhR mutation were established in order to generate homozygous knockout (KO) and wild-type (WT) individuals with a similar genetic background. qRT-PCR demonstrated that WT and KO animals express AhR mRNA. In contrast, Western blot analysis confirmed that WT animals express the 96 kDa AhR receptor, whereas KO animals do not. To test for loss of functional AhR activity in the KO, female AhR WT, 2-bp and 29-bp KO animals were treated with a single oral dose of either corn oil vehicle or 25 μg/kg TCDD and sacrificed 7 days later (n=3-4 / group). TCDD exposure decreased thymus/body weight ratio and increased liver/body weight ratio in WT animals, with no effects in either 2-bp or 29-bp KO animals. In addition, TCDD exposure resulted in induction of Cyp1a1, Cyp1a2, Cyp1b1 and Ahrr mRNA in WT animals, but not in either 2-bp or 29-bp AhR KO animals when compared to controls. These data suggest that AhR knockout animals lack a functional AhR receptor and may be an appropriate tool for investigating the role of AhR in TCDD-induced liver carcinogenesis.

2154 COMPARISON OF GENOME-WIDE GENE EXPRESSION IN TCDD-EXPOSED C57BL/6 MICE AND SPRAGUE DAWLEY RATS.

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a ubiquitous environmental pollutant that exerts most of its effects through the aryl hydrocarbon receptor (AhR). Although the structure and function of the AhR are conserved, emerging evidence suggests downstream effects are species-specific. In this study, rat hepatic gene expression data available from the DrugMatrix database (National Toxicology Program) were compared to a mouse hepatic whole-genome gene expression dataset. For the DrugMatrix dataset, male Sprague Dawley rats were gavaged daily with 20μg/kg TCDD for 1, 3 and 5d while the female C57BL/6 ovx mouse data set involved a single gavage at 3μg/kg for 1, 3 and 7d. Using the same data analysis, 932 mouse and 649 rat genes (fold change>1.5, P<0.05) were differentially expressed. HomoloGeneID identified 11654 orthologous genes represented across the rat Affymetrix 230 2.0 GeneChip (12310 total orthologs), and mouse 4x44K v.1 Agilent oligonucleotide array (15758 total orthologs). Comparative analysis found 584 and 525 orthologs differentially expressed in the mouse and rat, respectively. Thirty-five responses were in common to both species that exhibited similar patterns of expression and functionally associated with lipid metabolism, immune function, transcription, and metal/ion binding. However, the transcription factor Nfκb involved in cell-cell signalling was divergently expressed (induced in rat; repressed in mouse). Functional analysis of species-specific responses identified genes involved in lipid metabolism, immune response and carbohydrate metabolism in mice while rat-specific responses were associated with transcriptional and cell cycle regulation and carbohydrate metabolism. These results provide further evidence that TCDD elicits species-specific effects mediated by the AhR. Funded by SRP P42ES04911.

2155 ALTERATION OF GENE EXPRESSION PROFILES AND CYTO-GENOTOXIC EFFECTS INDUCED BY EXPOSURE OF PCB153 TO A HUMAN B LYMPHOBLASTOID CELL LINE.

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Polychlorinated biphenyls (PCBs) are ubiquitous, persistent pollutants found in the environment and human tissues. Exposure to PCBs is of great concern to human health because they have various deleterious effects, e.g. neurotoxicity, reproductive abnormalities, endocrine disruption, including cancers. In the present study, a human B lymphoblastoid cell line was exposed to three doses (25, 100, 200 μM) of non-coplanar 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153) for 24 h, and the gene expression profiles were analyzed using Illumina Human HT-12 expression bead-chips. After adjusting for background and housekeeping genes, genes with different expression between the exposure and control groups were determined by cluster analysis, and further confirmed by real-time RT-PCR, and the expression of differential genes was also studied when the cells were exposed to the same doses of PCB153 for 4h and 48h. In addition, the cell viabilities and DNA damage induced by PCB153 was detected with CCK-8 and comet assays. Our results indicated that the expression of 1 gene was up-regulated and that of 7 genes was down-regulated in all three exposure groups for 24 h, and three of them were confirmed by real time PCR in 4h, 24h, and 48h exposure groups, and also a time-dependent effect was found. However, the expression of 18 genes was up-regulated and that of 63 genes was down-regulated in the median and highest dose groups. The results of CCK-8 assay showed that exposure to 200 μM of PCB153 for 24 h could result in significant decrease of cell viability, but the cell viabilities decreased significantly at all three exposure groups when the exposure time was extended to 48 h. However, no significant DNA damage was induced by even the highest dose of PCB153. These genes could be used as molecular biomarkers for local residents exposed to PCBs, but it should be validated in the further well-designed population investigations.
Bone marrow (BM) hematopoietic stem cells differentiate to lymphoid, myeloid and erythroid lineages. Common lymphoid progenitors (CLP) emigrate to the thymus to form T cells, or differentiate into immature B cells that mature in the spleen. We provide evidence that in vitro suppression of BM progenitor cells by a single dose of 7,12-dimethylbenz[a]anthracene (DMBA) or benzo(a)pyrene (BP) subsequently decreases lymphoid populations in BM, spleen and thymus. Colony forming assays show that DMBA extensively suppressed BM lymphoid and myeloid progenitors within 6h. BP was impaired due to an early selective recovery. These relationships are profiled using congenic C57Bl/6j mice, Cyplb1/-/- or PAH-resistant Ah Receptor (AhR) d-type alleles. This acute suppression of BM progenitors by DMBA depends on constitutive Cyplb1, but not on AhR, which mediates the selective BP recovery. Rapid DMBA-mediated BM progenitor changes preceded coordinate losses (48h-168h) in total lymphocyte populations in BM, spleen and thymus, initially without affecting the ratios of lymphoid sub-populations. Thus, suppression and recovery of CLP in BM appears sufficient to account for changes in BM by acting on the progenitor that stimulates the re-emergence of CLP.

Microarray analyses on BM cells 6-24h after in vitro treatment by DMBA and BP identify AhR-mediated effects, equivalent to TCDD. Many AhR-mediated responses are selective to BP, transient and mediated by Cyplb1. An extra set of changes depends on Cyplb1, in absence of PAHs, indicating constitutive changes. Flow analyses of BM and thymus progenitor cells will compare Cyplb1-mediated constitutive and PAH-mediated changes from direct AhR-mediated effects of TCDD.

### 2157 REGULATION OF XENOBIOTIC TRANSPORTERS IN MOUSE KIDNEYS BY PERFLUOROCARBOXYLIC ACIDS.

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In recent years, perfluorochemicals have attracted great attention due to their persistence in the environment, detection in human tissues, and toxicities noted in the laboratory animals. Transporters play important roles in the disposition of chemicals. We previously reported that PFDA in mouse liver decreased organic anion transporting polypeptides (Oatps) but increased multidrug resistance-associated proteins (Mrps). However, it is not known whether perfluorochemicals similarly regulate transporters in kidney. Therefore, the present study was conducted to determine whether perfluorooctanoic acid (PFOA), perfluorooctanoic acid (PFNA), and perfluorodecanoic acid (PFDA) alter the mRNA of 38 critical transporters in kidneys of mice. The data showed that a single intraperitoneal administration of LO at mRNA levels in Cd-resistant (CdR) rat fetal lung fibroblasts (RFLF) following long-term Cd exposure. The cloned rat LO gene promoter -1/-804 (relative to ATG) free of the TATA box with the maximal promoter activity contains an Inr/DR1 element, four putative promoter response elements (PRES), one putative ARE (ARE), and one putative antioxidant response elements (ARE). ChiP assays reported here further characterize the rat LO gene promoter in response to Cd in an effort to understand mechanisms for Cd inactivation of the LO gene. CdR cells with different degrees of Cd-resistance exhibited enhanced methylation of Cpg at the Inr/DR1 region, inhibited hypoxia inducible factor 1 (HIF1) binding to the LO HRE as cells treated with CoCl2 that mimics hypoxia conditions in cultured cells, and MRE-binding transcription factor-1 (MTF-1) binding to the LO MRE, but increased NF-E2-related factor 2 (Nrf2) binding to the LO ARE in a dose-dependent manner. The collective effect of these factors in response to Cd to trans-inhibit the LO: ARE complex in vitro.

### 2158 METHYLSICIN AND 7, 8-DIHYDROMETHYLSICIN ARE TWO MAJOR KAVALACTONES IN KAVA EXTRACT TO INDUCE CYPIA1.


Kava is a plant traditionally used for making beverages in Pacific Basin countries and has been used for the treatment of nervous disorders in United States. The pharmacological activity of kava is achieved through the kavalactones in kava extract, which include kawain, 7,8-dihydrokawain, yangonin, 5,6-dehydrokawain, methysticin, and 7,8-dihydromethysticin. Recent studies have shown that kava extract induces hepatic CYP1A1 enzyme, however, the mechanisms of CYP1A1 induction have not been elucidated, and the kavalactones responsible for CYP1A1 induction have not yet been identified. Using a combination of biochemical assays and computational tools, we determined the functions of kava extract and kavalactones that delimited the underlying mechanisms involved in CYP1A1 induction.

The results showed that kava extract displayed a concentration-dependent effect on CYP1A1 induction. Among six major kavalactones, methysticin triggered the most profound inducing effect on CYP1A1 followed by 7,8-dihydromethysticin. The other four kavalactones did not show significant effects. Consistent with the experimental results, in silico molecular docking studies based on the aryl hydrocarbon receptor (AhR)-ligand binding domain homology model also revealed lower binding energies of methysticin and 7,8-dihydromethysticin compared to the remaining kavalactones. Additionally, results from a luciferase gene reporter assay suggested that kava extract, methysticin and 7,8-dihydromethysticin were able to activate AhR signaling pathway. Moreover, kava extract-, methysticin- and 7,8-dihydromethysticin- mediated CYP1A1 induction was blocked by an AhR antagonist and abolished in AhR-deficient cells. These findings suggest that kava extract induces the expression of CYP1A1 via the AhR-dependent mechanism, and that methysticin and 7,8-dihydromethysticin contribute to the CYP1A1 induction.
3,3',4,4',5-pentachlorobiphenyl (PCB 126) in an AhR-specific manner. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) was used as a positive control. Exposure of the cells to hypoxia (1% O2 for 8 hours) significantly inhibited the induction of CYP1A1 mRNA by up to 74%. CYP1A1 promoter/luciferase reporter assays to measure the transcriptional activities of AhR complexes further demonstrated a 75% inhibition of AhR-mediated luciferase expression following hypoxia treatment. Taken together, these data reveal that hypoxia significantly inhibits the induction of CYP1A1 after PCB 126 exposure and that hypoxia interferes with AhR-mediated transcriptional responses to PCBs. Our future studies will investigate the mechanistic nature of this crosstalk by analyzing the binding of AhR to CYP1A1 xenobiotic response element (XRE)-sequences after PCB exposure in normoxia and hypoxia.

2161 COMPARISON OF MICROARRAY GENE EXPRESSION PROFILES CORRESPONDING TO XENOBIOTIC RESPONSES TO OXIDATIVE STRESSES INDUCED BY IONIZING RADIATION AND BENZENE TREATMENT.

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To characterize global gene expression profiles corresponding to xenobiotic responses induced by ionizing radiation (IR) and benzene (Bz), we compared two different categories of gene expression profiles: i.e., one corresponds to common gene expression profiles (CEG) selected by one-way analysis of variance and/or the Welch-t-test, and the other to stochastic gene expression profiles (SEG) selected by principal component analysis. Because both IR and Bz are known to be oxidative-stress inducers and leukemogens, alterations of oxidative-stress-related gene expressions of bone marrow cells were focused on 4 weeks after the last treatment with IR or Bz: the IR group (5 mice) was irradiated with 3 Gy of 137Cs-gamma-ray whereas the Bz group (5 mice) was intermittently treated with 150mg/kg b.w./day Bz for two weeks by gavage. Oxidative-stress-related genes that showed altered expression levels followed the treatments were selected from an existing database, i.e., 151 (CEG, 34; SEG, 117) genes for the IR group and 182 (CEG, 32; SEG, 150) genes for the Bz group. Pathway analysis using these gene profiles and their expression levels in each mouse revealed the same pathways in 5 mice in each group, i.e., the NFKB and MAP kinase cascade for the IR group, and the Akt regulatory pathways including NFKB and the Bz group. Interestingly, in these pathways, SEG signals merged with a CEG and redistributed to SEGs. Moreover, different MAP kinase genes were included in each of these gene profiles: MAPK9/MAP2K3/MAP3K1/MAP3K4/MAP3K5 for the IR group and MAPK3/MAPK3/MAP3K2/MAP3K3 for the Bz group. In conclusion, different molecules within the same functional category were used for each profile.

2162 IDENTIFICATION AND CHARACTERIZATION OF TRANSCRIPTIONAL CONTROL ELEMENTS REGULATING A NOVEL RECEPTOR-MEDIATED SIGNALING REGULATOR, TNIP1.

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TNFalpha interacting protein 3 interacting protein 1 (TNIP1) is a novel nuclear receptor interacting protein, isolated and characterized as a co-repressor of RARs and PPARs in our lab. TNIP1 has also been shown to regulate a variety of other receptor-mediated events as diverse as programmed cell death and cell cycle stemming from TNF and EGF signaling, respectively. Changes in TNIP1 expression levels are likely to impact the biological endpoints of these different pathways. As the importance of TNIP1 becomes more apparent, it is crucial to determine what controls its expression levels, as can be determined by a study of its promoter. We isolated ~6kb of the human TNIP1 promoter and examined it both in silico and experimentally for transcriptional control elements with an eye directed at constitutive and inducible elements. Sequence analysis predicted two specificity protein (Sp) sites in the proximal region of the promoter and multiple NF-kB sites throughout the promoter. We predict the Sp family of transcription factors is responsible for much of TNIP1’s constitutive activity and NF-kB for its inducible expression. Transcriptional activation studies revealed NF-kB, Sp1 and Sp3 positively regulate TNIP1. Furthermore, EMSA and ChIP demonstrated the physical association between NF-kB, Sp1, Sp3 and specific regions of TNIP1 promoter. Decrease in protein via siRNA of Sp binding to cognate sites by mithramycin decreased TNIP1 mRNA while the potent NF-kB activator TNFalpha decreased TNIP1 expression. In summary, Sp1 and Sp3 contribute to the constitutive regulation of TNIP1 promoter through two proximal sites and NF-kB contributes to the inducible regulation of TNIP1 via two distal sites. Changes in endogenous Sp or NF-kB levels or pharmacological control of their activity would be expected to affect TNIP1 expression, which, in turn, could ultimately regulate TNIP1-related biological endpoints such as cell death, proliferation, and inflammation, and more globally, diseases such as psoriasis and rheumatoid arthritis.

2163 COEXPOSURE OF LOW-DOSE EXTRAPOLATED MODEL TESTICULAR TOXICANTS (X-RADIATION AND 2, 5-HEXANEDIONE) MODULATES GENE ALTERATIONS THROUGH TESTICULAR TRANSCRIPT PROFILING.

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Testicular toxic effects of chemical mixtures may be different than those of the individual chemical constituents. This study utilizes germ and Sertoli cell specific toxicants, X-radiation (x-ray) and 2,5-hexanedione (HD) respectively, to investigate their coexposure effect on spermatogenesis. X-ray induces germ cell apoptosis and has also been shown to attenuate the HD effects on Sertoli cells. Testis tissue from adult rats were exposed to single exposures of different levels of x-ray (0.1Gy, 0.5Gy), HD in the drinking water for 18 days (0.125%, 0.2% or 0.33%), or in combination (2Gy + 0.33% and 1%, 5Gy + 0.33% or 1%). Based on previous research in our lab using higher doses, we expected that a low-dose extrapolation of these toxicants would result in the same attenuating effect of x-ray induced genes with HD priming. Gene expression profiling using Affymetrix microarrays was used to identify through ranking and prioritization, target genes for further verification using more sensitive PCR-based expression arrays. Hierarchical clustering of the PCR array data identified the apoptosis-related genes, Fas, apoptosis enhancing nuclease (Aen), and Casp3 (Casp3), which possessed similar dose-dependent expression profiles. The independent gene expression analyses detected no significant change in Casp3, Fas and Aen following exposure to either x-ray or HD alone. However, the 5Gy + 1% HD coexposure induced Aen and Casp3 expression, with a maximum fold induction of 3.1 and 2.6, respectively. Additionally, Fas was significantly induced 2.6-fold at these coexposure doses. These results provide insight into environmentally relevant low-dose coexposures of model testicular toxicants and further identification of transcript profiles from low and high doses in our coexposure model may allow us to observe across the continuum of effects a threshold point between an adaptive and an adverse response.

2164 HEAT SHOCK PROTEINS A1A AND A6 ARE TRANSCRIPTIONALLY REGULATED BY TNIP1 IN KERATINOCYTES.


TNF alpha induced protein 3 interacting protein 1 (TNIP1) regulates multiple signaling pathways downstream of TNF alpha, EGF, and nuclear receptors leading to inhibition of some transcription factor involved toxic responses or cell stress. To gain a better understanding of the biological effects TNIP1 might have, we performed a gene expression microarray analysis of HaCaT keratinocytes overexpressing recombinant TNIP1. Significantly regulated genes included heat shock proteins A1A (HSP72) and A6 (HSP70B), with their mRNA downregulated 3.3- and 20-fold, respectively. The TNIP1-mediated HSP reduction seen in the HaCaT microarray study was confirmed via qPCR and western blot. Similar results were observed with human primary keratinocytes. HSP’s are key to maintaining cellular homeostasis in times of cellular stress and toxicity. To guide analysis of how HSP’s may be transcriptionally regulated by TNIP1, we performed in silico analyses of these genes’ promoters. Results showed putative response elements for transcription factors inhibited by TNIP1, including NF-kappaB, ERK2, RAR, and PPAR, some of which have yet been previously identified as HSP regulators. To determine the functionality of these putative sites, we isolated a 3 kilobase promoter fragment of HSPA1A and A6 for further analysis. These initial results suggest (1) a novel pathway, inhibited by TNIP1, possibly transcriptionally regulating these HSP’s and (2) TNIP1 could enhance cell stress and toxicological insults by reducing HSPA1A and A6 expression.

2165 IDENTIFICATION OF CYTOPLASMIC PROTEINS THAT COMPRISE THE PLASMID TRAFFICKING COMPLEX DURING GENE TRANSFER.

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Non-viral gene delivery requires the genetic material to cross several barriers to attain successful gene expression, making this approach less than optimal. One of the barriers is the dense, crowded cytoplasm, due to the fact that plasmids do not sim-
ply diffuse to the nucleus but require active transport. Very little is known about how this process occurs, but our lab and others have shown that the mitochondrial network and molecular motors are required for plasmid movement to the nucleus. To further investigate how plasmids exploit normal physiological processes to transfect cells, we are analyzing which protein adapters comprise the plasmid trafficking complex. We have developed a live cell DNA-protein pull-down assay in which biotin-labeled plasmids are transfected into human epithelial cell lines, and plasmid-protein complexes are isolated at certain time points post-transfection for analysis by mass spectrometry. From these results, we can develop a model of the plasmid trafficking complex as proteins bind and dissociate during gene transfer. Compared to our negative control plasmid (no promoter sequences), our positive control bound hundreds of unique proteins as early as 30 minutes post-electroporation. These include tubulin, microtubule-associated proteins, molecular motors, nuclear import proteins, and transcription factors, all previously hypothesized by us to play a role in plasmid transport. We are evaluating the significance of these proteins in plasmid trafficking by monitoring movement of microinjected fluorescently labeled plasmids via live-cell particle tracking. Prior to microinjection, we are analyzing which protein adapters comprise the plasmid trafficking network and molecular motors are required for plasmid movement to the nucleus. Very little is known about how cytoplasmic components influence intracellular trafficking of not only plasmids, but also molecules that rely on these processes for normal functioning.

2167 CUSTOM-DESIGNED MITOCOMP FOR INVESTIGATION OF DRUG- AND DISEASE-RELATED MITOCHONDRIAL TOXICITY.

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Mitochondrial dysfunction has been well recognized in a number of degenerative diseases and drug toxicities. The center has designed a custom-designed MitoChip to elucidate the role of mitochondrial toxicity. However, the molecular basis of altered mitochondrial function underlying these diseases and toxicities is not yet well defined. To advance our understanding of the basis of mitochondrial dysfunction associated with degenerative diseases and drug toxicities, we have developed a mitochondria-specific DNA microarray (MitoChip) in a mouse model. This custom-designed MitoChip was designed using eArray (Agilent Technologies, Inc.) and consists of 1,026 mitochondrial and nuclear genes associated with mitochondrial structure and function. There are 8 identical arrays on each slide (8x15k format). To further enhance the accuracy of this custom-designed MitoChip, we integrated 20 house-keeping genes for normalization and 12 ERCC (External RNA Controls Consortium) probes for monitoring fold changes. Total liver RNA samples from groups of mice (n=4) treated with vehicle or 600 ppm usnic acid, a mitochondrial uncoupler, were used to test this MitoChip. Artificial microarrays of ERCC RNA (Mix1 and Mix2; Life Technologies, Inc.) were also added to total RNA samples to test the performance of the MitoChip. The results showed that most of the MitoChips are highly reproducible with a median correlation value of 0.990 within each sample group. The experimental Mix1/Mix2 ratios of six ERCC probes with intensity levels above the 25th percentile are very close to theoretical ratios (1.25, 1.60, 2.0, 4.0, and 6.4) with four replications for each mixture. These results indicate that this custom-designed MitoChip provides accurate measurements of mitochondria-related gene expression changes and will be a useful genomic tool for investigating the mechanisms of drug- and disease-related mitochondrial toxicity.

2168 CHARACTERIZATION OF IN VITRO AND IN VIVO HEPATIC FUNCTIONAL RESPONSES USING AUTOMATED DOSE-RESPONSE MODELING.

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Pathways and over-represented functions identified in dose-response genomic and high throughput assays have been proposed to determine points of departure for hazard and dose assessment in cases where little or no safety data exist. Transcriptomic dose-response studies at 24 h were examined to investigate EC50 distributions for selected pathways and over-represented functions using IPA and DAVID. Mouse (Mm), rat (Rn) and human (Hs) hepatocytes were treated with 0.001-100nM 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) while C57BL/6 mice were gavaged once with 0.01-100μg/kg TCDD. ToxResponse modeler identified the best-fit model on a per probe basis and calculated EC50s for genes with a sigmoidal dose-response. EC50s exhibited broad distributions across species, models and functional categories/pathways. For example, although median EC50s associated with lipid metabolism were comparable (2.2, 1.3 and 0.4nM TCDD for Hs, Mm and Rn primary hepatocytes, respectively) they ranged over 2-3 orders of magnitude (Hs: 0.6-8.2nM; Mm: 0.4-30.8nM; Rn: 0.09-2.6nM), and result in dramatically different in vivo phenotypes. 2,2',4,4',5,5'-Hexachlorobiphenyl (PCB153) also elicited broad EC50 distributions. Moreover, there was a weak negative correlation between EC50s and ED50s for 62 (hepatocyte vs. liver, r=0.06) and 37 (hepatomas vs. liver, r=-0.001) overlapping differentially expressed genes suggesting in vitro gene expression is a poor predictor of in vivo responses. Collectively, this suggests that median in vitro gene expression EC50s may not accurately reflect the in vivo responsiveness of a pathway or over-represented function. Partially funded by SRP P42ES04911. The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the US EPA.
Microsomal epoxide hydrolase (mEH, EPHX1) is an important biotransformation enzyme that catalyzes the hydrolysis of many epoxy metabolites. EPHX1 substrates vary from the endogenous steroid epoxides, such as estrone and androstenedione, to the pro-carcinogenic epoxy intermediates such as benzo[a]pyrene-7,8-epoxide derived from the xenobiotic metabolism of polycyclic aromatic hydrocarbon. Previous studies from our laboratory suggested the involvement of post-transcriptional regulation in modulating EPHX1 expression, mediated by the presence of complex secondary structure and two upstream open reading frames (uORFs) within the 5′-untranslated region (5′-UTR) of an EPHX1 transcript variant termed E1-b'. Utilizing a lentivirus-based transduction system to over-express the E1-b' transcript in mammalian cell lines, we found that the presence of E1-b' inhibits overall EPHX1 expression. E1-b' transcripts containing mutations of the respective uORFs' start codons were devoid of this inhibitory activity. The E1-b' transcript uORFs are predicted to code for short peptide sequences of 26 and 17 amino acids in length, respectively. We hypothesized that these peptides contribute regulatory function in EPHX1 expression. Synthetic 26 and 17 amino acid peptides were titrated into an in vitro transcription/translation system utilizing either rabbit reticulocyte lysate or wheat-germ extract programmed with the EPHX1 E1-b' construct. The results obtained demonstrated that these peptides can inhibit the production of EPHX1 protein in both the mammalian and plant-derived systems. Transfection of these peptides into mammalian cell lines further confirmed their EPHX1-specific inhibitory effect. In addition, kinetic analysis of mRNA stability indicated that the percent of these Cyps respond by two-fold or more to this diet change (25 up, 3 down). Cyp1b1 is not expressed in mouse hepatocytes, but deletion dramatically redirects liver gene expression (>1000 genes FC>2.5; p<0.02), even more than the dietary change. The same Cyp genes are affected in a coordinate manner, often as clusters within sub-families (2a, 2b, 2c, 3a and 4a). Cyp2c genes are stimulated by dietary fat and Cyp1b1 deletion, whereas fat-responsive genes in each of the 2a, 2b, 3a and 4a sub-families are highly suppressed. Cyp1b1 deletion appears to affect a general suppression of endogenous PPAR, CAR and PXR activities that extends to non-Cyp genes. This does not extend to external ligands, as PPAR induction is effective stimulated by WY14623 in Cyp1b1-/- mice. One possibility is that endogenous ligands are either not modified or are sequestered by endogenous proteins. An extensive set of such proteins are expressed by chromosomally adjacent lipocalin and Mup families. Six such genes are highly induced by Cyp1b1 deletion. The diet change and Cyp1b1 deletion also suppress genes in the liver that are associated with oxidative stress and inflammation. Paradoxically, Cyp1b1 deletion in endothelia causes NF-κB activation. The external source of the Cyp1b1 regulation remains elusive. These studies show that diet change and Cyp1b1 deletion exhibit similar selectivity for effects on several families of genes. Dietary flavonoids can inhibit Cyp1b1 and may affect metabolism of endogenous or dietary substrates thus altering hepatic glucose and fatty acid metabolism.

The toxicity of complex mixtures is a significant and growing problem that needs to be addressed by applied toxicology. While valuable techniques for the assessment of single compounds, such as non-metabolizable halogenated aromatic hydrocarbons, in a mixture based on relative toxicity exist such approaches are inadequate for the assessment of complex mixtures with several different types of components. In complex mixtures, multiple exposures generate specific changes in gene expression that cannot be attributed to a single mechanism. Data on these kinds of mixtures are scarce and often contradictory. Our previous work has demonstrated that alteration of epigenetic regulation of gene transcription plays an important role in chromium's toxic response, both alone and in a complex mixture with BaP. Here we begin to dissect the mechanism of mixture toxicity using long-term low-concentration exposure to chromium with and without acute BaP exposure. We find that mouse hepatoma cells exposed to long-term low concentrations of sodium chromate, in the range of 0.1 – 0.5 μM, accumulate double-strand DNA breaks, and that the percentage of cells undergoing apoptosis increases concurrently with either increased concentration or longer period of exposure to Cr. Despite increased apoptosis and an increased number of DNA double-strand breaks the cell cycle progression parameters of these cells are unaffected by Cr concentration or exposure time. At a transcriptional level, qRT-PCR revealed that long-term low-concentration exposure to Cr changes the expression of many genes, most commonly of genes important for DNA damage response and apoptosis. We also find that the pattern of gene expression associated with BaP exposure is altered if cells have been exposed to long-term Cr and those differences are concentration dependent. These results begin to tease out the mechanism by which exposure to one toxicant, Cr, can affect the cellular responses to another, BaP, shedding light on the toxic responses to complex toxic mixtures. Supported by NIH grant ES010807.
Cigarette smoke exposure has been associated with the incidence of lung cancers. The role of microRNAs in the transition to malignancies has been studied extensively over the last decade. As a result, a number of microRNA biomarkers in lung cancer have been identified. Also, the kelch-like ECH-associated protein 1 (Keap1) nuclear factor erythroid-derived 2-like 2 (Nrf2) pathway is implicated in lung cancer. In this study, the role of microRNAs in the transition from non-tumor to malignant status in lung tissue is examined with a focus on the Keap1/Nrf2 pathway. Data from the Human Whole miRNome Project version 1 was analyzed for insights. Some, such as miR142-5p and miR-193a-5p have increased expression; others, such as miR144 have decreased expression. Quantitative PCR data curated in the PhenomIR database validate a number of the differentially-expressed microRNAs identified in the high throughput approach. Using a bicluster acquisition algorithm, functional clusters of microRNAs in lung cancer were identified. Furthermore, results of a gene set enrichment analysis indicate that, relative to non-malignant tissue, gene targets of mir27A, mir-27B, mir-142-5p, mir-144, mir-153, mir-199A, and mir-522 have suppressed expression in Kras mutation-induced tumor tissue in mice in which either Nrf2 or Keap1 expression is diminished. As documented in the Comparative Toxicogenomics Database, a number of the gene targets of these microRNAs are impacted by components of cigarette smoke including acrolein and nicotine. The novel findings lend further insights into microRNA regulation of the Keap1/Nrf2 pathway perturbations associated with the transition to lung cancer.

Xenoestrogens (XEs) in the environment have been linked to the occurrence of reproductive abnormalities in many aquatic species, including largemouth bass (Micropterus salmoides; LMB). Steroidogenic acute regulatory protein (StAR), a protein vital to steroid hormone synthesis, has been shown to be a target of a number of XEs in LMB. Investigators working with higher vertebrate models have reported similar effects following exposure to XEs as well as endogenous estrogen (E2); however, the mechanisms underlying XE/E2 regulation of StAR are not understood. To determine whether E2 elicits a response in StAR transcriptional activity in LMB, a segment of the LMB StAR promoter was transfected into MA-10 Leydig cells and promoter activity was measured following E2 exposure under basal and gonadotropin (hCG)-induced conditions. hCG stimulated promoter activity +4 fold above basal level; concomitant E2 exposure reduced hCG-induced activity by ~25% (p<0.001). Effects of E2 on promoter activity were limited to hCG-induced conditions only. To determine whether the observed reduction in hCG-induced StAR promoter activity may be mediated by genomic estrogen receptor (ER) signaling, transfected cells were treated with a potent ER inhibitor (ICI); ICI reversed the observed decrease in hCG-stimulated activity. In situ analysis of the LMB StAR promoter revealed a putative ER-binding element (ERE/-1745); functionality of ERE/-1745 was verified by chromatin immunoprecipitation (ChIP). ChIP results confirmed that ERα and ERβ bound to ERE/-1745; ER β was enriched under hCG-induced conditions only and exposure to E2 diminished ERβ binding to the promoter. These studies are amongst the first to determine that ERs binds directly to the StAR promoter and that ER beta may play a non-classical role in hCG-induced activation of the LMB StAR promoter. NIEHS SBIR R01 ES015449.
To demonstrate a constitutive role for AHR in this regulation, qPCR and western blot analysis were used to analyze liver extracts from C3Alb/Arf/HepF1 mice, which are deficient in hepatic AHR, and AHR-silenced human hepatoma cells, Hep 3B. Both demonstrated enhanced constitutive expression of the genes of interest. Conversely, downregulation of AHR-heterodimer partner ARNT in Hep 3B cells showed no effect. Inhibition of the cholesterol synthesis pathway by statins, the most important class of lipid-lowering agents, leads to an increased expression of all its enzymes due to a compensatory mechanism. Following primary human hepatocyte treatment with lovastatin coupled with an AHR ligand, qPCR data indicated the ability of the receptor to inhibit the statin-mediated up-regulation of the genes of interest. These data firmly establish a role for AHR as regulator of cholesterol biosynthesis gene expression independent of its DRE-binding ability and suggests that AHR may be a previously unrecognized therapeutic target.

### 2179 RNA-SEQ REVEALS DIFFERENT ABundance OF TRANSPORTERS AND PRESENCE OF NOVEL ISOFORMS DURING LIVER DEVELOPMENT.

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During liver development, maturation of transporters is essential to maintain normal cellular uptake and elimination of chemicals in children. RNA-Seq provides a "true-quantification" of transcripts, and an unbiased detection of novel mRNA isoforms. The purpose of the present study was to compare the mRNA abundance of critical liver transporters using RNA-Seq and identify novel mRNA isoforms of these genes during development. Male C57BL/6 mice livers were collected from GD17.5 to 60 days of age. The transcriptome was determined using an Illumina HiSeq2000 with 200 cycles paired-end (n=3), and the mRNA abundance was estimated by Cufflinks. The total transcripts of both uptake and efflux transporters increased during development. A hierarchical clustering dendrogram showed three patterns of transporters in liver, namely perinatal-, adolescent-, and adult-enriched patterns. Before birth, the highest expressed transporter was Ent1 (30%), followed by Bcrp (26%). Right after birth (1-day of age), the bile acid uptake transporter Ntcp became the major transporter (52%). From adolescent to adult age, Ntcp retained the highest transporter in liver, followed by Oat1, which gradually increased during development. Three mRNA isoforms of Bcrp were identified that have alternate leading exons (Exa1, 1b, and 1c). RT-PCR showed that Elb is the major isoform throughout liver development. Exon 2 skipping was observed for Oatp1b2 mRNA, and intron retention (between exon 1 and 2) was observed for Bsep mRNA during liver development. In conclusion, the present study was the first to compare the real abundance of liver transporters during development, and identified novel mRNA isoforms of transporters using RNA-Seq. (Supported by NIH ES019487 and RR-021940)

### 2180 MULTIPlicITY OF 5′-END OF HUMAN HEPATIC MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 4 TRANSCRIPTS.


Multidrug resistance-associated protein 4 (MRP4, ABCG4) is an efflux transporter localized in the basolateral membrane of hepatocytes. Hepatic MRP4 expression is inducible by toxic acetonitrophen exposure. Our previous work showed that the transcription activity of human MRP4 promoter is constitutively active and that the activity of the promoter-proximal region is controlled by many activating (e.g., E2F1, NRF1, TATAFAP2A) and repressive (e.g., HES1, PATZ1, ELK1, ZFP161) transcription factors. However, knowledge on transcription initiation of the MRP4 gene is very limited. In this study, we characterized the 5′-end of the human hepatic MRP4 transcript. Total RNA from the human hepatic cell line HCO4 was processed for RNA sequence identification using rapid and identity of DNA 5′ ends. Nested PCR products were resolved on agarose gels and sequenced through cloning. The band obtained from the nested PCR product covered an area in the agarose gel of approximately 100 bp in width. The upper half of this 100 bp region contained a major band. After sequencing the upper and lower portions of the 100 bp region through cloning, the length of first exon for the different MRP4 transcripts was found to be in the range of 152 to 240 bp. Analysis of site distribution of the 5′-end of the MRP4 transcripts in single-nucleotide resolution with representative nested PCR products identified 13 sites in the range of -77 to -165 bp relative to the codon sequence start site among 27 valid sequencings out of 33 clones randomly sampled. The frequency of 152 bp and 187 bp was 7 and 3, respectively. These results indicate the presence of multiple 5′-ends of MRP4 transcripts. Interestingly, a novel exon was found between exon 1 and 2 (NM_005845). In silico translation analysis showed that the insertion of this novel exon results in a truncated 40-aa peptide at the N-terminal region of MRP4 (NP_005836). The mechanism underlying the multiplicity of 5′-ends for human MRP4 transcripts in hepatocytes remains to be further investigated. Supported by NIH Grant DK069557.

### 2181 EVOLVED METAL TOLERANCE IN DAPHNIA RESULTS FROM VARIATION IN GENOME ARCHITECTURE.

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Daphnia, or the water flea, is a sentinel species of freshwater ecosystems. Their populations are defined by the boundaries of ponds and lakes, are sensitive to modern toxicants in the environment, and thus are used to assess the ecological impact of environmental change. A hallmark of the genome sequence is a large number of duplicated genes that are most responsive to ecological challenges and are specific to the Daphnia lineage. Natural populations of Daphnia pulex living near smelters in the Sudbury region of Ontario have faced severe metal stress for over 100 years. We identified genotypes living in lakes from this region that have genetically adapted to cadmium stress. These isolates show no differences in their life history parameters when comparing control and cadmium exposed Daphnia. By contrast, cadmium exposures significantly decrease reproductive success in non-adapted Daphnia. Our studies also indicate that no fitness costs are imparted on these adapted Daphnia in the absence of metals, which differs from our observations of animals physiologically acclimated to cadmium through multiple generations. Adaptation produced different patterns of gene expression in metal exposed Daphnia. To explore the genomic basis for gene-expression differences, gene copy number was mapped across the entire genome to determine if it is due to multiple copies of the same gene adapted to cadmium and these were compared to the sequenced reference genome. A large amount of copy number variation (CNV) was observed between individuals, including CNV that comprised functionally relevant networks and pathways of genes contributing to the adapted phenotype (i.e., oxidative stress, stress response). These studies detail the genomic basis for adaptation in natural populations.

### 2182 PROTECTION OF HYDROQUINONE-INDUCED APOPTOSIS BY DOWNTREGULATION OF FAU IS MEDIATED BY NQO1.

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Using functional expression cloning, the Fau gene (Finkel-Briske-Reilly mouse sarcoma virus (PB-RMuSV)-associated ubiquitously expressed gene) was identified as a potential tumor suppressor gene. Downregulation of Fau by overexpression of its reverse sequence has been shown to inhibit apoptosis induced by DNA damaging agents. To address a potential role of Fau in benzene toxicity, we investigated the apoptotic effects of hydroquinone (HQ), a major benzene metabolite, in W7.2 mouse thymoma cells transfected with either a plasmid construct expressing the anti-sense sequence of Fau (rfau) or the empty vector (pcDNA3.1) as a control. HQ induced apoptosis via increase production of reactive oxygen species (ROS) and DNA damage as assessed using dihydroethidium (HE) staining and Alkaline Comet assay in W7.2 pcDNA3.1 cells. In contrast, when Fau was downregulated by the antisense sequence in W7.2 rfa cells, HQ treatment did not cause DNA damage and oxidative stress and these cells were markedly more resistant to HQ-induced apoptosis. Further investigation revealed that there was an upregulation of NAD(P)H: quinone oxidoreductase 1 (NQO1), a detoxification enzyme for benzene-derived quinones, in W7.2 rfa cells. Compromising cellular NQO1 by use of a specific mechanism based inhibitor (MAC 220) and NQO1 siRNA re-sensitized W7.2 rfa cells to HQ-induced apoptosis. These data suggest that downregulation of Fau results in NQO1 upregulation which protects against HQ-induced apoptosis in W7.2 cells.
The role of toxicology and toxicity testing in legislation and regulatory decision-making continues to change and an understanding of the opportunities, as well as challenges, that accompany the consideration of alternative test methods in a legislative framework is critical for forward progress in public health protection. It is well-established that toxicology in the twenty-first century has taken on many faces, ever-evolving complex hazards that pose health risks. These demands must be met to keep pace with expanding global economies, global threats to public health, and hazards advance at a blinding rate. There are new demands on toxicologists to ever-widen exposure control options, occupational exposure limit factors, and risk management options.

Glucagon-like peptide 1 receptor (GLP1R) is a G-protein coupled receptor of the glucagon/secretin/vasoactive intestinal peptide receptor subfamily. GLP1R has been targeted for treatment of type-2 diabetes using GLP1R agonists. However, GLP1R agonists have been linked to post-marketing reports of drug-induced acute pancreatitis. The risk for both type-2 diabetes and pancreatitis is elevated by the consumption of a high-fat, high-caloric diet. In vitro studies were undertaken to investigate the effects of glucose, fatty acids, and GLP1R modulation on GLP1R expression and function in the major pancreatic cell types. Oleic acid at 0.4mM was added into the culture medium of rat pancreatic acinar cells (AR42J), ductal cells (DSL-6A/C1) and islet cells (RIN-m5F). After three days of incubation, each cell type was exposed to glucose at 30mM for 1h. After glucose exposure, other cell types were unaffected while oleic acid-treated ductal cells demonstrated a decrease in GLP1R expression as measured by western blot. This decrease was exacerbated by co-incubation with exenatide, a GLP1R agonist. The decrease of GLP1R in fat-treated ductal cells was time and oleic acid/glucose-concentration dependent. After glucose exposure, exenatide-induced increases in cAMP were reduced depending on the concentration of the glucose. In addition, the inhibition of proteinase activity and lysosome formation by their respective inhibitors rescued GLP1R expression, suggesting that the decreases in GLP1R expression involved protein degradation in the lysosome. The co-localization of GLP1R with endosomes/lysosomes confirmed the internalization, translocation and degradation of GLP1R. The results suggest that ductal cell regulation might be altered in people with diabetes and elevated triglycerides and that this change may be potentiated by GLP1R agonists.

Hydroxylated polychlorinated biphenyls (OH-PCBs), major metabolites of PCBs, have been reported to act as estrogen receptor α (ERα) agonists or antagonists. However, little concern has been paid to the ability of OH-PCBs to interfere with other steroid hormone receptors such as ERβ, androgen receptor (AR) or glucocorticoid receptor (GR). In this study, we characterized the agonistic and antagonistic activities of available 100 OH-PCBs (39 ortho-, 24 meta-, and 37 para-OH compounds), including some congeners identified in humans, against human ERα, AR, and GR using in vitro reporter gene assays. In the ERα assay, 45 and 9 of the 100 OH-PCBs tested showed agonistic and antagonistic activities, respectively. In the ERβ assay, 45 and 15 compounds showed agonistic and antagonistic activities, respectively. In the AR and GR assays, although none of the compounds tested showed agonistic activity, 83 and 31 of the 100 OH-PCBs showed antagonistic activity, respectively. These AR and/or GR antagonistic compounds had various patterns of substitution in the structure, while relatively potent ERα agonists and
antagonistic compounds possessed para- and ortho-OH structures, respectively. Three OH-PCBs, predominantly identified in human tissues, showed little ERα/β or AR activities, apart from the weak ERs and/or GR antagonistic activity observed in 4-OH-CB107 and 4-OH-CB187. Taken together, these results suggest that a large number of OH-PCBs might act as agonists and/or antagonists against ERα/β, AR and GR, and that para-substituted OH-PCBs with no chlorine substitution are not able to bind to the GH group appear to possess more potent ERα/β agonistic and AR/GR antagonistic activities.

2188 MISFOLDING OF MUTATED VASOPRESSIN causes ER-RETENTION AND DECREASE OF VASOPRESSIN SECRETION.

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Arginine-vasopressin (AVP) is a peptide hormone normally secreted from neuroendocrine cells via the regulated secretory pathway. In Familial Neurohypophyseal Diabetes Insipidus (FNDI), an autosomal dominant form of central diabetes insipidus, mutations of vasopressin appear to increase in the endoplasmic reticulum (ER) causing a lack of biologically active AVP in the blood. To investigate the effect of pro-vasopressin mutations regarding intracellular functions of protein targeting and secretion, we created two FNDI-associated amino acid substitution mutants, e.g., G14R, and G17V in frame with green fluorescent protein (GFP) and control pro-vasopressin (VP) in frame with GFP (VP-GFP) and/or with red fluorescent protein (VP-RFP). Fluorescence microscopy of Neuro-2a cells revealed colocalization of VP-GFP and VP-RFP to punctate granules along the length and accumulating at the tips of neurites, characteristic of regulated secretory granules. In contrast, the two FNDI-associated amino acid substitution mutants, e.g., G14R-GFP and G17V-GFP showed very little to no colocalization to a perinuclear region of the Neuro-2a cells characteristic of the endoplasmic reticulum. Co-expression of these mutants with VP-RFP showed VP-RFP was retained in the ER suggesting the formation of heterodimers as found in FNDI. Stimulated secretion experiments indicated that VP-GFP was secreted in an indiscernible manner whereas, G14R-GFP and G17V-GFP were retained to nearly 100% within the cells. Analysis using GFP pull-down followed by western blotting indicated an increased protein expression for an ER resident molecular chaperone, BiP. In conclusion, G14R-GFP and G17V-GFP mutants are retained in the ER of Neuro-2a cell through up-regulation and directly associated with the molecular chaperone BiP resulting in significantly decreased AVP secretion.

2189 THYROXINE (T4) CATABOLISM IN HUMAN AND RAT HEPATOCYTES INCREASES FOLLOWING EXPOSURE TO HEPATIC ENZYME INDUCERS.

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Nuclear receptor agonists phenobarbital (PB), 3-methylcholanthrene (3MC), and 2,2',4,4'-tetrabromodiphenyl ether (BDE 47) decrease serum thyroxine (T4) in vivo. CAR agonists phenobarbital (PB), 3-methylcholanthrene (3MC), and 2,2',4,4'-tetrabromodiphenyl ether (BDE 47) decrease serum thyroxine (T4) in vivo. CAR agonists phenobarbital (PB), 3-methylcholanthrene (3MC), and 2,2',4,4'-tetrabromodiphenyl ether (BDE 47) decrease serum thyroxine (T4) in vivo. CAR agonists phenobarbital (PB), 3-methylcholanthrene (3MC), and 2,2',4,4'-tetrabromodiphenyl ether (BDE 47) decrease serum thyroxine (T4) in vivo.

Testing and evaluation of endocrine disrupting properties of chemicals becomes a regulatory need under the REACH regulation and the EU regulations on plant protection products and on biocidal products. The OECD test guidelines (TGs) form a basis for regulating endocrine disrupting chemicals (EDCs). How to use these TGs for risk assessment and testing strategy of EDCs, however, is an unsolved issue. This paper evaluates the fish short term reproduction assay (FSTR) and the Daphnia 21d reproduction test by compiling 137 studies (35 chemicals) for fish and 123 studies (77 chemicals) for Daphnia. Chemicals included are both EDCs and non-EDCs, with various modes of action (MOAs). Conclusions from this analysis include: (i) a first step is to consider the most sensitive endpoint for both fish and Daphnia, whereas sex determination shown by secondary sex characteristics (SSC) in fish and sex ratio in Daphnia is a less sensitive endpoint. (ii) Environmental Toxicology - Wageningen, Netherlands and 2RIKILT—Institute of Food Safety, Wageningen, Netherlands. Sponsor: Z. Dang.

Introduction: With a view to REACH and the need of reducing, refining and replacing the use of experimental animals for safety testing, this project aims to develop an alternative in vitro test battery for estrogenicity, which can replace current in vivo assays and has an enhanced predictive capacity. These in vitro models will be selected from presently available and newly developed in vitro cell-based assays for optimal representation of the different modes of action that lead to estrogenic activity. Methods: A set of twelve compounds was selected representing different modes of estrogen action. Subsequently these compounds were tested in: (i) four cell proliferation assays based on different estrogen-sensitive tissues, i.e. breast (MCF-7 and T47D), endometrium (ECC-1) and ovary (BO-1); (ii) three reporter gene assays based on both mammalian (U2OS and T47D) and yeast cells; (iii) a xenobiotic-dedicated DNA microarray test, measuring 13 different estrogen-responsive genes in MCF-7 cells exposed to the test compound. Results: (i) The seven proliferation of all four cell lines showed a good correlation with the uterotrophic assay based on the 12 compounds tested in the MCF-7 cell line (R2=0.85). (ii) Reporter gene induction also showed good correlations with the uterotrophic assay, R2=0.89 for both the U2OS and T47D based ER-CALUX bioassays. (iii) The 7 best marker genes on the DNA microarray produced fingerprints correctly predicting the (anti)estrogenic activities of the 12 compounds tested. Conclusions: Our study showed that the currently selected in vitro bioassays showed very good correlations with the uterotrophic assay based on the 12 compounds tested. However, there are still compounds of which the estrogenic properties cannot be correctly predicted by the current panel. Other in vitro assays are needed to fill the gaps, e.g. the H295R assay in order to detect effects of compounds that affect steroidogenesis and are not detectable by testing direct interaction with the estrogen receptor.

2190 EVALUATION OF FISH AND DAPHNIA REPRODUCTION TEST FOR DETECTING ENDOCRINE DISRUPTORS: REGULATORY IMPLICATIONS.

Z. Dang. RIVM, Bilthoven, Netherlands.

POTENTIAL OF EXTRACTS OBTAINED FROM EASTMAN TRITAN™ COPOLESTERS.

M. Eldridge, F. Menn and G. Sayler. The Center for Environmental Biotechnology, University of Tennessee, Knoxville, TN. Sponsor: M. Eldridge.

Eastman’s Tritan™ copolymers are a family of plastics from Eastman Chemical Company manufactured utilizing three monomers: dimethylterephthalate, 1,4-cyclohexanediol and 2,2,4,4-tetramethyl-1,3-cyclobutandiol. Tritan™ polymers are used for the manufacture of various types of reusable food and beverage containers.
age containers, kitchen appliances, as well as medical devices. In light of recent pub-
lic attention over the presence of endocrine active compounds that may be able to
leach out of some plastics, studies were conducted to understand whether extracts
from Tritan™ copolymers possess either estrogenic (EA) or androgenic activity
(AA). Studies were conducted on extracts obtained from finished pellets and
molded bars of Tritan copolymers. The extraction procedures utilized were based
on FDA and European guidelines for safety assessment of plastics used for food
contact materials and exaggerate typical end-use conditions. Specifically, conditions
utilized to obtain extracts consisted of incubating the various products in water and
ethanol/water (10/90 and 50/50) solutions for 240 hrs at 40°C, and in a 50/50 so-
lution for 2 hrs at 70°C. To simulate harsh dishwasher conditions samples were in-
cubated in Cascade™ (10 g/L) for 240 hrs at 70°C followed by immersion for 240
hours at 40°C in the same three extracting solutions listed above. Extracts ob-
tained from these solutions were concentrated 500X using Hydrophilic-Lipophilic-
Balance SPE (solid phase extraction) disks as outlined in EPA Method 1694 to en-
dance the detection of chemicals that may have activity. Assessment of EA and AA
activity was determined using a biosynthetic yeast-based detection method as out-
lined by Sanseverino et al. (2009). Results showed no evidence of either EA or
AA for any Tritan™ product under any of the extraction conditions. Accordingly,
and in conjunction with data indicating the monomers do not possess estrogenic
or androgenic activity, these results further reaffirm the safety of Tritan™ polystyrenes.

However, when islets were incubated in high glucose buffer there was a 10 fold de-
crease in insulin release compared to 1000 nM Cd. Similar decreases in glu-
cose-stimulated insulin release were observed in islets incubated in buffer contain-
ing 100 and 10 nM Cd. To further determine the effects of Cd on islet cell biology
and function, male Sprague Dawley rats were injected subcutaneously with either
saline (control) or Cd (0.6 mg Cd/kg/day, 5 days per week). After 6, 9 and 12
weeks of Cd treatment, pancreatic tissue samples were removed then fixed in for-
malin. Later, pancreata were sectioned and immuno-stained for beta catenin. Co-
immuno-labeling of insulin was performed to ensure that the changes in beta
catenin labeling occurred in islets and not the more ubiquitous acinar cells.
Interestingly, all islets from animals exposed to Cd, at all time points examined,
showed increased staining for beta catenin. The current study shows that the envi-
ronmental contaminant, Cd, disrupts glucose-stimulated insulin release at very low
levels and is associated with increased beta-catenin labeling, a protein associated
with cell-cell adhesion.

A wide variety of environmental chemicals alter the function of the thyroid system
in many animal species. Thyroperoxidase (TPO), the enzyme that synthesizes thy-
roid hormone, is one of the known biochemical targets for thyroid disrupting
chemicals (TDC). The majority of the in vivo toxicological research on TDCs is
conducted in rats whereas most TPO research has been conducted using porcine
thyroid microsomes. The sensitivity of porcine and rodent microsomes for TPO in-
hibition using known TDCs and chemicals of unknown activity was compared to
establish the species concordance. Microsomes were isolated from rat and pig thy-
roid glands and the TPO assay was performed based on the guaiacol oxidation
method. Concentration response curves were derived for the following chemicals:
mehtimazole (MMZ); dibutyrylphosphate; diethylhexylphosphate; diethylphosphate;
Triclosan; p-nonylphenol; 6-proplyphiouracil (PTU); sodium perchlorate (PERC);
iodoacetic acid, 4-propoxyphenol (4POP); 5,5-dimethylpyrazole-1-methanol (DPM)
and 2-mercaptoethanol (MBT). Of these tested chemicals, MMZ, MTU, PTU, DPM,
and MBT inhibited TPO activity. Results demonstrate a qualitative concordance of
response between the two species. All chemicals that inhibited TPO in porcine microsomes also inhibited TPO in rat microsomes. The derived IC50 values revealed slight differences in relative potency between species.
MBT, PTU, and DPM exhibited greater relative potency in rats than pigs, but rank
order persisted for inhibition of TPO with PTU<MBT<PERC<4POP. These results
support the extrapolation of porcine TPO data on environmental chemicals to po-
tential activity in vivo rodent studies. This abstract does not nec-
essarily reflect the policy of the US EPA.

There is increasing interest in how environmental contaminants can contribute to
the onset of diabetes. Multiple epidemiological studies show that exposure to the
metal cadmium (Cd), is associated with diabetes and reduced serum insulin. One
goal of this study was to examine the acute effects of Cd on insulin release. Islets
were isolated from rats then allowed to recover until the next day when the experi-
ments were conducted. Individual islets were incubated in physiological buffer for
4 hours containing either low (0.5 mg/ml) or high (3.0 mg/ml) glucose with or
without Cd (1000, 100 and 10 nM) present. In the low glucose buffer, Cd caused a
nearly two fold increase in insulin release at all concentrations of Cd examined.

The sudden onset of menopausal symptoms is a common side effect of breast can-
cer treatment with e.g. the selective estrogen receptor (ER) modulator tamoxifen
(TAM) or aromatase inhibitor letrozole (LET). To alleviate these symptoms, women
often use alternative medicines that can contain the phytoestrogens genistein
(GEN) or 8-prenylnaringenin (8PN). In this study, the potential interaction
with TAM or LET was determined for GEN, 8PN and four commercially available
menopausal supplements. For that, human adrenocorticotropin H295R cells
were co-cultured with MCF-7 cells. In this co-culture, interaction with theestro-
gen-producing enzyme aromatase (CYP19, in H295R), ER (in MCF-7) and subse-
dent effect on cell proliferation were studied and relative proliferative potencies
(RPP) were calculated. In addition, a reporter-gene assay was used to determine ER

2195 A COMPARATIVE QUALITATIVE ANALYSIS OF PORCINE AND RODENT THYROPEROXIDASE—EFFECTS OF ENVIRONMENTAL CHEMICALS.
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2196 MENOPAUSAL SUPPLEMENTS, GENISTEIN AND 8PN INDUCE ESTROGEN-DEPENDENT TUMOR CELL GROWTH AND COUNTERACT THE EFFICACY OF TAMOXIFEN AND LETROZOLE IN IN VITRO BREAST CANCER MODEL.
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activation and calculate relative estrogenic potencies (REPs), GEN, 8PN and all supplements concentration-dependently increased cell proliferation and activated the ER. The RPP of GEN and two supplements was 5- to 185-fold higher than the REP. This suggests that these compounds affect cell growth both through interaction with CYP19 and the ER. This was corroborated by the induction of CYP19 activity, CYP19 expression and estrogen production in the co-culture model. All tested compounds reduced cell growth inhibition by LET. Conversely, the growth inhibition by 4-OH-TAM could only be negated at concentrations higher than 3 nM (E2), 1.6 μM (GEN) and 0.2 μM (8PN), but not by the supplements. The effect concentrations of GEN and 8PN were within the range of plasma levels that can be found upon consumption of GEN or 8PN supplements. These data indicate that using phytoestrogen-containing menopausal supplements can potentially reduce the efficacy of breast cancer treatment.

2197 BIOMARKERS OF PERCHLORATE EXPOSURE ARE CORRELATED WITH CIRCULATING THYROID HORMONE LEVELS IN THE 2007–2008 NHANES.

The relationships between biomarkers of perchlorate exposure, other covariates, and levels of circulating thyroid hormones in subjects from the 2007–2008 NHANES were investigated using generalized additive mixed models (GAMMs). Consistent, statistically significant relationships were found between biomarkers of exposure to perchlorate, and serum thyroid hormone levels in male and non-pregnant female subjects age 12 years and older. These relationships were detected in models which also included age, ethnicity, income, and behavioral covariates (smoking, prescription medication consumption). The models also adjusted for the presence of biomarkers of phthalate ester exposures, which have previously been shown to influence thyroid hormone measurements in these same subjects. (Meeker and Ferguson, 2011) Urinary biomarker measurements were highly correlated with one another, and significant relationships between perchlorate and T3/T4 levels could only be detected when urinary biomarker values were individually adjusted for creatinine secretion. Perchlorate regression coefficients were significant in one or more regressions. However, perchlorate was not shown to influence thyroid hormone measurements in these same subjects. The relationships between urinary biomarker measurements, other covariates, and levels of circulating thyroid hormones were highly correlated with one another, and significant relationships between perchlorate and T3/T4 levels could only be detected when urinary biomarker values were individually adjusted for creatinine secretion. Perchlorate regression coefficients were significant in one or more regressions. However, perchlorate was not shown to influence thyroid hormone measurements in these same subjects.

2198 NO EVIDENCE OF ENDOCRINE DISRUPTION BY GLYPHOSATE IN HERSHEYBERGER AND UTEROTROPIC ASSAYS.
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The Food Quality Protection Act and Safe Drinking Water Act amendments (1996) required the USEPA to develop the Endocrine Disruptor Screening Program (EDSP), which currently consists of 11 Tier 1 screening assays to evaluate potential for a chemical to interact with the endocrine system. The first list of 67 compounds subject to the EDSP, which included glyphosate, were selected for screening based on their potential for exposure rather than suspected interference with the endocrine system. The potential for glyphosate (G) to induce endocrine disruption has now been evaluated in the Hersherberger and Uterotrophic assays, the two most well validated in vivo EDSP Tier 1 assays. The Hersherberger assay screens for androgen agonists, androgen antagonists and 5α-reductase inhibitors. Nine groups of 6 orchidoepididymectomized rats were dosed for 10 days: vehicle control, testosterone propionate (TP) 20 mg/kg/d, flutamide/TP, G 100, 300 and 1000 mg/kg/d, GT-TP 100, 300 and 1000 mg/kg/d. TP treatment induced an expected increase in all five androgen sensitive organ (ASO) weights (bulbourethral gland, glans penis, ventral prostate, LABC muscle group, seminal vesicles) while co-treatment with the anti-androgen flutamide induced the expected decrease in ASO weights compared to the TP-only treatment. No evidence of androgenicity, anti-androgenicity or 5α-reductase inhibition was noted in any G treated group. All ASO weights were comparable to the respective control following 10 days of treatment. The Uterotrophic assay evaluates estrogenicity. Five groups of 6 ovariectomized rats were dosed for 3 days: vehicle control, 17β-Ethynylestradiol (E) positive control at 0.003 mg/kg/d, G 100, 300 and 1000 mg/kg/d. Absolute uterine wet and blotted weights in the positive control group were respectively 356% and 859% of the vehicle control. Absolute wet and blotted uterine weights in the glyphosate treated groups were comparable to the vehicle control. Based on these results, glyphosate does not exhibit endocrine disruption in the Hersherberger and Uterotrophic assays.

2199 WEIGHT-OF-EVIDENCE EVALUATION OF ENDOCRINE DISRUPTION POTENTIAL FOR CHLORPYRIFOS.

Chlorpyrifos, a non-systemic insecticide used on a variety of crops, has one of the most extensive toxicological databases for any regulated chemical. Based on EPA exposure-based prioritization, chlorpyrifos was included on the 1st list of chemicals to be screened under the Endocrine Disruptor Screening Program (EDSP) which consists of 11 in vitro and in vivo screening level assays. Following completion of EDSP Tier 1, a weight of evidence (WOE) evaluation was conducted to determine if chlorpyrifos has the potential to interact with estrogen, androgen or thyroid (EAT) endocrine systems. All relevant lines of evidence, including the EDSP Tier 1 data-set, peer-reviewed literature and GLP registration studies, were systematically evaluated for quality, consistency, specificity, and reproducibility. Next, hypotheses were generated and lines of evidence were constructed into a WOE framework based on the 5-level OECD endocrine conceptual framework. Single end-point in vivo studies (e.g. Hersherberger) received greater weight than in vitro assays, and those receiving the greatest weight were apical in vivo studies (e.g. level 5 multi-generation reproduction). To explain observations in the in vivo EDSP screens that could be considered ‘false positive’ responses, alternative hypotheses involving a well-studied chlorpyrifos mode of action, cholinesterase inhibition, were explored. For example, in the amphibian metamorphosis assay, delayed developmental stage and reduced growth were explained by and correlated with cholinesterase inhibition in the absence of effects on thyroid histopathology. In level 5 studies in mammals, adverse effects have not been observed on key endpoints including male or female fertility, litter size or viability, or number of embryos implanted in the uterus. Results of this evaluation will be presented and conclusions drawn on whether chlorpyrifos has the potential to interact with EAT hormone systems. It is important to conduct a WOE evaluation across data levels as this provides critical insight on the utility of the EDSP.
with the analytical chemistry data (GC/MS/MS analysis). Analytical chemistry results and EEQ results were consistent with vitellogenin induction in male fish. Disclaimer: This abstract does not necessarily reflect US EPA policy.

**2201 ATP-BINDING CASSETTE (ABC) TRANSPORTERS PRESENT IN THE BLOOD-TESTIS BARRIER ARE POTENTIAL DETERMINANTS IN ENDOCRINE DISRUPTION.**

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Endocrine-disrupting chemicals (EDCs) are exogenous agents that interfere with steroid hormone function. In males, the testis is highly susceptible for EDC-induced toxicity. Efflux transporters expressed in the blood-testis barrier might influence the exposure to chemicals. Among these are the ABC transporters, P-glycoprotein (P-gp/ABC1), breast cancer resistance protein (BCRP/ABC2G) and multidrug resistance protein 1 (MRP1/ABC1). Here, we hypothesized that these efflux transporters are key determinants in EDC-induced testis toxicity. Selected EDCs included bisphenol A (BPA), bis(2-ethylhexyl) phthalate (DEHP), mono-(2-ethylhexyl) phthalate (MEHP), perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS). Their interaction with human P-gp, BCRP and MRP1 transport activity was tested in membrane vesicles of HEK293 cells, overexpressing the transporters. Next, ATP-dependent transport of radiolabeled substrates was measured. Our data show that BPA, PFOA and PFOS inhibit P-gp, BCRP and/or MRP1 activity with 81.4 ± 2.2%, 4.2 ± 3.2% and 31.0 ± 2.1% respectively. The inhibitory effects of PFOA (100 µM) were most pronounced, with 60.6 ± 4.5%, 73.3 ± 2.6% and 61.7 ± 2.2% reduction in the activity of P-gp, BCRP and MRP1, respectively. This is in line with the analytical chemistry data (GC/MS/MS analysis). Analytical chemistry methods are used to identify the effect of different intracellular ERTs/ERβ ratios on estrogen induced cellular responses. The aim of the present study was to compare ERTα/ERβ expression levels in rat and human breast and other estrogen sensitive tissues in vivo with levels found in the T47D-ERβ cell line exposed to variable tetracycline concentrations, and to determine at which tetracycline levels the T47D-ERβ model mimics the ERTα/ERβ ratio in these tissues. Both RNA (qPCR) and protein (Western blot) levels of ERTα and ERβ were analyzed in T47D-ERβ cells exposed to a range of tetracycline concentrations and compared to the levels found in breast, prostate, and uterus from Sprague Dawley rat and human origin. The ERTα/ERβ ratio in T47D-ERβ cells exposed to 50 mg/ml tetracycline and higher is comparable to the ratio in rat mammary gland. The ERTα/ERβ ratio in human breast tissue is mimicked in T47D-ERβ cells exposed to 35 mg/ml tetracycline or more. The ERTα/ERβ ratio of other estrogen sensitive rat and human tissues can also be mimicked in the T47D-ERβ cells, although the use of the T47D breast cancer cells with tetracycline dependent ERTα/ERβ expression as a model for estrogen sensitive tissue other than breast may be hampered by differences in the role of coactivators and corepressors and possible differential ERTα and ERβ expression between different cell types in these tissues. The results of the present study gives insight in experimental conditions under which the ERTα/ERβ ratio of the T47D-ERβ cell model is comparable to breast and other estrogen sensitive tissues in vivo which is an important step in the validation of the model to mimic especially rat and human breast tissue.

**2202 HUMAN T47D BREAST CANCER CELLS WITH TETRACYCLINE-DEPENDENT ERα/ERβ EXPRESSION AS AN IN VITRO MODEL FOR ERTα/ERβ RATIO DEPENDENT RESPONSES IN BREAST TISSUE.**

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The T47D-ERβ breast cancer cell line with tetracycline dependent ERβ expression and constant ERTα expression is used to investigate the effect of different intracellular ERTs/ERβ ratios on estrogen induced cellular responses. The aim of the present study was to compare ERTα/ERβ expression levels in rat and human breast and other estrogen sensitive tissues in vivo with levels found in the T47D-ERβ cell line exposed to variable tetracycline concentrations, and to determine at which tetracycline levels the T47D-ERβ model mimics the ERTα/ERβ ratio in these tissues. Both RNA (qPCR) and protein (Western blot) levels of ERTα and ERβ were analyzed in T47D-ERβ cells exposed to a range of tetracycline concentrations and compared to the levels found in breast, prostate, and uterus from Sprague Dawley rat and human origin. The ERTα/ERβ ratio in T47D-ERβ cells exposed to 50 mg/ml tetracycline and higher is comparable to the ratio in rat mammary gland. The ERTα/ERβ ratio in human breast tissue is mimicked in T47D-ERβ cells exposed to 35 mg/ml tetracycline or more. The ERTα/ERβ ratio of other estrogen sensitive rat and human tissues can also be mimicked in the T47D-ERβ cells, although the use of the T47D breast cancer cells with tetracycline dependent ERTα/ERβ expression as a model for estrogen sensitive tissue other than breast may be hampered by differences in the role of coactivators and corepressors and possible differential ERTα and ERβ expression between different cell types in these tissues. The results of the present study gives insight in experimental conditions under which the ERTα/ERβ ratio of the T47D-ERβ cell model is comparable to breast and other estrogen sensitive tissues in vivo which is an important step in the validation of the model to mimic especially rat and human breast tissue.

**2203 ANALYSIS OF HORMONE LEVELS IN H295R CELL CULTURE SUPERNATANTS USING LC-MS/MS ANALYSIS IN AGREEMENT WITH CURRENT OECD AND OPPTS TESTGUIDELINES.**

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Endocrine disruption (ED) is a topic under current scientific and political debate. Therefore, much effort has been done to establish in vitro methodologies that could give reliable insights into the ED mechanisms of compounds without the use of animals, including those proposed in the US Endocrine Disruptor Screening Program (EDSP). Amongst those methods, the steroidogenesis assay aims to identify compounds that interfere with the steroid hormone synthesis. It uses the human adrenocarcinoma cell line H295R, which harbors the genes encoding key enzymes for steroidogenesis. The read-out of the assay is the change in estradiol (E2) and testosterone (T) levels in the cell culture supernatants. In contrast to; most commonly used RIAs or ELISA, analytical methods such as LC-MS/MS can determine several hormones in parallel and largely avoid cross-reactivity of the test substance. Herein, we report our cross-validation analysis of ELISA and LC-MS/MS data obtained from measurement of E2 and T produced in H295R cells exposed to substances with known effects on hormone synthesis. The linear regression factor exhibited a great correlation of Rs 0.9. In summary, the LC-MS/MS measurement is an efficient alternative to the ELISA analysis for the measurement of steroid hormones produced by H295R cell system not only because it reduces costs, time and avoids cross-reactivity artefacts, but also it enables us to enhance the read-out of the steroidogenesis assay, since in parallel to E2 and T, we can determine further steroid hormones, which can be of importance to identify specific targets of test substances and hence may provide useful information for elucidation of putative ED mode of action.

**2204 EFFECT OF ENDOCRINE DISRUPTORS ON METABOLITE PROFILES.**

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BASF SE and metanomics GmbH launched a project measuring metabolite profiles in rat serum to predict toxicological risks with one repeated dose screening study. For that purpose, a comprehensive database (MetaMap® OTox) has been established with about 500 pharmaceutical, chemical and agrochemical compounds. About 300 metabolites were measured in plasma samples of dosed Wistar rats after administration for 7, 14 and 28 days. Sets of common metabolite level changes (metabolite pattern) were arranged to characterize several toxicological modes of action. Endocrine disruption is a main toxicological issue in the development of chemicals and agrochemicals. Reliable detection of endocrine disruptors at an early development phase is important. Therefore, we established a metabolite pattern in male rats after administration of compounds decreasing endogenous androgen synthesis (i.e., Trenbolone, 17a-Methyltestosterone, 17a-Ethinylestradiol and Tamoxifen) and opposed it to that of androgen synthesis increasing compounds (i.e., Cyporsteron acetate, Flutamide, Aromatase inhibition). Metabolite level changes apart from hormones can be explained mechanistically as consequence of disruption of the steroid synthesis homeostasis.

**2205 EVALUATION OF POTENTIAL ENDOCRINE ACTIVITY OF ISOPHORONE IN THE TIER 1 IN VIVO MAMMALIAN ASSAYS.**


Isophorone, a solvent and crop protection inert, was included on the US EPA 1st priority list for the Endocrine Disruptor Screening Program (EDSP). EDSP Tier 1 includes 4 in vivo mammalian assays (uterotrophic, Hershberger, male and female pubertal assays) to examine a chemical’s potential to interact with estrogen, androgen or thyroid signaling. Isophorone high dose levels were selected to avoid significant acute irritation (subcutaneous (sc) dosing at 75 mg/kg/day (mkd)) or to avoid excessive systemic toxicity (oral dosing at 1000 mkd). For the uterotrophic assay, ovx-ovariomized CD rats (6/dose) were treated with 0-50 mkd isophorone sc for 3 days. Body weight gains were decreased at 50 mkd, but neither wet nor bloated uterine weights were increased. For the Hershberger assay, castrated male CD rats
Bisphenol-A (BPA) is a well known endocrine disruptor that is able to mimic the effects of physiological estrogens via membrane bound estrogen receptors (mERs, mERβ, GPER/GPR30) thereby initiating non-genomic signaling (e.g. mitogen activated protein kinases, Ca2+ release) and functional responses (prolactin release). More stringent government regulations on the amount of BPA permitted in the food, and lead to the development of alternative and more heat stable bisphenol compounds as substitutes; one such alternative is bisphenol-s (BPS). This study aimed to characterize the effects of BPS on kinase signaling pathways, alone and together with a physiological estrogen, estradiol (E2). Using the G3H/B6/F10 rat pituitary cell line we quantified phosphorylations of E2-induced extracellular signal-regulated kinases (ERKS) and c-Jun-N-terminal kinase (JNKs) by a quantitative high-throughput plate immuno-assay. BPS as a lone compound was able to elicit phospho-activation of ERK in a nonmonotonic dose-mimic (mM) and time-dependent (2.5-60min) manner. When combined with E2 (1mM) the physiological estrogen’s response was attenuated. In both instances BPS (alone and in a mixture) featured non-monotonic dose responses. BPS was unable to activate JNK. In contrast, the combination of E2 and BPS generated a non-mono tonic dose-response. The ability of BPS to activate the ERK signaling pathway and deactivate the JNK pathway suggests that BPS may influence and promote cell proliferation. Dose-dependent studies revealed that BPS caused cell proliferation with similar proliferative effects as E2 while the combination of the two chemicals resulted in a decline in cell numbers implicating an active apoptotic response. Higher levels of caspase 8 vs. 9 activities over a 24hr exposure period demonstrated that BPS is more likely to initiate cell death via the extrinsic pathway. These results indicate that BPS, a novel compound proposed as a substitute for BPA, possesses the ability to disrupt membrane initiated E2 cell signaling leading to changes in cell number.

Correct placental-fetal communication is essential for healthy pregnancy and sex steroid hormones play an important role in maintenance of pregnancy and fetal development. Concerns exist about effects of contaminants and medications on fetal development. Concerns exist about effects of contaminants and medications on fetal development. Evaluation of the reproductive system indicated delayed sexual maturation for both males and females indicated delayed sexual maturation. Decreases in sperm count and mobility and uterus and/or vaginal atrophy were noted. In conclusion, the findings indicate that letrozole has the same effect in both males and females resulting in a delay in bone maturation and prevention of pubertal progression. These results provide unique insight into the effects of sex steroid hormones on bone growth and development in male and female rats.
Conazole fungicides are widely used antifungal chemicals to which especially workers in agricultural professions are exposed. Several effects (e.g. anti-androgenic, disturbance of sex steroid biosynthesis, developmental, embryo- and hepatotoxicity) have been suggested. At present, no appropriate in vitro alternative assay is available to screen for effects on key targets in male toxicity. In this study, we evaluated effects of three conazole fungicides, flusilazole (FLU), ketoconazole (KET), and tebuconazole (TCBZ) on sex steroidogenesis and androgen receptor activation using the human adrenocorticotrophic carcinoma cell line (H295R) and T47D-ARE cell line (ARE), a breast cancer cell line transfected with an androgen responsive element with a firefly luciferase reporter gene. Enzyme activity of cytochrome P450 17 (CYP17) and 19 (CYP19, aromatase) were measured using a commercially available kit for DHEA and the triitated water release assay, respectively. Androgen response was measured via luciferase assay. FLU and KET showed a concentration-dependent inhibition of DHEA production in H295R cells (IC50 = 48.4 and 1.2 μM, respectively), whereas TCBZ did not. On the other hand, KET and TCBZ increased CYP19 activity in a concentration-dependent manner (EC50 = 0.6 and 1.1 μM, respectively). FLU increased CYP19 activity at concentrations up to 0.3 μM but inhibited CYP19 activity at higher concentrations. Androgen receptor (AR) activation was inhibited by FLU and KET in a concentration-dependent manner as compared to the testosterone control with an IC50 of 5.6 and 5.4 μM, respectively. TCBZ did not affect AR activation. Our results show that conazole fungicides might cause male toxic effects through inhibition of androgen synthesis and acting as AR antagonist, two key steps in male fertility and spermatogenesis.

ESTROGENIC EFFECT OF CADMIUM ON MALE HEALTH: THE ROLE OF ESTROGENIC AND ANTIOESTROGENIC GLUCOCORTICOSTEROIDS.

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Homosalate, 2-ethylhexyl-p-dimethyl aminohenozoate (padimate O), and ensulizole are common ingredients found in commercial sunscreens. Due to their widespread exposures, these chemicals are under investigation for their impact on human health. Oral dose formulations of homosalate, padimate O, and ensulizole in a common vehicle of corn oil were developed for use in animal toxicity studies. GC methods were developed and validated for analysis of homosalate and padimate O formulations with concentrations ranging 2.4-240 mg/mL for each analyte. Formulations of ensulizole were analyzed by LC over the validated range 2.5-100 mg/mL. Validation parameters included linearity, accuracy, precision, stability, and detection and quantitation limits. Formulations of each chemical with concentrations up to ~33 μg/mL were diluted into the validated ranges and analyzed with acceptable precision and accuracy. Ensalizole formulations were homogeneous suspensions at 33 and 333 mg/mL. Formulations of all three test chemicals were found to be stable under appropriate storage conditions (ambient and refrigerated for ~42 days) as well as under simulated dosing conditions. The three chemicals were all evaluated in DMSO for use in a complementary in vitro toxicity assay. The DMSO solutions were analyzed using a common LC method which was validated over the approximate range 0.2-15 mM for each analyte. Solutions of homosalate and padimate O with concentrations up to 30 mM
were diluted into the validated ranges and analyzed with acceptable precision and accuracy. For enusilzone, solutions of concentrations up to 20 mM were diluted successively into the range, but enusilzone is insoluble above this level. The methods were evaluated using the same criteria as for the corn oil formulation analyses. DMSO solutions of the three chemicals were found to be stable under appropriate storage conditions (ambient and refrigerated for -42 days) as well as under simulated dosing conditions.

2215 DEFICIENCY IN THE NUCLEAR FACTOR-ERYTHROID 2-RELATED FACTOR 2 REDESIGNS PANCREATIC BETA-CELLS VULNERABLE TO ARSENIC-INDUCED CELL DAMAGE.


Chronic human exposure to inorganic arsenic (iAs), a potent environmental oxidant stressor, is associated with increased incidence of diabetes, where impairment of pancreatic beta-cell function is a key pathogenic factor. Nuclear factor-erythroid-2-related factor 2 (Nfr2) is a central transcription factor regulating cellular adaptive response to oxidative stress. However, persistent activation of Nfr2 in response to chronic oxidative stress, including inorganic arsenite (iAs3+) exposure, blunts glucose-triggered reactive oxygen species (ROS) signaling and impairs glucose-stimulated insulin secretion (GSIS). In the current study, we found that pancreatic islets isolated from Nfr2-knockout (Nfr2-KO) mice and MIN6 beta-cells with stable knockdown of Nfr2 (Nfr2-KD) by lentiviral shRNA exhibited reduced expression of many antioxidant and phase 2 detoxification enzymes in response to acute iAs3+ exposure. As a result, Nfr2-KO islets and Nfr2-KD MIN6 cells were more susceptible to iAs3+ and monomethylarsonic acid (MMA3+)-induced cell damage, measured by decreased cell viability and augmented apoptosis. In contrast, pretreatment of MIN6 cells with Nfr2 activators, such as sulforaphane, protected the cells from iAs3+-induced cell damage in an Nfr2-dependent fashion. Our studies demonstrate that Nfr2-mediated antioxidant response is critical in pancreatic beta-cell defense mechanisms against acute arsenic cytotoxicity. Considering the potential inhibitory effect of persistent Nfr2 activation on ROS signaling in GSIS, the current study shows that Nfr2 plays paradoxical roles in pancreatic beta-cell dysfunction induced by environmental arsenic exposure.

2216 GENETIC PATHWAYS UNDERLYING THE IMMEDIATE AND LIFESPAN IMPACTS OF A DEVELOPMENTAL EXPOSURE TO THE ENDOCRINE-DISRUPTING HERBICIDE ATRAZINE.

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There is a growing body of evidence which indicates continual exposure to endocrine-disruptors over the lifespan of an individual or during increased sensitivity windows (such as development) substantially elevates the risk to develop multiple types of diseases. Atrazine, an herbicide commonly applied to agricultural areas throughout the Midwest and a common contaminant of potable water supplies, is implicated as an endocrine-disruptor and potential carcinogen. The specific adverse health effects associated with atrazine exposure and the underlying molecular mechanisms of these effects are not well defined. In an effort to delineate the mechanisms of atrazine toxicity, we exposed zebrafish embryos to environmentally relevant concentrations of atrazine shortly after fertilization through 72 hours post fertilization (hpf). Genomic analysis immediately following the embryonic atrazine exposure has identified enrichment of genes with altered expression patterns that are involved in neuroendocrine development, cell cycle regulation and carcinogenesis. A subset of individuals was permitted to mature under normal conditions to assess estrogen dependent in rats and the critical period for this action spans gestation and early neonatal life. Here we determined the effects of prenatal BPA exposure on the sex-specific gene expression of nuclear estrogen receptors (ERα and ERβ) in the preoptic area (POA), the medio basal hypothalamus (MBH), and the medial amygdaloid nucleus (MeA). Pregnant Sprague-Dawley rats were orally gavaged with vehicle, 2.5 or 2.0 μg/kg BPA (LBPA or HBPA) or 5.0 or 10.0 μg/kg ethinyl estradiol (LEE or HEE) on gestational days 6-21. Gene expression in postnatal day 1 brains (3-8/sex/group) was assessed by in situ hybridization. Overall, sex effects were apparent and as expected at this age (e.g., increased ERβ mRNA signal in female MPOA, increased ERα mRNA signal in male AVPV). Treatment effects on anterior hypothalamic ER expression were mild: LBPA decreased ERα mRNA signal in the female AVPV and MPOA by approximately 36%. ERβ expression in the AVPV, but not MPOA, was mildly increased by EE, particularly in males. These data suggest that ER expression in neonatal POA is sensitive to prenatal BPA treatment. These effects could lead to organizational changes within sexually dimorphic neuroendocrine pathways which, if permanent, might then alter adult reproductive physiology and socio-sexual behavior.

2217 PROFILE OF HYPOTHALAMIC ESTROGEN RECEPTOR EXPRESSION IN NEONATAL MALE AND FEMALE RATS FOLLOWING PRENATAL BISPHENOL A (BPA) EXPOSURE.

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Human exposure to BPA, a component of polycarbonate plastics and epoxy resins, is ubiquitous. As an endocrine disruptor, BPA may perturb the sex-specific organization of the neuroendocrine system and compromise later adult reproductive endocrinology and behavior. Sexually dimorphic hypothalamic organization is largely estrogen dependent in rats and the critical period for this action spans gestation and early neonatal life. Here we determined the effects of prenatal BPA exposure on the sex-specific gene expression of nuclear estrogen receptors (ERα and ERβ) in the preoptic area (POA), the medio basal hypothalamus (MBH), and the medial amygdaloid nucleus (MeA). Pregnant Sprague-Dawley rats were orally gavaged with vehicle, 2.5 or 2.0 μg/kg BPA (LBPA or HBPA) or 5.0 or 10.0 μg/kg ethinyl estradiol (LEE or HEE) on gestational days 6-21. Gene expression in postnatal day 1 brains (3-8/sex/group) was assessed by in situ hybridization. Overall, sex effects were apparent and as expected at this age (e.g., increased ERβ mRNA signal in female MPOA, increased ERα mRNA signal in male AVPV). Treatment effects on anterior hypothalamic ER expression were mild: LBPA decreased ERα transcript in the female AVPV and MPOA by approximately 36%. ERβ expression in the AVPV, but not MPOA, was mildly increased by EE, particularly in males. These data suggest that ER expression in neonatal POA is sensitive to prenatal BPA treatment. These effects could lead to organizational changes within sexually dimorphic neuroendocrine pathways which, if permanent, might then alter adult reproductive physiology and socio-sexual behavior.

2218 USE OF A NORMAL HUMAN 3-DIMENSIONAL (NHU-3D) ORGANOPTHIC VAGINAL TISSUE MODEL TO IDENTIFY ENDOCRINE-DISRUPTING CHEMICALS.


Chemicals used in the agricultural, food processing, and pharmaceutical industries, along with environmental contaminants can block, alter, or modulate the endocrine system. Exposure to these agents, known as endocrine disrupting chemicals (EDC), can adversely affect: 1) reproduction 2) fetal development, and 3) cancer development. Animal tests are expensive and require a large number of animals and the European Cosmetics Directive bans the use of animals for studies involving a broad variety of cosmetic and personal care products. In this study, we investigated the use of the NHu-3D EpiVaginalTM tissue for Tier 1 screening of chemicals that may be estrogen receptor (ER) agonists or antagonists. MTT, histology, RT-PCR, and ELISA assays were used to define tissue viability, structure, gene expression, and estrogen release patterns, respectively. The functionality of ER in the differentiation of EpiVaginal tissues were assessed by a 7 day culture in medium supplemented with non-toxic concentrations of two ER agonists (17α ethinyl estradiol and Equilin) and two ER antagonists (Flutamide and 4-hydroxytamoxifen). Results showed that ER antagonist resulted in: 1) thinned/disorganized tissue structure and 2) reduced tissue viability and transepithelial electrical resistance (TEER). In contrast, ER agonists resulted in a thicker tissue and a similar degree of tissue viability and TEER values to untreated controls. RT PCR showed an increase in progesterone receptor B (PRB) levels for 3 of 3 agonists and a decrease or no change for 6 of 8 antagonists, when compared to negative controls. ELISA assays showed increased estrogen release for 9 of 11 ER agonists while 17 of 17 ER antagonists were identified as negative. Based on estrone release (n=28 test articles), a prediction model (PM) for ER agonists was established. The PM identifies ER agonists with high sensitivity (81.8%), specificity (100%), and accuracy (92.9%). In conclusion, the EpiVaginal tissue appears to be a useful in vitro model to screen for chemicals with endocrine disrupting potential.

2219 EVALUATION OF STEROIDGENIC PATHWAY DISRUPTION AND ADRENAL STEROID HORMONE SECRETION IN THE HUMAN ADRENOCORTICAL H295R CELLS BY LOWER CHLORINATED PCBs, THEIR METABOLITES AND COMMERCIAL MIXTURES.

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PCBs have been shown to accumulate in the adrenal glands when incorporated into the body. To assess the effects of different lower chlorinated PCBs (PCB3, PCB11), their metabolites (PCB3-2',OH, -4',OH and -2',5'-HQ), as well as complex mix-
tures of halogenated biphenyls like Aroclor 1242, Aroclor 1254 and firemaster FF-1 on the endocrine system. We employed the Hansen 95R steridogenosis assay. This assay system is part of the OECD level 2 battery of in vitro assays for endocrine disruption and is mandatory in the U.S. EPA Endocrine Disruptor Screening Program. We tested each compound/mixture at concentrations which were non-cytotoxic and determined the secretion of testosterone, estradiol and cortisol with an ELISA. In addition, we analyzed the expression of 10 genes (CYP1A1, CYP1B1, CYP19, CYP21, CYP1B2, SfAR, HMGR, BfHSD2, 17fHSD1 and 17bHSD4) involved in steriodogenesis using qRT-PCR. Estradiol production was the most sensitive target and was altered by almost all compounds, often in a biphasic way with lower concentrations (0.1 μM) causing a diminished and higher concentrations (10 μM) an increased production. Only PCB3, PCB3-4’OH and firemaster FF-1 altered the testosterone and cortisol secretion as well, and PCB11 only diminished the cortisol secretion. Interestingly the results of the gene expression analysis showed that PCB11 and the commercial mixtures (Aroclor 1242, Aroclor1254, and firemaster FF-1) up-regulated CYP19, CYP11B2 and SfAR gene expression and down-regulated CYP21 expression; the PCB3 metabolites interfered with CYP21 and CYP19 expression as well. Thus the effects of our test compounds on hormone secretion can at this point only partly be explained by the altered expression of steriodogenic genes. (Supported by NIEHS P42 ES013661, DAMD17-02-1-0241, and the Center for Health Effects of Environmental Contaminants (CHEEC) of the University of Iowa).

**2220 ARYL HYDROCARBON RECEPTOR 2 MEDIATES THE INHIBITION OF VITELLOGENESIS BY 2, 3, 7, 8-TETRACHLORDIBENZO-P-DIOXIN IN ZEBRAFISH (DANIO RERIO).**

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Zebrafish (Danio rerio) were used to test the hypothesis that 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) inhibits the mRNA induction of 3 vitellogenins (zVtg type 1) by 17α-ethynylestradiol (EE2). Vitellogenin is a hepatic protein required for oogenesis (yolk-formation) and is often down-regulated in oviparous organisms inhabiting aquatic systems contaminated by aryl hydrocarbon receptor (AhR) agonists, such as 2,3,7,8-TCDD. To investigate the effect of 2,3,7,8-TCDD on zVtg regulation, zebrafish embryos were either treated with EE2 (1000 ppo) from 6 hours post fertilization (hpf) to 4 days post fertilization, or were pre-treated with 2,3,7,8-TCDD (50 and 400 ppo) for 1 hr at 3 hpf and then exposed to EE2. When treated with EE2, zVtg 1, 2 and 3 were significantly induced 225, 220 and 68-fold, respectively, over control (p ≤ 0.05). Exposure to 400 ppo TCDD for 1 hr prior to EE2 treatment inhibited induction of zVtg 1, 2, and 3, by 99, 99.7 and 98.8%, respectively. Exposure to 50 ppo 2,3,7,8-TCDD down-regulated induction of zVtg 1 and 3 by 81, 87, 85%, respectively. Similar findings were reported using 1,2,3,7,8-pentachlorodibenzo-p-dioxin. Further studies tested the hypothesis that the aryl hydrocarbon receptor 2 (zAhR2) mediates the inhibition of vitellogenesis by 2,3,7,8-TCDD. A splice-variant morpholino oligonucleotide was used to knock-down expression of zAhR2. Zebrafish injected with zAhR2 morpholino had significantly less inhibition of zVtg (1-3) induction by 2,3,7,8-TCDD (70-75%), relative to a control morpholino group (95-100%). These results are the first to demonstrate that acute exposure to 2,3,7,8-TCDD can inhibit vitellogenin mRNA induction through an AhR2-mediated mechanism in vivo. This work implicates the role of the zAhR2 in estrogen receptor pathway cross-talk and offers insight into reproductive impacts observed in oviparous species inhabiting aquatic systems heavily contaminated by AhR agonists.

**2221 OPTIMIZATION OF A CELL MICROELECTRONIC SENSING TECHNIQUE FOR PROFILING OF ESTROGENIC CHEMICALS.**

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With a public concern about the adverse effects of environmental contaminants with endocrine activity on reproduction of humans and wildlife species, the aim of our study was to validate a technique to detect potentially estrogenic chemicals by using a commercial MCF-7 breast cancer cell line. A cell microelectronic sensing technology known as RTCA (Real-Time Cell Analyzer, Roche xCELLigence system) was used based on the ability of estrogenic chemicals to induce proliferation of MCF-7 cells. The optimization was tested by observing cell growth under several conditions including defacement cell seeding numbers and starvation conditions and the presence or absence of growth factor; insulin. In addition to RTCA, MT assay was also performed to investigate cell proliferation. We used 17β-estradiol (E2) and IRI (estradiol 6,6’-dimethylbenzo[a]pyrene (BAP)), a known environmental mutagen and carcinogen. mRNA abundance of genes involved in estrogenic and diethylstilbestrol (DES) and bisphenol A (BPA). We found that cell growth factor recommended by the vendor, insulin, did not increase cell proliferation in our assay. Overnight of MCF-7 cell starvation in medium supplemented with Charcoal-Dextran stripped fetal bovine serum improved the sensitivity of the assay. We were able to detect the increased cell proliferation in response to E2, DES and BPA exposure at concentrations as low as 1 nM, 1 pM and 100 nM, respectively. In conclusion, we have developed a cell microelectronic sensing assay with high sensitivity and reproducibility, to screen for estrogenic chemicals. This assay was established to use with the incremental MCF-7 cell line which will be practical for researchers. In addition, continuous cell population monitoring of RTCA throughout the entire experiment significantly reduced workload and technical-time when compared to the traditional endpoint assays.

**2222 ESTROGENIC ACTIVITY OF THE RED CLOVER ISOLAVONE IRILONE IN HUMAN IISHIKAWA AND MCF-7 CELLS.**

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Human exposure to irilone (IRI) results from consumption of red clover-based food supplements containing mainly formononetin and biochanin A, which can be converted into the well-investigated isoflavones daidzein (DAI) and genistein (GEN). Therefore up to now, the biologic activity of such food supplements has been attributed to these isoflavones. However, IRI is detected at similar concentrations as DAI in human plasma after intake of red clover-based products. Thus, IRI may contribute to their biologic activity as well. Therefore, we investigated the estrogenic activity of IRI by means of reporter gene expression in Ishikawa cells and cell proliferation in MCF-7 BUS cells. Experiments were carried out in the absence and presence of both the positive control 17β-estradiol (E2) and the estrogen receptor (ER) antagonist ICI182,780. In Ishikawa cells, the activity of alkaline phosphatase (AIP) was determined by spectrophotometric quantitation of the hydrolysis of 4-nitrophenolphosphate. In addition, relative mRNA levels of AIP and progesterone receptor (PR) were determined by RT/TAqman PCR. MCF-7 cell proliferation was determined by electronic determination of cell numbers and by flow cytometric determination of cell cycle distribution. IRI significantly induced AIP activity as well as AIP and PR mRNA levels in Ishikawa cells; however maximum stimulation was not achieved in the non-cytotoxic concentration range. The relative stimulating activity was 0.02% of that of E2, thus placing its activity between that of the soy isoflavones DAI and GEN. Moreover, IRI significantly induced MCF-7 BUS cell proliferation in the same concentration range as DAI and maximum stimulation was achieved. Neither E2-induced AIP activity nor MCF-7 cell proliferation was affected by IRI and ICI 182,780 antagonized IRI-induced effects on AIP activity and MCF-7 cell proliferation, suggesting an ER agonistic mode of action. Thus for the first time, we demonstrated the estrogenic activity of IRI, which is likely to contribute to the bioactivity of red clover-based food supplements.

**2223 COEXPOSURE TO XENOESTROGENS AND BENZO(a)PYRENE ENHANCES DNA DAMAGE RESPONSE.**

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Many ubiquitous environmental contaminants have been identified as endocrine disrupting compounds (EDC) in recent scientific literature. However, much controversy still exists regarding their potential effects on ecological and human health, and mechanistic studies of various forms of EDCs remain under investigation at the cellular and genetic level. Numerous xenobiotics have been connected to adverse effects in humans and wildlife at low concentrations, and studies have also linked them to potential carcinogenesis. One potential mechanism underlying adverse effects of xenobiotics is DNA damage and aberrant DNA repair processes. Nucleotide excision repair (NER) is an essential DNA repair mechanism required for the removal of bulky DNA adducts. To investigate the effects of known xenoestrogens on NER, we exposed killifish (Fundulus heteroclitus) to varying concentrations of arsenic and ethynylestradiol (EE2), a heavy metal that exhibits estrogen-like effects and a synthetic pharmaceutical estrogen, respectively. After 48 hours fish were intraperitoneally injected with benzo[a]pyrene (BaP), a known environmental mutagen and carcinogen. mRNA abundance of genes involved in NER, as well as CYP families of genes were examined due to their known induction by EDCs and
toxicant exposure. Xenoestrogens alone hindered transcriptional response to the NER damage recognition gene XPA, which is consistent with previous literature. BaP alone induced a dose-response in mRNA abundance of CYP1A1 and XPA, while the addition of EE2 and arsenic further induced XPA up-regulation. Our data yield insight into combinations of environmental mutagens and xenoestrogen contaminants commonly found in aquatic systems. Such mutagens can potentially hinder accurate DNA replication processes or enhance inaccurate replication and potentially lead to genetic instability, which is crucial for proper function and survival. We aim to use these data to further investigate the potential effects of these and other xenoestrogens on DNA repair.

**2224 A NOVEL SENSITIVE AND SELECTIVE REAL-TIME CELLULAR ASSAY FOR DETECTION OF ENDocrine DISRUPTORS USING NATIVE ENDocrine SIGNALING PATHWAYS.**

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A cell based assay for detection of endocrine disruptors has been previously developed using real time impedance monitoring of native endocrine signaling pathways in T47D breast cancer cell line. Stimulation of T47D cells with respective endocrine hormones (e.g., estrogen or progesterone) leads to distinct time dependent cellular changes (cell number change and/or cell morphological change) which can be detected by gold microelectrodes embedded in the bottom of the well. This assay is highly sensitive and allows one cell based co-culture assay to be performed under the USEPA’s Endocrine Disruptor Screening Program (EDSP), to screen for contaminants commonly found in aquatic systems. Such mutagens can potentially lead to genetic perturbations in the early embryonic development. We have used a roboticized MCF-7 cell proliferation assay to quantify the estrogenic activity (EA) and antiestrogenic activity (anti-EA) in an ethanol extract of each personal care product. These products included such items as shampoos, hair conditioners, and hand lotions. They were selected for testing because they were found to be frequently used products in a population under study for uterine fibroids. The EA of extracts was quantified as the relative maximum response to 17β estradiol (EC50, a measure of response amplitude) defined as the percentage of the maximum DNA/well produced by an extract at any dilution with respect to the maximum DNA/well produced by E2 at any dilution, corrected by the DNA response to the vehicle (negative control). Anti-EA is calculated relative to IC50 suppression of EA as EC50/IC50. Our series of the eight personal care products produced one that had high level of EA (63% RME2) and four that had detectable EA. E2O4 extracts of all three of the products that had no detectable EA had high levels of anti-EA: 52, 63, and 79% (RME2). Our results indicate that at least some personal care products contain components that exhibit easily detectable EA or anti-EA when tested on a human cell line in vitro. These results may lead to in vivo studies suggesting hormonal perturbations in the early onset of puberty in girls, development of uterine fibroids in adult females, and other adverse health effects (e.g., breast cancer) remains to be determined.

**2225 ENDOCRINE DISRUPTION POTENTIAL OF BENEFIN: WEIGHT OF EVIDENCE EVALUATION.**

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Benefin is a pre-emergent herbicide with a complete toxicological database required for any regulated pesticide. Benefin was included on the 1st list of chemicals to be screened under the USEPA’s Endocrine Disruptor Screening Program (EDSP), which consists of 11 in vitro and 14 in vivo screening assays. Following completion of EDSP tier 1, a weight of evidence (WoE) evaluation was conducted using all available data to determine if benefin has the potential to interact with the estrogen, androgen or thyroid (EAT) systems following the 5 tier OECD conceptual framework. In this framework, limited single endpoint in vitro studies (e.g. Hershberger) received greater weight than in vitro assays and apical in vivo studies received the greatest weight (e.g. level 5 multi-generation reproduction assays). To explain observations in the in vitro EDSP screens that could be considered ‘potentially positive’ responses, additional mode of action work was explored. While previous data indicated the thyroid gland as a target organ for benefin, further studies demonstrated the non-human relevance of the mode of action of these effects. Additional information on the thyroid activity is also available from the tier 1 amphibian metamorphosis assay (AMA), designed to identify hypothalamus - pituitary - thyroid (HPT) pathway agonists and antagonists. Benefin was considered “likely thyroid inactive” with no treatment-related effects on development and no histopathological effects observed in the thyroid glands of the benefin-exposed tadpoles. Existing level 5 multigeneration reproduction studies in mammals did not demonstrate a negative mode of action different from that observed in rodents with adverse endocrine-mediated effects on reproductive organs or processes. Thus, it becomes critical to evaluate the utility of the EDSP tier I testing, specifically de-

**2226 PERSONAL CARE PRODUCTS HAVE DETECTABLE ESTROGENIC AND ANTIESTROGENIC ACTIVITY.**

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We have used a roboticized MCF-7 cell proliferation assay to quantify the estrogenic activity (EA) and antiestrogenic activity (anti-EA) in an ethanol extract of each personal care product. These products included such items as shampoos, hair conditioners, and hand lotions. They were selected for testing because they were found to be frequently used products in a population under study for uterine fibroids. The EA of extracts was quantified as the relative maximum response to 17β estradiol (EC50, a measure of response amplitude) defined as the percentage of the maximum DNA/well produced by an extract at any dilution with respect to the maximum DNA/well produced by E2 at any dilution, corrected by the DNA response to the vehicle (negative control). Anti-EA is calculated relative to IC50 suppression of EA as EC50/IC50. Our series of the eight personal care products produced one that had high level of EA (63% RME2) and four that had detectable EA. E2O4 extracts of all three of the products that had no detectable EA had high levels of anti-EA: 52, 63, and 79% (RME2). Our results indicate that at least some personal care products contain components that exhibit easily detectable EA or anti-EA when tested on a human cell line in vitro. These results may lead to in vivo studies suggesting hormonal perturbations in the early onset of puberty in girls, development of uterine fibroids in adult females, and other adverse health effects (e.g., breast cancer) remains to be determined.

**2227 DIFFERENTIAL RECRUITMENT OF ESTROGEN RECEPTOR COACTIVATORS BY XENOESTROGENS.**

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Xenoestrogens are environmental contaminants that interfere with endocrine activity by modulating estrogen receptor (ER) transcriptional activity. One hypothesis that may explain contaminant-specific impacts of estrogen (E2) signaling is that coactivators are differentially recruited to the ER. Using Time Resolved-Fluorescence Resonance Energy Transfer (FRET) co-assembly screening assays we revealed that E2 weakly recruited a peptide specific to SRC-3, a known ER coactivator, to the human (h)ERα. Conversely, greater recruitment of the same peptide to the hERα was observed upon stimulation with the phytoestrogen genistein (Gen, EC50 17nM). To begin to assess the functional role of SRC-3 in ER activation by these compounds, human embryonic kidney cells were transfected with an ER-driven luciferase reporter and wild-type (w)SRC-3, or mutant (m)SRC-3, which impairs interaction with the ER through mutated phosphorylation sites. Cells were exposed to 10μM E2, or 1μM Gen for 24 hours and luciferase activity was measured. Results showed a significant increase in hERα activation in response to Gen (16 fold) with the addition of wSRC-3 over baseline values (3 fold). Although E2 had higher baseline activation (8 fold), addition of wSRC-3 did not significantly increase the response. In the presence of mSRC-3, ER activation was ablated by both compounds. These results suggest that although SRC-3 is essential for ERE-driven responses by both agents, the enhanced response of Gen in the presence of additional SRC-3 may play a role in xenoestrogen-specific effects on ER activation. Mechanisms driving these observations are under current investigation but may involve SRC-3 site specific modifications that are ligand dependent.

**2228 VALIDATION OF T47D-KBLUC CELL ASSAY FOR DETECTION OF ESTROGEN RECEPTOR AGONISTS AND ANTAGONISTS.**


There is growing concern of exposure to fish, wildlife, and humans to environmental estrogens and their potential impact on reproductive health. Cell-based assays are useful tools to determine the estrogenic activity of chemicals. Confidence in in
vitro assay results is strengthened by the use of a well-characterized assay. The T47D-KBluc estrogen receptor transcriptional activation (ERTA) assay utilizes a stable cell line run in a 96-well plate format which contains both human estrogen receptors alpha and beta (ERα and ERβ) and an estrogen-responsive luciferase reporter. The cell line was transferred from the EPA lab to CeeTox, Inc for an external validation. Initially about 60 well-characterized estrogenic or anti-estrogenic compounds of thyroid toxicity were tested. Testing of this validation set of compounds indicated that the assay produced the expected results and confirmed assay performance. About 50 "unknown" chemicals of interest were then tested. Several compounds were found to be weakly estrogenic such as diethylphthalate and cyclotrisilazane. Only one displayed weak anti-estrogenic effects: N,N-Dimethyltriacrylamide. To further characterize the ERTA assay with respect to ERα and ERβ their respective specific agonists, PPT and DPN were tested. The calculated EC50 of PPT was 4.9e+11M and the EC50 of DPN was 1.5e-8M, compared to that of estradiol which is typically 2.2e-12M. We also tested an ERβ specific antagonist, MPP, in competition with either PPT or DPN. Both PPT and DPN-luciferase induced activity were reduced by MPP. Therefore, it cannot be ruled out that DPN, while more specific for ERβ is not also activating primarily ERα. Results demonstrate that the T47D-KBluc cell line responds to both ERα and ERβ agonists but support the assumption that ERα is inducing more reporter expression than ERβ. Future studies will be conducted to further examine the relative proportion of ERα and ERβ in reporter gene induction. This abstract does not necessarily reflect EPA policy.

**2229 COMPARISON OF IN VITRO CYTOTOXICITY, ESTROGENICITY, AND ANTIESTROGENICITY OF TRICLOSAN, PERFLUOROOCTANE SULFONATE (PFOS), AND PERFLUOROOCTANOIC ACID (PFPAO).**


Concern with increasing levels of emerging contaminants exists on a global scale. Three commonly observed emerging environmental contaminants: triclosan (2,4,4-trichloro-2'-hydroxydiphenyl ether), a synthetic, broad-spectrum antibacterial agent, and perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFPAO), used stain and water resistant treatments, have become distributed ubiquitously across ecosystems and have been detected in wildlife and humans. MCF-7 human breast cancer cells were used to investigate the potential for cytotoxicity, estrogenicity, and antiestrogenicity of these three compounds at environmentally relevant concentrations using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt assay (MTS) and the E-SCREEN bioassay. The doses used ranged from 0.002-200 μg ml-1 for triclosan and 0.03-30 μg ml-1 for PFOS and PFPAO. Quantitative results from the MTS assay revealed no significant cytotoxicity at lower concentrations for any of the test compounds; however, both triclosan and PFOS were cytotoxic at the highest concentrations examined (100-200 μg ml-1 and 30 μg ml-1, respectively), while PFOS showed no significant cytotoxicity at any of the concentrations tested. Positive estrogenic responses (p < 0.05) were elicited from the MTS assay at all concentrations examined for triclosan and PFOS and at 30 μg ml-1 for PFOS. Further, significant antiestrogenic activity (p < 0.05) was detected for all compounds tested at all concentrations when cells were co-exposed with 10-9 M 17-β estradiol (E2). The overall results demonstrated that triclosan, PFOS, and PFPAO have estrogenic activities and that co-exposure to contaminants and E2 produced antiestrogenic effects. Each of these compounds could provide a source of xenobiotics to humans and wildlife in the environment.

**2230 REASSESSMENT OF THE CRITICAL EFFECT OF PERCHLORATE TOXICITY IN THE HUMAN THYROID TO INFORM ON DRINKING WATER REGULATIONS.**

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In 2005, the National Research Council (NRC) issued a report that identified iodide uptake inhibition (IUI) in the thyroid as the critical first step in the mode of action for perchlorate thyroid toxicity in rodents and humans. Based on the NRC findings, drinking water standards in Massachusetts and California were derived based on developmental effects from impaired thyroid function in pregnant mothers and newborn children as the critical effect. Since the release of the NRC report, multiple occupational studies in perchlorate workers and epidemiological studies in perchlorate-exposed pregnant women and their children have been published. We assessed whether occupational and epidemiology data published before and since the NRC report support IUI as the first critical effect in perchlorate toxicity in adults and children. The data fail to show deficiency in thyroid function of perchlorate workers and pregnant women or increased incidence of developmental effects in children exposed to perchlorate at levels that resulted in IUI. In fact, the data indicate that the human thyroid adapts to perchlorate-induced IUI to maintain proper thyroid health and adequate thyroid hormone levels. Human thyroid toxicity may result from perchlorate exposures that are sufficiently high to overcome the adaptive upregulation of iodide uptake. The occupational, epidemiological, and therapeutic clinical data indicate a marked difference in perchlorate exposure levels required to initiate IUI or overwhelm the adaptive mechanisms and reduce thyroid hormone below healthy levels. Thus, the perchlorate literature support derivation of drinking water standards based on a critical endpoint other than thyroid IUI.

**2231 EFFECT OF GREEN TEA EXTRACT ON THYROTROPHIC EFFECT OF LEAD ACETATE IN MALE WISTAR RATS.**

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Green tea is known for its antioxidant and anti-carcinogenic effects. However, its effects on the thyroid gland have not been adequately investigated. The present investigation has been designed to evaluate the effect of green tea extract (GTE) on the thyrotrophic effect of lead acetate (LA) in adult male Wistar rats. Four groups of rats, 10 animals each, were used in this study (control, LA, LA + GTE alone). Rats received a daily dose of LA (100mg/kg) by stomach tube for 30 days. GTE at a dose of 0.5g/100ml was provided in the drinking water ad-libitum for the same time period. Administration of LA induces marked effect on thyroid function in the treated group compared to control. Co-administration of LA and GTE attenuates the toxic and inhibitory effect of LA alone on serum levels of T3, T4 and TSH. These results were confirmed by microscopic examination of the cellular structure of the thyroid gland of treated rats. LA caused cystic dilatation with flattened lining epithelium in the follicles. Us-ing a standard alkaline comet assay procedure, LA caused significant DNA damage as indicated by visible tail lengths. However, thyroid damage was significantly reduced in animals received LA and GTE. In addition, GTE reduced DNA migration in of LA treated animals compared to LA alone. In conclusion the present study suggests that green tea may be useful in combating the thyroid damage due to LA toxicity. However, this is limited study in concentration and duration. More investigation is needed to understand the effect of various concentration of GTE and the chronic effect of exposure.

**2232 IN VITRO ENDOCRINE-DISRUPTING POTENCIES OF PHOSPHATE COMPOUNDS USED AS FLAME RETARDANTS AND PLASTICIZERS AND ANTIFOAMING AGENTS ON HUMAN OSTEOSARCOMA (U2OS) CELL-BASED REPORTER GENE ASSAYS.**

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Phosphate compounds (PCs) are added to commercial products as additives such as flame retardants, plasticizers and antifoaming agents. As a result, PCs have been ubiquitously found in various matrices such as indoor dust, surface water, sewage sludge and biota. Our recent study has reported that some of PCs were detected in indoor dusts on the order of micrograms per gram, which are equivalent to or exceed polybrominated diphenylethers (PBDEs) containing congeners controlled as persistent organic pollutants (POPs) that indicate endocrine-disrupting toxicities. In this study, a panel of human cell-based reporter gene (CALUX®) bioassays was utilized to evaluate androgen receptor (AR), estrogen receptor alpha (ERα), proges-
antagonistic activities against all tested nuclear receptors were observed in some of the PCs. Especially, AR and PR antagonistic activities were observed at a higher rate than other receptors. On the other hand, some of PCs also indicate ER antagonistic activities against all tested nuclear receptors were observed in some of PCs which indicate in vitro endocrine-disrupting potentials.

The disruption of androgen receptor (AR) mediated androgen signaling played very important roles in several androgen related diseases and symptoms. In order to screen the androgen modulating potency of chemicals, we developed stable AR-GreenS cell lines to AR mediated transcriptional activation and optimized the protocol. Stable AR-GreenS cell line was stably transfected with pGL4-MMTV/Hygro, which is a firefly luciferase reporter vector bearing androgen responsive element (ARE) for detecting androgenic activity of endocrine disrupters, in 22RV1 cells which is human prostate cancer cells contained functional AR. In this stable cell line, AR agonist, 5α-dihydrotestosterone (DHT), was dose-dependently induced the luciferase activity and the activity was significantly increased at 1.0 x 10^(-13) M and started to reach to plateau 10^(-12) M with maximum about 15 folds compared with the vehicle control. This DHT induced luciferase activity was inhibited by the treatment of AR antagonist, bicalutamide. AR-GreenS cells have maintained their growth rate, morphology and responsiveness to DHT until now for 46 passages over five months culturing. The inter variation of assay was relatively small with about 5.1 ± 1.3 mean value of CV (the coefficient of variation). From these results suggested that AR-GreenS cells were stable and sensitive to develop the AR-mediated transcriptional activation assay. Using AR-GreenS cells, we established the test protocol, and optimized the testing condition for AR transcriptional activation assay. Now we are validating the stable cell line in inter- and intra laboratory system using 20 compounds among 78 substances which are recommended for 46 passages over five months culturing. The inter variation of assay was relatively small with about 5.1 ± 1.3 mean value of CV (the coefficient of variation). From these results suggested that AR-GreenS cells were stable and sensitive to develop the AR-mediated transcriptional activation assay. Using AR-GreenS cells, we established the test protocol, and optimized the testing condition for AR transcriptional activation assay. Now we are validating the stable cell line in inter- and intra laboratory system using 20 compounds among 78 substances which are recommended for validating the in vitro androgen receptor transcriptional activation assay as says by ICCVAM. This research was supported by a grant (11162KFDA728) from Korea Food & Drug Administration in 2011.

Endocrine disruptors can cause non-receptor mediated effects either indirectly by altering common signal-transduction pathways or directly via (non-)competitive inhibition of enzymes involved in the steroidogenic pathway. The H295R steroidogenesis assay provides an in vitro cell-based assay to evaluate the potential interference of compounds with steroid hormone production. Current endpoints of this assay are limited to measuring a selected set of hormones using targeted analytical methods such as LC- and GC-MS or EIAs. Recent developments in LC-MS and bioinformatics however, allow more comprehensive approaches to evaluate changes in steroid profiles. In the current work, a metabonomics approach was developed that monitors changes in metabolic profiles in both a targeted and untargeted way. Therefore, H295R cells were exposed for 48h to forskolin, aminoglutethimide, prochloraz, trilostane and atrazine, which are compounds known to affect steroidogenesis. After exposure, the culture medium was subjected to a solid phase extraction clean-up procedure and analyzed by Ultra Performance Liquid Chromatography Time-Of-Flight Mass Spectrometry (UPLC-TOF/MS). Generated profiles were compared to profiles obtained from DMSO blanks using sophisticated preprocessing and alignment software (MetAlignTM). Differential mass signals (p-value <0.05 and fold change >2) were selected and profiles typical for the selected reference compounds were constructed. The observed differences in metabolite profiles were mainly caused by alterations in levels of free and sulfated steroids. These alterations were considered to be very relevant as the identity of the differential metabolites could be related to the expected effect of the compound under investigation. In conclusion, it can be stated that application of a comprehensive metabolite profiling methodology provides a promising analytical approach to screen compounds for steroidogenic modulating properties as well as chemical class prediction.
The objective of this study was to evaluate the potential systemic and local effects induced by 25 repeated intramuscular injections of the full human dose of recMAGE-A3-AS15 ASCI, and to evaluate their reversibility 3 months after the last injection. Cynomolgus was selected based on MAGE-A homology with humans. The ASCI was administered at 2-week intervals and compared to a saline control group. A total of 5 males and 5 females were allocated to each group. 3/6 from each group were sacrificed 3 days post last dose, and remaining animals were kept for a 3-month treatment-free period. A MAGE-A3 specific antibody response was measured in all monkeys injected with the ASCI, confirming exposure of the animals. No toxicologically significant effects on clinical signs, body weight, body temperature, electrocardiography, ophthalmology, hematology, clinical chemistry, gross pathology, organ weights or histopathology were observed. Single or repeated injections of the MAGE-A3 ASCI up to four times at the same injection sites were well-tolerated. Five to seven repeated injections at the same site triggered marked local swelling of the injected limb. These local reactions disappeared completely within 4-7 days after injection. Transient and reversible increases in neutrophils and monocytes, and increased fibrinogen on the days following dosing were considered to be part of an expected immune response to treatment. Three days after the last injection, slight mononuclear inflammatory cell infiltrates were noted in several injected muscles, along with immune stimulation of some draining lymph nodes. After the treatment-free period, a complete recovery was observed. Under the conditions of this study, 25 repeated injections of recMAGE-A3-AS15 ASCI were considered to be systematically and locally well tolerated by cynomolgus monkeys.

Recombinant human lactoferrin (rhLF) is produced by utilizing genetically modified rice plants as the expression system for protein production. Lactoferrin is believed to be a key factor that promotes the growth of beneficial microflora in the GI tract and inhibits the growth of selected pathogens and promotes intestines cell growth and migration. This suggests that rh LF may be able to protect individuals from severe diarrhea resulting from treatment with broad spectrum antibiotics. Since the biological and pharmacological activity of rhLF for this indication occurs in the GI tract, clinical trials and supporting toxicology studies utilize the oral route of administration. In the 28-day oral toxicology study in rats, four treatment groups of 10 male and 10 female CD® [Crl:CD®(SD)] rats were administered the vehicle (trisodium citrate in distilled water) or the test article at respective dose levels of 0, 200, 600, and 200 mg/kg/day (dose volume of 10 ml/kg). Additionally, four groups of four or eight animals/group served as toxicokinetic (TK) animals and received the vehicle or test article in the same manner as the main study groups. Cynomolgus monkeys were used because of their similar body weights, food consumption, ophthalmology, clinical pathology, and macroscopic and microscopic evaluations of tissues. Tissues were microscopically examined for animals at 0 and 2000 mg/kg/day and any gross lesions were microscopically examined for all animals at all dose levels. No test article-related findings were noted in any parameter examined. Based on the lack of toxicological findings, the No Observed Effect Level (NOEL) was 200 mg/kg for this compound. The maximum tolerated dose (MTD) was 2000 mg/kg/day for the compound tested, which is the limit dose for this species. This study supported an IND and the initiation of a pivotal trial in patients with antibiotic-induced diarrhea.

The cynomolgus monkey represents an important primate model for preclinical safety evaluation and the study of age-related disease. The present work examines the feasibility of using computer tomography scans for identifying. To further enhance imaging capabilities in the field of toxicology research two geriatric cynomolgus monkeys (≥10 years, 4.0 and 6.7 kg) underwent computer tomography evaluation. In this feasibility study a GE LightSpeed VFX 16 (CT5007), a modern 16 slice computer tomography device with a 120 seconds spiral-class performance at a 4.3 software level in a fully air-conditioned trailer was used. The monkeys were sedated with 1 mg/kg ketamine and 0.06 ml/kg medetomidine for the entire duration of the examination. They were placed within the scanner in a prone position with no further fixation. To perform the examinations of the skeleton a 6.3 MUH Performix Ultra

**2238 REPEATED-DOSE ORAL TOXICITY STUDIES TO EVALUATE HISTAMINE H3-RECEPTOR ANTAGONIST LENS-RELATED CHANGES IN RATS.**

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The ocular safety profile of SAR110894, a histamine H3-receptor antagonist, was assessed in Sprague-Dawley (albino) and Long Evans (pigmented) rats. Non-reversible lens opacities in rats were observed in repeat-dose oral toxicity studies up to 6 months in duration. Reversible corneal changes were also observed in these studies. Microscopically, the changes were characterized as swelling/regeneration of subcapsular posterior cortical lens fibers, which extended to the anterior portion of the lens at higher doses, and corneal inflammation and erosion. While the SAR110894 has been shown to bind to melanin, no decrease in compound-related incidence or severity of lens opacities was observed in a study in which the compound was administered to pigmented rats for 28 days. Lens concentrations of sodium, potassium, and calcium were evaluated in a time-course study in which SAR110894 was administered by oral gavage for 7, 14, 21 or 28 days to albino rats. This study showed that an ion-related osmotic imbalance, with an increase in lens calcium (+192%) and sodium (+143%) content, was associated with the microscopic lens changes. While the compound-related lens changes did not reverse during a 12-week treatment-free period, no delayed treatment-related development of lens opacity was observed. Transmission electron microscopic examination of the lens confirmed that the compound-related lens changes were not associated with cytoplasmic accumulation of phospholipids. Interestingly, there were no compound-related changes in the cornea or lens in dogs. In conclusion, while the oral administration of SAR110894 did result in the development of lens opacities in rats, species differences in structure and membrane physiology suggest this is unlikely to occur in the human lens.

**2239 COMBINED VIDEO-EEG AND CARDIOVASCULAR SCREENING USING TELEMETRY-IMPLANTED, FREELY MOVING BEAGLE DOGS.**

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Although EEG recordings are commonly used to assess the proconvulsant risk of novel drugs, in-depth EEG investigations are frequently conducted only as follow-up studies to the safety pharmacology core battery mandated by the ICH S7A. To expand the range of feasible core preclinical assessments of the CNS function, we have tested a large animal telemetry model (freely moving beagle dog) implanted with DSI TL11M3-D70-CCCTP transmitters capable of recording arterial pressure, body temperature, ECG and EEG data. Here we outline possible directions for the analysis of continuous EEG data. EEG leads were surgically implanted 1 cm from midline and 5 mm anterior to the intra-aural line, and ECG leads were placed in a lead I configuration. After recovery, these males were administered the psychomotor- activator pentylentetrazole (PTE) intravenously at a rate of 1.5 mg/kg/min via a time delayed ambulatory pump, while the animals were observed directly for paroxysmal activity. Once onset convulsive activity was noted, the PTZ infusion was discontinued and 1 mg/kg Diazepam was administered immediately. For evaluation of behavior and confirmation of EMG artifacts on the EEG trace, time-matched video recordings were performed using the same system. High quality EEG and cardiovascular data were collected using this model. Automated (using NeuroScore Version 2.1) and direct (expert review) EEG data analyses were conducted and the results compared, while video data were used for confirmation of events. Various protocols for automated spike train detection were developed for individual animals and their use discussed. Detection of seizure activity can be performed using this model either by direct review of the signals or by automated analysis (both methods supported by time-matched video recordings). In conclusion, this telemetry collection paradigm is recommended for simultaneous evaluation of the seizure potential and cardiovascular risk of new drug candidates.

**2421 DIAGNOSTIC COMPUTER TOMOGRAPHY IN NONHUMAN PRIMATES—A FEASIBILITY STUDY IN GERIATRIC CYMONOLGUS MONKEYS.**

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The cynomolgus monkey represents an important primate model for preclinical safety evaluation and the study of age-related disease. The present work examines the feasibility of using computer tomography scans for identifying. To further enhance imaging capabilities in the field of toxicology research two geriatric monkeys (≥10 years, 4.0 and 6.7 kg) underwent computer tomography evaluation. In this feasibility study a GE LightSpeed VFX 16 (CT5007), a modern 16 slice computer tomography device with a 120 seconds spiral-class performance at a 4.3 software level in a fully air-conditioned trailer was used. The monkeys were sedated with 1 mg/kg ketamine and 0.06 ml/kg medetomidine for the entire duration of the examination. They were placed within the scanner in a prone position with no further fixation. To perform the examinations of the skeleton a 6.3 MUH Performix Ultra

**2237 REPEATED DOSE TOXICITY AND LOCAL TOXICITY STUDY WITH RECMAE-A3-AS15 ANTIGEN-SPECIFIC CANCER IMMUNOTHERAPEUTIC (ASCI) GIVEN INTRAMUSCULARLY TO CYMONOLGUS MONKEYS.**

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The objective of this study was to evaluate the potential systemic and local effects induced by 25 repeated intramuscular injections of the full human dose of recMAGE-A3-AS15 ASCI, and to evaluate their reversibility 3 months after the last injection. Cynomolgus was selected based on MAGE-A homology with humans. The ASCI was administered at 2-week intervals and compared to a saline control group. A total of 5 males and 5 females were allocated to each group. 3/6 from each group were sacrificed 3 days post last dose, and remaining animals were kept for a 3-month treatment-free period. A MAGE-A3 specific antibody response was measured in all monkeys injected with the ASCI, confirming exposure of the animals. No toxicologically significant effects on clinical signs, body weight, body temperature, electrocardiography, ophthalmology, hematology, clinical chemistry, gross pathology, organ weights or histopathology were observed. Single or repeated injections of the MAGE-A3 ASCI up to four times at the same injection sites were well-tolerated. Five to seven repeated injections at the same site triggered marked local swelling of the injected limb. These local reactions disappeared completely within 4-7 days after injection. Transient and reversible increases in neutrophils and monocytes, and increased fibrinogen on the days following dosing were considered to be part of an expected immune response to treatment. Three days after the last injection, slight mononuclear inflammatory cell infiltrates were noted in several injected muscles, along with immune stimulation of some draining lymph nodes. After the treatment-free period, a complete recovery was observed. Under the conditions of this study, 25 repeated injections of recMAGE-A3-AS15 ASCI were considered to be systematically and locally well tolerated by cynomolgus monkeys.
Tube 24 x 912 Hilight Detector VariSpeed 0.5–4.0s scan time (360) was used. The axial scanning of both upper and lower extremities was performed with following parameters: 0.625mm Helix – 120 KV – MA 50-100 – FOV 70 – Matrix 512x512. The raw data were reconstructed in 3mm maximum intensity projection (MIP) thin range in axial, sagittal and coronal planes. Additionally shaded surface display (SSD)s reconstructions were performed. Examined monkeys showed severe degenerative changes in multiple joints of computer tomography appearance typical for arthritis. Narrowing of the involved joint spaces, large osteophytes along the joint margins, bone cysts and sclerosis as well as loose bodies were identified. Both large joints (e.g. knee) and small joints (e.g. interphalangeal, metacarpophalangeal, carpometacarpal) were involved. The obtained results represent a good example on how enhanced imaging capabilities might improve in vivo diagnostics as an add on study tool for regulatory safety assessment. In addition radiology screening might allow establishment and in vivo characterization of suitable primate disease models (such as arthritis, Alzheimer, etc.).

**2242** PROLONGED AND CONTINUOUS INTRAVENTRINE INFUSION TOXICITY STUDIES IN THE MARMOSET MONKEY USING A PORT CATHETER SYSTEM.


The common marmoset, Callithrix jacchus, is one of the smallest nonhuman primate (300-500g) commonly used in biomedical research. In some pre-clinical studies, where cynomolgus monkeys (Macaca fascicularis) as nonhuman primate models are not fulfilling investigators needs, marmosets are successfully used as an alternative nonhuman primate species. As many test items need to be administered intravenously, prolonged or continuous infusion are required regularly. The recommended volume for daily intravenous bolus administration is 2.5mL/kg, with a maximum of 10mL/kg. Most recent technology now enables researchers to equip the marmoset with an independent, not tethered, infusion system for long term administration (24 hrs/day), in combination with an extra-cutane port catheter (3.0 French) system. The weight of the back pack (17g; Lomir Biomedical, USA), together with the gas driven infusion pump (12g plus 5mL reservoir) should not exceed 1.7 of the animal body weight. The Infu-Disk™ technology by Med-e-Cell (USA) fulfills these requirements. The pre-set pump with delivery rates ranging from 0.03 to 4.0mL/hour has a reservoir of 5mL. Infu-Disk™ is gas driven and pre-set by the supplier. The external pump carried by the marmoset can be connected to the port system and is replaced regularly. Using the activity monitoring system Actiwatch® (CamNtech Ltd., UK) it could be demonstrated that the animals activity is reduced by only approx. 10% when wearing the 30g infusion back pack system. All continuously infused marmosets (n=38) survived the post surgery period as well as up to 5 weeks of continuous intravenous delivery. Microscopically, epidermal scabs, subcutaneous edema, hemorrhage, acute and subacute inflammation, fibrosis and ulcer were observed in single animals at the catheter site. This back-pack system now enables daily or weekly continuous intravenous infusion in the marmoset and therefore enriches the administration regime for this species.
range of blood pressure in monkeys, whereas conventional oscillography displayed the same characteristics in dogs. It can be concluded that both can be considered as alternatives to telemetry in toxicology studies.

**PS 2246 VALIDATION OF A UV-LLNA PROTOCOL BASED ON LYMPH NODE WEIGHT AND CELL COUNT QUANTIFICATIONS TO ASSESS PHOTOALLERGIC POTENTIAL BY TOPICAL OR ORAL ADMINISTRATION TO MICE.**

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Photosafety testing of pharmaceuticals may ultimately include an in vivo test. The UV-LLNA assay originally developed by Ulrich et al (2001) is suitable for testing compounds after topical cutaneous application or systemic administration by oral route. The photoallergic assay is based on quantification of both local reactions and local lymph node stimulation without the use bromodeoxyuridine or tri-thymidine. As recommended by OECD guideline 429, we have validated this assay by performing four consecutive assays with two known phototoxic and photosensitizing compounds applied either orally or topically.

Seven groups of six BALB/cJ mice were treated for four days with vehicle or three different doses of either sparfloxacin (10, 30, 100 mg/kg/day) by oral gavage, or 3,3',4',5 tetrachloroacetylcytamine (0.02, 0.1 and 0.5%) by topical application to the ears. One group at each dose level was irradiated with 10 J/cm2 UVA shortly after treatment each day, while the other was not. Erythema was graded each day. The mice were sacrificed on day 5 and ear punch biopsy weights, cervical lymph node weights and lymph node cell counts were quantified. Histopathology of the eye was performed to examine degeneration of the photoreceptor cell layer and external nuclear cell layer. Both compounds, sparfloxacin given orally, or 3,3',4',5 tetrachloroacetylcytamine applied topically, reproducibly increased ear weight, lymph node weight and lymph node cell count in a dose dependent manner and sparfloxacin induced retinal degenerative lesions following exposure to UVA. The UV-LLNA assay presented here allows a quantitative in vivo assessment of phototoxicity, and phototoxicity in four days.

**PS 2247 USE OF AWAKE ANIMAL IMAGING TO FINGER PRINT FOR CNS LIABILITY.**

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The objectives of safety pharmacology studies include but are not limited to identifying undesirably pharmacodynamic properties of a substance that may have relevance to its human safety and to investigate the mechanism of the adverse pharmacodynamic effects observed and/or suspected. In this study we hypothesized that drugs which carry a Black Box warning for suicide share a common distributed neural circuit involved with cognitive function. Using phMRI (pharmacological magnetic resonance imaging), we sought to identify brain activity common to selected drugs with FDA warnings for risk of suicide. Awake rats, acclimated to the scanning procedure, were analyzed for changes in brain activity using BOLD (blood oxygen level dependent) imaging. The activation patterns were compared for drugs known to have a risk of suicide (venlafaxine, ramoxabant and gabapentin) to those for controls (dopamine and buspirone). BOLD signal changes across 154 brain areas demonstrated a difference in neural activity between drugs at risk and those used to treat suicidal ideation and aggression. Brain areas comprising this putative neural circuit characteristic of drugs at risk include prelimbic cortex, secondary somatosensory cortex, medial dorsal striatum, central thalamus, CA1 hippocampus and others. Interestingly, the integrated neural circuit identified for drugs at risk match many of brain areas involved in schizophrenia.

**PS 2248 PRECLINICAL CARDIAC RISK ASSESSMENT OF HDAC INHIBITORS: INVESTIGATIONS OF HDAC-INDUCED QT PROLONGATION.**

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In the clinical setting a number of HDACi are associated with slight dose- and schedule-dependent prolongation of the QTC interval and isolated cardiac deports of torsade de pointe at high doses. To identify the potential mechanism(s) responsible for these effects we performed an extensive assessment of non-clinical cardiac safety studies. HDACi of diverse structures, specificities and potencies were assessed in numerous assays including: HDAC isotype profiling, cytotoxicity, hERG, Nav1.5 and Cav1.2 binding and patch clamp, hERG trafficking and maturation, comparative dog telemetry and toxicogenomics analyses. The results indicate that HDACi effects on cardiac repolarization are likely due to target mediated epigenetic changes of the myocardium. Common themes of HDACi on the hERG channel and cardiac repolarization and myocardial toxicogenomic signatures will be presented and discussed.

**PS 2249 DETECTION OF SUBTLE THYROIDAL EFFECTS OF AMIODARONE BY MEANS OF METABOLOMICS.**

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Metabolomics is a tool to measure treatment-induced changes of endogenous metabolite levels in biological samples. BASF SE and metanomics GmbH have developed a data base (MetaMap®Tox) containing the plasma metabolite profiles in rats of more than 500 data rich chemicals, agrochemicals and drugs. This metabolome database has been built based upon four weeks studies (adapted to OECD 407 guideline) with blood sampling and measuring of about 300 metabolites after 7, 14 and 28 days. Sets of common metabolite level changes (metabolite patterns) were arranged to characterize several toxicological modes of action (MOAs). Metabolite patterns were established to differentiate between a direct effect (e.g., thyroperoxidase inhibition) and an indirect effect (increased thyroid hormone conjugation by microsomal liver enzyme induction) leading to hypothyroidism.

We created the metabolome of Amiodarone to create a metabolic pattern for anti-arrhythmic drugs in a group with other pharmacuetica. Later, based on the results of a publication of J. Cohen-Lehman et al. (2010): Effects of amiodarone therapy on thyroid function, Nat Rev Endocrinol 6 (1), 34-41) we did re-evaluate the 2007 metabolome data for potential thyroid effects of Amiodarone. We noted that the Amiodarone metabolome correlated with the pattern for direct inhibiting thyroid effects. There was also a very good match with the pattern for indirect inhibiting thyroid effects. However, T4 levels were not decreased but rather elevated suggesting a different or at least additional effect on the thyroid. This fact demonstrates the potential of metabolomics to identify subtle toxicological changes.

**PS 2250 HEPATIC LIPIDOSIS MEDIATED BY OFF-TARGET ACTIVATION OF CANNABINOID 1 RECEPTOR.**


Activation of the cannabinoid (CB) 2 receptor represent an attractive new approach for the broad-spectrum treatment of pain. During the toxicological characterization of A-001, an experimental CB2 agonist, hepatic lipidosis was observed in rats after 5 days of dosing at exposure levels 30 fold above those required to achieve in vivo efficacy. The histopathological changes were accompanied with increases of triglycerides levels in the liver. A toxicogenomics analysis of the liver from these rats showed an up-regulation of genes involved in fatty acids and cholesterol biosynthesis (including SREBP, acetyl-CoA carboxylase, fatty acid synthase, and HMG-CoA reductase) and a down-regulation of genes involved in beta-oxidation of fatty acids (such as carnitine palmitoyltransferase and hydroxyacyl-CoA dehydrogenase). These genomic profiles were consistent with the downstream molecular effects expected after activation of the CB1 receptor, suggesting that the hepatic lipidosis
may have been induced by an off-target interaction with CB1 receptor. To further investigate this hypothesis, CB1-null and wild-type mice were treated with either vehicle or A-001 for 5 days at dosage levels resulting in exposures similar to those in rats. The CB1 null mice did not develop hepatic liposnosis in contrast to what was observed in wild-type mice treated with A-001. Collectively, these findings coupled with the in vitro selectivity profile suggest that A-001-induced hepatic liposnosis was mediated by off-target activation of CB1 receptor, and not by activation of the CB2 receptor.

**2251 ASSESSMENT OF SKELETAL MUSCLE MASS AND NERVE CONDUCTION VELOCITY IN RAT PRECLINICAL TOXICITY STUDIES.**

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New techniques are now available to evaluate skeletal muscle and peripheral nerve conduction in preclinical studies. The purpose of this study was to evaluate muscle by peripheral Quantitative Computed Tomography and nerve conduction velocity in orchidectomized (ORX) testosterone depleted mature Sprague-Dawley rats over a 3 month period. Six-month-old male rats were randomly assigned to 2 groups by body weight (BW). Ten rats underwent either ORX or Sham operation and were observed for 13 weeks. Bone densitometry and fat/lean analysis by pQCT was measured at 0, 6 and 13 weeks at the proximal tibia metapysis and diaphysis. BW and food consumption were measured weekly and muscle weights were recorded at necropy. Nerve conduction velocity was recorded for the mixed caudal nerve, the sensory digital nerve, and the distal motor branches of the tibial nerve innervating planar muscles of the foot prior to ORX and at 6 and 12 weeks post ORX. After surgery, food consumption of ORX rats was lower than Shams by up to 10% between Weeks 5 and 13, resulting in lower BW gains and a reduced BW of 5% at the end of the study. pQCT muscle area was decreased relative to Shams of 6 and 13% at Weeks 6 and 13, with no significant changes in muscle density or fat area. Trends for lower gastrocnemius and extensor digitorum longus weights by 4.5% and 4.0% for ORX rats compared to shams were noted at necropy. The changes in muscle were not associated with slowing of either sensory or motor nerve conduction velocity.

pQCT and nerve conduction velocity provide reproducible and repeated non-invasive measurements of skeletal muscle and peripheral nerves in rats. These data, combined with biochemical, histology evaluations and muscle functionality assessment (dynamic weight bearing and grip strength), provide a comprehensive assessment of muscle and peripheral nerve conduction, supporting the use of these endpoints in preclinical studies for drug development.

**2252 REPRODUCTIVE AND DEVELOPMENTAL TOXICITY STUDIES DEMONSTRATE THAT AN2728, A NOVEL BORON-BASED PARENTAL MALIGNANCY TREATMENT, IS NEITHER A REPRODUCTIVE TOXICANT NOR A TERATOGEN.**

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AN2728 is a new chemical entity which represents a new class of boron-based compounds that are being developed for the topical treatment for psoriasis. A fertility and reproductive toxicity study was conducted in rats via oral gavage at doses up to 600 mg/kg/day. Males were dosed 28 days before cohabitation, through cohabitation and continuing through the day before sacrifice; females were dosed 15 days before cohabitation and continuing through Day 7 of presumed gestation (DG 7). No effects on mating and fertilization in male or female rats or Caesarean-sectioning and litter parameters of female rats, and as a result, the respective no-observed-adverse-effect-level (NOAEL) was 50 mg/kg/day. Developmental toxicity (teratology) studies were conducted in rats and rabbits. In the rat teratology study, pre- and maternal-pregnant rats were dosed with AN2728 at 150, 300 and 600 mg/kg/day by oral gavage from DG 7 to 17. AN2728-related findings were limited to decrease in BW and food consumption of muscle and peripheral nerve conduction velocity.

After surgery, food consumption of ORX rats was lower than Shams by up to 10% between Weeks 5 and 13, resulting in lower BW gains and a reduced BW of 5% at the end of the study. pQCT muscle area was decreased relative to Shams of 6 and 13% at Weeks 6 and 13, with no significant changes in muscle density or fat area. Trends for lower gastrocnemius and extensor digitorum longus weights by 4.5% and 4.0% for ORX rats compared to shams were noted at necropy. The changes in muscle were not associated with slowing of either sensory or motor nerve conduction velocity.

**2253 NONCLINICAL SAFETY EVALUATION OF XOMA 3AB, A NOVEL MONOCLONAL ANTI-BOTULINUM TOXIN TOXIN TYPE A, IN SPRAGUE DAWLEY RATS.**

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XOMA 3AB is being evaluated for the treatment of botulinum toxin type A (BoNT/A) poisoning. XOMA 3AB is composed of an equimolar mixture of three human or humanized IgG1 monoclonal antibodies (mAb), referred to as NX01, NX02 and NX11, that target unique non-overlapping regions on BoNT/A, resulting in rapid clearance of the toxin from the systemic circulation. Nonclinical safety evaluation pharmacokinetics (PK) and toxicology studies were conducted in Sprague-Dawley rats to support the clinical development of XOMA 3AB. The PK of XOMA 3AB was evaluated in rats for 71 days following a single IV injection at dose levels 0.1, 1 and 10 mg/kg. XOMA 3AB was administered weekly for five weeks by IM injection at doses up to 50 mg/kg and by IM injection at a dose level of 3 mg/kg for toxicology assessment. Parameters evaluated included clinical observations, food consumption, body weights, clinical pathology, ophthalmology, urinalysis, macroscopic and microscopic evaluation. Electrocardiogram-luminescence (ECL)-based assays were developed to measure the individual mAbs in rat serum. An ECL assay was also developed to measure total anti-drug antibodies (ADA) to XOMA 3AB. The results show that NX01, NX02 and NX11 have similar PK profiles and demonstrate a dose proportional bi-exponential decline in initial and terminal half-lives, clearance and volume of distribution. The half-lives of NX01, NX02 and NX11 ranged from 12.4 to 17.2 days. Animals with ADA showed increased clearance of XOMA 3AB from the serum. There were no toxicologically significant findings related to administration of XOMA 3AB to rats. The no-observable-adverse-effect-level (NOAEL) following intravenous administration was ≥ 50 mg/kg, the highest dose tested. This project has been funded in whole or in part with federal funds from the NIAID, NIH, DHHS contract numbers HHSN266200600080C and HHSN26620060011C.

**2254 RESPIRATORY SAFETY PHARMACOLOGY IN RATS, CANINES, AND NONHUMAN PRIMATES: COMPARISON OF MINIMAL DETECTABLE DIFFERENCE.**

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Respiratory safety pharmacology can be included as part of repeat toxicity studies in rats, canines and nonhuman primates (NHPs). Various parameters that impact sensitivity were evaluated by retrospective evaluation of historical control data from various species commonly used in toxicology. Respiratory data was obtained from head-out and whole body plethysmography in rats and with a pneumotachometer connected to a mask in canines. Respiratory data obtained with a helmet and bias flow or from freely moving animal were used for nonhuman primate analysis. The minimal detectable difference (MDD) was calculated for n=8 per group for respiratory rate (RR), tidal volume (TV) and minute ventilation (MV).

The MDD in restrained NHPs when compared with data from freely moving animals was 12%, 25% and 21% higher for RR, TV and MV respectively. The MDD in restrained NHPs when compared with nonhuman primates was 16%, 29.9% and 33.2% for rats, NHPs (helmet) and canines, respectively. In conclusion, our results suggest that individual percent of baseline provide increased sensitivity when compared with absolute values. In NHPs, respiratory data obtained from freely moving animals was associated with increased sensitivity when compared with a restrained models. In rats, sensitivity was comparable with head-out or whole body plethysmography.

**2255 TEMPORAL ASSESSMENT OF ACCLIMATION PERIOD WITH JACKETED EXTERNAL TELEMETRY: IMPACT ON HEMODYNAMIC STABILITY ON STUDY DESIGN.**

S. Tichenor, H. Holzgrefe, D. Regalia and D. Meyer. Toxicology, Charles River Laboratories, Reno, NV.

Jacketed external telemetry (JET) has allowed for the periodic non-invasive acquisition of surface ECGs in repeat dose toxicity studies. The recent addition of minimally invasive blood pressure (MIBP) has further expanded the scope and use of
JET. While non-invasive, the use of JET in non-human primates is characterized by transient stress-induced increases in heart rate and blood pressure which adversely affect both data quality and interpretation. Multiple JET acclimation schemes have been described, but the temporal hemodynamic stability of JET acclimation has not been systematically characterized. Following a graded 2 wk acclimation procedure, 10 male cynomolgus monkeys (cynos) were instrumented with JET and ECGr and MIBP monitored for 48 h post baseline for 14 days. During baseline (day 0-1), 4/10 cynos (group A) exhibited marked increases in heart rate (232±25 vs. 165±28 bpm) and blood pressure (156±10 vs. 134±13 mmHg) compared to 6 cynos (group B) which exhibited sustained acclimation. During the second JET session (day 6) heart rates (169±23 vs. 165±28 bpm) and MIBP (132±11 vs. 134±13 mmHg) demonstrated normal post acclimation values in all animals (day 6 n-10) vs. (day 1 n-6). These results demonstrate that cyno acclimation responses are an individual characteristic which cannot be assumed to remain stable, even during a brief quiescent period prior to study initiation. Importantly, the group A animals exhibited sustained increases in heart rate and blood pressure over the entire 48 hour day 1 acquisition period, allowing for the objective identification of animals which required an additional acclimation renewal period prior to study start. These data demonstrate that the routine inclusion of an immediate prestudy acclimation characterization will allow the selection of animals with a stable hemodynamic background, thus increasing both the power and sensitivity of a given study to detect significant treatment-associated hemodynamic and ECG responses.

**2256 MECHANISMS OF ACUTE PHOTOTOXICITY AND PHARMACOKINETIC PROPERTIES IN SKIN OF BALB/C MICE.**


Photosensitization—sunlight-induced toxicity—is a common phenomenon mediated by many natural and synthetic compounds. There are well-established and routinely used test methods to investigate phototoxicity related to drug substances. However, these test methods frequently do not provide sufficient mechanistic insight. The oral UV-Local Lymph Node Assay (UV-LLNA) in Balb/c mice as a well-established reference model for acute phototoxicity. Typically, animals were treated orally once daily followed by subsequent irradiation (e.g. 10 J/cm2 of UVA) for three consecutive days. In addition to clinical signs e.g. at the ear, acute reactions of auricular lymph nodes are assessed. In order to investigate the co-localization of histopathological signs of acute phototoxicity and the distribution of phototoxic compounds within skin after oral administration early sampling time points were added. Initially, Sparfloxacin was used as a well-known _in vivo_ phototoxic compound. After 2, 4, 12 and 24 hours post-UV exposure, mice were euthanized and ear punch-out biopsies were taken, weighed and further processed for histopathological and pharmacokinetic analyses. A significant, time-dependent increase of the ear weight at 2 and 4 hours post-UV exposure was seen, indicative of edema formation, which decayed at 12 hours already. These results correlated well with the H&E staining of mouse ear skin showing edema, congestion and inflammatory infiltrate. Specific analyses of involved inflammatory pathways are ongoing. In parallel, analytical imaging techniques were evaluated. Sparfloxacin was applied to a reconstructed skin model via the subcutaneous (Phenol®) and skin samples were analyzed by mass spectrometry imaging (MALDI-SDM-QsTOF). Applying this method to skin samples from the murine UV-LLNA is expected to provide corresponding localization data. Following Sparfloxacin a set of phototoxic reference compounds will be profiled in order to enlarge the mechanistic understanding of the underlying phototoxic mechanism _in vivo_.

**2259 GLYTI INHIBITOR REDUCES OSCILLATORY POTENTIALS OF ELECTRORETINOGRAM IN RATS.**


Selective inhibitors of glycine transporter type 1 (GlyT1) are being developed to treat cognitive and negative symptoms in schizophrenia. Reduced neurotransmission at the NMDA subtype of the glycine transporter (GlyT) subtype 1 (GlyT-1) has been identified as a primary neurochemical deficit in schizophrenia. Since glycine is a required co-agonist for glutamate activation of the NMDA receptor, increasing synaptic glycine concentration by inhibiting its reuptake from the synapse should enhance NMDA signaling. In support of this hypothesis, clinical studies have demonstrated evidence of improvements in symptoms of schizophrenia following a high dose of glycine to patients. However, large increases in systemic glycine (when used as an irrigating fluid during prostate surgery) have been associated with visual and electroretinogram (ERG) disturbances. Thus, we conducted the current study in order to determine if
an orally delivered, selective GlyT1 inhibitor causes changes in ERG responses in rats. Male Sprague-Dawley rats (6/group) were administered PT-03463275 (PF) subcutaneously at 1, 3 and 10 mg/kg one hour prior to ERG acquisition. Scotopic and photopic luminance responses, photopic adaptometry and flicker responses were measured. Plasma and vitreous samples were obtained for determination of PF concentrations at the end of the ERG session. A dose-dependent reduction (up to ~70%) in the amplitude of the scotopic ERG oscillatory potentials (OP) was observed following PF administration. The amplitude of OP was also negatively correlated to the concentration of PF in the vitreous humor (r = −0.64, p < 0.0001). With the exception of a small increase in scotopic ERG a-wave amplitude and latency no effects were observed on other ERG parameters tested. Our findings are consistent with reports of decreased OPs in humans (Cred et al, 1987) and rabbits (Korol, 1973) following systemic or intravitreal glycine administration, respectively. Thus, inhibition of the GlyT1 transporter may cause ERG changes in the rat and underlie recent reports of visual disturbance with GlyT1 inhibitors in the clinic (Ouellet, 2011).

2260 EVALUATION OF RESPIRATORY INDUCTIVE PLETHYSMOGRAPHY USING THE EMKABELT SYSTEM IN THE CONSCIOUS DOG AND PRIMATE.


To date, respiratory measurements are typically obtained from conscious large animals using a pneumotachograph attached to a face mask. However, this method requires restraint of the animals, restricts measurements to a relatively short time and recordings cannot be performed during inhalation administration of test substances. Respiratory Inductive Plethysmography (RIP) is reported to allow accurate measurement of ventilatory parameters in freely moving animals. The purpose of this study was to evaluate the data obtained from the non-invasive telemetry EMKABELt (jacket) RIP system to that obtained using the face mask and pneumotachograph system. 4 dogs and 4 primates implanted with DSITM telemetry transmitters were habituated to EMKABELt jackets and face masks over 14 days. Preliminary respiratory recordings were obtained in order to evaluate the most appropriate restrained posture (standing or sitting) for calibration of the EMKABELt system with the pneumotach/mask system. Respiratory recordings from both mask and RIP were obtained prior to and following CO2 (via a re-breath manoeuvre), Doxapram (i.v.) or inhaled Methacholine (5 min). Cardiovascular parameters were monitored by telemetry during all procedures. The data obtained showed a high degree of correlation between the EMKABELt (jacket) RIP system and the pneumotach/mask system. The EMKABELt system provides viable respiratory data and has the potential to be used on stand-alone safety pharmacology studies or as a functional end point on toxicology studies.

2261 SAFETY EVALUATION OF RECOMBINANT HUMAN LACTOFERRIN FROM RICE: 28-DAY TOXICOLOGY STUDY IN BEAGLE DOGS.

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Recombinant human lactoferrin (rHLF) is produced by utilizing genetically modified rice plants as the expression system for protein production. Lactoferrin is believed to be a key factor that promotes the growth of beneficial microflora in the GI tract and inhibits the growth of selected pathogens and promotes intestine cells growth and migration. This suggests that rHLF may be able to protect individuals from severe diarrhea resulting from treatment with broad spectrum antibiotics. Since the biological and pharmacological activity of rHLF for this indication occurs at ~70% in the amplitude of the scotopic ERG oscillatory potentials (OP) was days. Preliminary respiratory recordings were obtained in order to evaluate the most appropriate restrained posture (standing or sitting) for calibration of the EMKABELt system with the pneumotach/mask system. Respiratory recordings from both mask and RIP were obtained prior to and following CO2 (via a re-breath manoeuvre), Doxapram (i.v.) or inhaled Methacholine (5 min). Cardiovascular parameters were monitored by telemetry during all procedures. The data obtained showed a high degree of correlation between the EMKABELt (jacket) RIP system and the pneumotach/mask system. The EMKABELt system provides viable respiratory data and has the potential to be used on stand-alone safety pharmacology studies or as a functional end point on toxicology studies.

Pazopanib is an antiangiogenic drug approved for advanced renal cell carcinoma. Severe hepatotoxicity has been observed in clinical studies. To investigate potential mechanisms, in vitro and in vivo studies were performed. The effects of pazopanib on cellular viability, mitochondrial function, oxidative stress and calcium homeostasis were investigated in HepG2 cells. Cells were exposed to pazopanib in excess of clinical exposures (up to 4 μM, limit of solubility) for up to 24h. Except for a transient minimal ATP reduction at 2h, pazopanib did not induce any significant changes in endpoints related to viability, mitochondrial membrane potential and mitochondrial respiration, reactive oxygen species generation, glutathione content and lipid peroxidation nor calcium homeostasis (intracellular free calcium). In a repeat dose toxicity study in rats, a dose comparable to clinical exposures caused slight but significant increases in BSEP mRNA levels in liver, concordant with mild increases in ALT, GLDH, bilirubin and total bile acids. Slight increases in CYP7A1 mRNA were also observed, which is typically decreased in bile stasis. There was no histopathological evidence of hepatocellular or biliary toxicity in any preclinical studies.

To rule out potential inhibition of hepatobiliary transport as a mechanism of hepatotoxicity, pazopanib was interrogated for its potential to inhibit rat and human bile salt export pump (BSEP) and sodium taurocholate cotransport polypeptide (NTCP). Using sandwich cultured hepatocytes, pazopanib was not found to inhibit human BSEP. Modest inhibition was observed for rat NTCP at 30 μM, but was insufficient to calculate an IC50. Based on these results it appears unlikely that inhibition of BSEP or NTCP contribute to the observed hepatotoxicity following pazopanib administration. In conclusion, results from a battery of assays have helped rule out several potential mechanisms of clinical hepatotoxicity.

2263 INCLUSION OF MICROCHIP TRANSPONDER BODY TEMPERATURE MEASUREMENTS IN RAT TOXICOLOGY STUDIES.


In preclinical studies, the simplest method of measuring body temperature is via a rectal probe. Measurements can cause stress and hyperthermia when repeated over many hours/days and disturb animals in their home cages. A microchip transponder system for recording body temperature (IPTT-300, BMD5) is an alternative non-invasive method. This was included in rat MTD/DRF toxicity studies for 6 compounds from one project where changes to temperature were previously observed, or were expected. Transponder microchips were subcutaneously implanted in the interscapular region of male Han Wistar rats (3/group) during surgery for placement of intravenous cannulae. Following 7 days recovery, each group received a 1 h infusion of a single dose level of compound, on 2 consecutive days (MTD phase). Microchip temperatures were recorded pre-dose then at 0.5, 1, 2, 4, 6 and 24 h post-dose on both days. Further groups received a 1 h infusion of a single dose level of compound on 7 consecutive days (DRF phase), with microchip temperatures recorded as above on days 1, 2, 5 and 7. During both the MTD and DRF phases, decreases in temperature (from 0.5 to 5°C) were detected and monitored for all 6 compounds. Differentiation between the compounds was possible on the basis of magnitude of change in body temperature, other clinical signs and plasma exposures. Microchip measurements were quick to perform and required no handling of the animals, were user-independent, and data transferred directly to Microsoft Excel. No microchip-associated histopathological effects were noted at necropsy.

In conclusion, the microchip transponder method facilitates the monitoring of body temperature at multiple timepoints over the extended period of toxicity studies, providing useful data from low numbers of animals. This is also a 3Rs refinement to standard rectal measurements, with the potential to provide more data from a less-invasive method and should be routinely included when enhanced temperature data would be beneficial.
chronic use of medications such as antipsychotics and antidepressants can induce certain metabolic disorders including insulin resistance, glucose intolerance and non-alcoholic steatohepatitis (NASH) in a subset of patients. To identify the mechanism driving these adverse events, we used an integrated in silico approach based on a proprietary set of in vitro pharmacological profiles of a number of drugs (all known to cause liver lipodosis), genomic databases, and statistical sampling and interrogation techniques to identify previously unknown off-target activities of these compounds, specifically histamine receptor subtype 2 (H2) antagonism. This prediction, based on our in silico approach, of H2 antagonism as the most probable mechanism associated with this spectrum of drug-induced metabolic disorders was later independently confirmed by receptor (H2-R) knock-out transgenic animal studies. Histamine antagonist activity has been a suspected mechanism underlying these complications. Considering that antihistamines are an important treatment strategy for seasonal allergic reactions, identifying the specific histamine receptor subtype(s) (H1, H2 and/or H3) that may be mechanistically related to these observed side effects would be a valuable finding with respect to development strategies for new safer antihistamine drugs.

The purpose of this study was to determine the target organ toxicity and reversibility of decitabine (DAC) and tetrahydrodronodine (THU); administered 1 hour prior to DAC in mice treated orally twice/week for up to 9-weeks (total of 18 doses) followed by a 28-day recovery period. Two hundred sixty-six animals (101 main groups + 156 TK groups) received either both THU and DAC vehicles, 167 mg/kg THU followed by 0.2, 0.4, or 1.0 mg/kg DAC or THU vehicle followed by 1.0 mg/kg DAC. Cmax values were attained within 1 hr at all dose levels. The plasma DAC level increased with dose in groups treated with THU/DAC. Pretreatment with THU increased DAC plasma levels. There was an overall trend for higher plasma levels in females when compared to males. Due to severe toxicity, treatment for the high dose + THU group of female mice was discontinued at 5 weeks. Significant microscopic findings were bone marrow hypocellularity, thymic lymphoid depletion, intestinal epithelial apoptosis and testicular seminiferous tubule degeneration. Bone marrow hypocellularity correlated with hematology findings. Bone marrow micronucleus analysis also confirmed bone marrow cytotoxicity, suppression of erythropoiesis, and genotoxicity in all treatment groups. Following the recovery period, only the testicular findings were observed (at reduced severity) indicating probable resolution of the bone marrow, lymphoid tissue, intestinal tissue and female reproductive findings. Due to the adverse findings noted at all dose levels, a no-observed-adverse-effect level (NOAE) could not be established. The target organs identified under the conditions of this study included the hematopoietic tissue (bone marrow), lymphoid tissue (thymus and spleen), small intestines, testes, and liver. Multiple prior therapies. A total of 209 doses were administered, mean of 4.5 (range 1-30). Treatment was generally well tolerated with no dose dependent changes in liver function tests. The most common adverse events were grade 1-2 fatigue (24%), nausea (17%) and fever (15%) with no clear dose-dependence. Grade 2 infusion-related reactions were noted in 15% of patients (3% of doses administered) and all responded to slowing of infusion. Grade 1-2 chills/rigors were observed at 1.25 mg/kg and were associated with transient IL-6 induction (resolution by 24hrs). The dose-limiting toxicities were liver failure and death in a patient with extensive hepatic metastases and prior splenectomy/partial hepatectomy at 0.7 mg/kg (possibly related), transient grade 3 thrombocytopenia at 1.25 mg/kg, and grade 3 hypokalemia in 1 patient at 1.5 mg/kg. Findings associated with complement activation and transient cytokine elevations were anticipated from preclinical studies. Disease control (stable disease or better) was observed in 13 of 31 evaluable patients (42%) treated at doses of 0.4 to 1.5 mg/kg. Collectively, the results demonstrate the clinical safety of LNPs for systemic delivery of RNAi therapeutics and highlight areas of investigation for ‘next generation’ LNPs.
In the dog study, there were no test article-related findings, except for a reversible, dose-dependent increase in resting pupil diameter, without corresponding changes in ocular histopathology, electroretinogram, or intraocular pressure. The mydriatic effect could be related to the activity against the μ-opioid receptor, and was not deemed adverse in nature. The high dose level at a predicted safety margin of 180-fold was considered to be less than the HNSTD in dogs. Statistically significant target modulation by PF-05727485 was observed in both rat and dog studies, determined for animals of the high dose group relative to the vehicle control group. Taken together, these data demonstrate the safety and efficacy of a selective SMO inhibitor well-suited for the treatment of Hh-dependent tumors.

NKTR-102, a novel topoisomerase I inhibitor-polymer conjugate, has demonstrated a favorable tolerability profile in nonclinical studies with improved safety over irinotecan, a first-generation topoisomerase I inhibitor. Toxicology studies were conducted in rats to further evaluate NKTR-102 safety and to support the combination of NKTR-102 in combination with PLD in humans. A single dose of NKTR-102 was administered as an IV infusion to rats at doses up to 540 mg/m² (90 mg/kg) irinotecan equivalents followed by a single infusion dose of PLD at 30 mg/m² (5 mg/kg). Clinical observations, clinical chemistry, hematology, and histopathology were performed and toxicokinetic samples were collected for NKTR-102, irinotecan, SN38, PEG-SN38, and doxorubicin levels. As anticipated, PLD treated rats had depressed body weight gain by 7 days post-dose administration and decreased white blood cell counts due to a decrease in lymphocytes compared to the concurrent vehicle control. These effects completely recovered by 21 days post dose. PLD-related splenic hypocellularity was observed 21 days after dosing. Of note, NKTR-102 at up to 540 mg/m² did not exacerbate the hematologic or body weight changes caused by PLD. NKTR-102 in combination with PLD (30 mg/m²) increased the incidence and severity of splenic hypocellularity observed with 30 mg/m² PLD alone only at the highest dose of NKTR-102 at 540 mg/m². Exposure levels of PLD and NKTR-102 and NKTR-102 metabolites were similar to that observed in previous studies. In conclusion, NKTR-102 does not exacerbate the hematologic effects (up to 540 mg/m²) or splenic hypocellularity (up to 180 mg/m²) caused by a single 30 mg/m² dose of PLD at SN38 levels that are more than 70-fold (based AUC at 180 mg/m²) of that observed at NKTR-102 dose of 145 mg/kg in humans.


d'isolement deSu témoin de la dose optimaleIVO de NKTR-102 aminé d'une demande au moins 70% de la dose maximale recommandée. L'hémogramme a montré une diminution des leucocytes et des plasmocytes, avec une augmentation des granulocytes et des plaquettes.

2270 TOXICOLOGICAL ASSESSMENT OF A SPLEEN TYROSINE KINASE INHIBITOR, CC-118, IN CYMOMOLGUS MONKEYS.


Inhibition of the spleen tyrosine kinase (Syk) enzyme has been advocated as therapy for autoimmune disease, due to the important role of Syk in signaling downstream from immunoglobulin receptors on cells integral to the inflammatory process. CC-118 is a small molecule selective kinase inhibitor with high potency against the Syk target (enzyme IC50 of 7 nM) and slightly less potency for the Janus kinase 2 (Jak2; enzyme IC50 of 30 nM) and colony stimulating factor-1 receptor (CSF1-R, enzyme IC50 of 28 nM) targets. The objective of this study was to determine the effects of CC-118 in cynomolgus monkeys (1/sex/group) when dosed once daily via oral gavage for 7 days. In male and female monkeys dosed with 0, 100, 500, and 1000 mg/kg/day, doses of 500 and 1000 mg/kg/day were well-tolerated and results were generally similar between males and females. In both sexes, colored skin (red) and hunched posture were noted at ≥100 mg/kg/day. Hematological changes included decreased red blood cell count, hemoglobin, hematocrit, lymphocytes, and eosinophils and increased neutrophils and platelets at ≥100 mg/kg/day. Increased serum alanine and aspartate transaminases and decreased alkaline phosphatase, calcium, and phosphorous were observed at ≥100 mg/kg/day. No alterations in coagulation tests were observed. At necropsy, red discoloration was observed on multiple organs in all dose groups and microscopic changes consisted of multi-organ acute hemorrhage, gastric erosion/ulceration, and lymphoid depletion (thymus and spleen) at ≥100 mg/kg/day.

2271 PRECLINICAL DEVELOPMENT OF BIOMIMETIC ACTINIDE DECORPORATION AGENTS.


In the event of incidents associated with a radiological dispersal device or after a natural disaster affecting nuclear power plants or nuclear material storage sites, therapy to a large population at risk may require the administration of chelating agents that are effective in decorporating a variety of radionuclides. Independent of the route of contamination, radionuclides, including actinides such as plutonium, americium, curium, uranium and neptunium are likely to be absorbed and transported to target organs, such as bone, liver or kidney, in which they are stored or slowly excreted through the urine or feces. These alpha particle emitters pose significant health hazards, especially in the target tissues, due to their ionizing radiation properties. A family of sequestering agents has been identified by a team of scientists at the Glenn T. Seaborg Center in the Chemical Sciences Division of the Lawrence Berkeley National Laboratory, using a biomimetic design based on bio-chemical transport properties of plutonium and iron. Among the family members, two lead hydroxypyridinone-based (HOPO) ligands, 5-LIO(Me-3,2-HOPO) (a tetradentate ligand) and 3,4,3-LI(1,2-HOPO) (an octadentate ligand) have emerged showing superior efficacy in chelating actinides with excellent preclinical properties. These chelators possess targeted selective properties for a number of actinide metal ions with specific higher affinity for plutonium compared to the current therapeutic alternative available in the marketplace. A large number of decorporation efficacy studies were conducted in mice and dogs showing superior nuclide-specific decorporation when compared to the currently approved DTPA. Results from the preclinical safety studies, including Ames and CHO genotoxic in vitro studies, as well as in vivo maximally tolerated dose (MTD), pharmacokinetics (PK), and 28-day safety toxicology studies will be presented at the meeting. This work is supported by NIH/NaID Grant # 1R21AI087604-01.

2272 THE DESIGN OF CHRONIC TOXICOLOGY STUDIES OF MONOCLONAL ANTIBODIES: IMPLICATIONS FOR THE REDUCTION IN USE OF NONHUMAN PRIMATES.


The changing environment of monoclonal antibody (mAb) development is impacting on the cost of drug development and the use of experimental animals, particularly nonhuman primates (NHPs). The drive to reduce these costs is huge and involves rethinking and improving nonclinical studies to make them more efficient and more predictive of man. As our knowledge base on mAbs expands, the information can be used to improve drug development and maximise the output of experimen-mental data. Cross-company data-sharing of nonclinical study decisions and designs in a noncompetitive way can establish an evidence base to influence regula-tory data. On GLP regulatory toxicology studies for 58 mAbs were shared from ten companies covering a wide range of therapeutic areas. These data have been used to investigate current practice and identify study designs that minimise NHP use. Our analysis shows that there is variation in the number of animals used for similar studies. This information has been used to develop practical guidance and make recommendations on the use of science-based rationale to design studies using fewer animals taking into account the current regulatory guidance. These changes can be used to improve animal welfare and reduce costs. These changes have been implemented in the NC3Rs guidelines.

2273 NONCLINICAL SAFETY EVALUATION OF MEDI-573, A DUAL-TARGETING ANTI-INSULIN-LIKE GROWTH FACTOR (IGF)-I AND IGF-II MONOCLONAL ANTIBODY IN CYMOMOLGUS MONKEYS.

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MEDI-573, a human immunoglobulin G2 lambda (IgG2a) monoclonal antibody (Mab), is a dual-targeting human antibody that neutralizes the insulin-like growth factor (IGF)-I and IGF-II ligands, thus inhibiting insulin-like growth factor recep-
tor (IGF-1R) as well as insulin receptor A isoform (IR-A) signaling pathways, but not insulin receptor B (IR-B) signaling, in cancer cell lines expressing these receptors. MEDI-573 is the only mAb currently in clinical development that targets IGF ligands instead of the IGF receptor. The results of in vitro pharmacology studies have shown that MEDI-573 inhibits both IGF-I and IGF-II-stimulated phosphorylation of IGF-1R and that of downstream signaling proteins, including protein kinase B (Akt) and mitogen-activated protein kinase (MAPK), in a number of engineered NIH-3T3 cell lines transfected to express human IGF-1R and either human IGF-I or -II. MEDI-573 has a 10-fold reduced affinity for murine IGF-I and adult mice do not produce IGF-II, thus limiting the assessment of in vivo anti-tumor activity in mouse tumor models. In support of clinical development, the potential toxicity, toxicokinetics, and pharmacodynamics (decrease in serum free IGF-I and IGF-II levels) of MEDI-573 were evaluated in single- and multiple-dose toxicity studies in cynomolgus monkeys which were the relevant toxicology species. Following 30-minute intravenous (IV) infusion administration of up to 60 mg/kg/week of MEDI-573 for 13 weeks in cynomolgus monkeys, there were no adverse changes attributable to dose or drug treatment; no toxicity was observed in serum or urine level attributed to MEDI-573 administration. A dose-dependent decrease in serum free IGF-I and IGF-II levels was observed, demonstrating that MEDI 573 was pharmacologically active at or above the serum concentration needed to fully suppress the free IGF-I and IGF-II ligands. 

**FUNCTIONAL MEASUREMENTS AND REPEAT-DOSE RELATIONSHIPS BETWEEN CARDIOVASCULAR FUNCTIONAL EFFECTS AND OCULAR PHOTOTOXICITY.**

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The eye, by its very function, can be a target of xenobiotic-associated phototoxicity caused by ultraviolet radiation (UVR)in some instances visible light (VL). While cutaneous phototoxicity is easily identified by erythema, edema and flaking of the skin, phototoxicity to the eye, preclinical ocular phototoxicity can be assessed using ophthalmological and histopathological evaluation of the eye is necessary to accurately access this damage. Absorption of light by the various ocular structures also alters the incident spectrum on successive structures, as UVR is absorbed during its passage through the eye, with the outer structures providing protection for the deeper structures, and affects potential ocular damage in a deeper structure. Using the Long-Evans rat model and the phototoxin 8-methoxypsoralen, characteristic lesions were identified in eye tissues that are predictive and diagnostic of ocular phototoxicity. In the cornea, degenerative epithelial changes and changes consistent with inflammatory responses are common, and may be associated with extension of inflammation into the anterior chamber and iris. Damage to the lenticular epithelium and lens fibers frequently results in evidence of early cataract formation. Also, damage due to UVR exposure alone (photic) or procedural changes must be recognized and figured into evaluation of changes in the eye that may be related to test material-induced phototoxicity. Foci of retinal degeneration are not uncommonly found in the target area of the UVR in both vehicle control and treated rats—these are considered to be indicative of photic, rather than phototoxic, injury. Little, if any, damage is seen using standard fixation and processing in the ciliary body, vitreous chamber, choroid, sclera and optic nerve. The totality of the histopathological changes in the eye, along with ophthalmological and the cutaneous observations, are critical in evaluation of the phototoxic potential of a test material and assessing its overall phototoxic risk.

**MULTIVARIATE PRECLINICAL OBSERVATION PROFILES—TWO FINDINGS ARE BETTER THAN ONE.**

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All compounds reaching clinical trials and finally the market have a rigorous amount of supporting preclinical data. Still, safety related late stage attrition continues to be a major hurdle in drug development. Here we present how observational profiles derived from public and internal sources can be used to derive translational models for different endpoints, exemplified with nausea and dizziness. Nausea is a subjective human sensation, and little is known about any preclinical biomarkers. Preclinical findings such as vomiting and salivary hypersecretion have been suggested as markers for nausea. While often accurately predicting the true nausea compounds, there is a high proportion of non-nausea compounds also associated with these observations. To investigate if a set of preclinical observations could better predict the presence or absence of nausea, a set of 86 marketed drugs were collected together with their publicly available preclinical gastrointestinal (GI) observations. The observations were transformed into a preclinical profile and the drugs were then clustered based on these profiles. A clear separation of nausea and non-nausea compounds was then achieved, and it was seen that the risk of nausea was greatly increased if vomiting was observed together with salivary hypersecretion. To validate the findings a set of 20 AstraZeneca compounds reaching clinical trials was created and the associated preclinical GI observations collected. The set was blinded before prediction and a prediction accuracy of 90% was achieved for both nausea and non-nausea compounds, a prediction made without any need for additional animal testing. We also present the results from a similar approach applied to the prediction of dizziness. The results from these studies highlight some of the benefits of looking at combinations of preclinical observations.
2278 CONCORDANCE BETWEEN NONCLINICAL AND CLINICAL TOXICITY DATA DETERMINED BY A SURVEY OF APPROVED PHARMACEUTICALS IN JAPAN.


Great attention has been paid on the concordance between nonclinical and clinical toxicity data among toxicologists. There have been, however, only few publications, and the latest one is reported by Olson et al. (2000) using data from pharmaceutical candidates mainly with development being terminated. Toxicity data of pharmaceuticals which were approved as new ingredient during the period from 1996 to 2010 in Japan have been collected in this study. Adverse events with the incidence of > 5% in humans were selected from package inserts and their corresponding toxicity findings in animals were explored in CTDs (Common Technical Documents) and review reports for marketing approval application. The results demonstrated that the concordance rate was approximately 36% in the approved pharmaceuticals. Interesting findings from the investigation include 1) Concordance of common toxicity parameters between humans and animals is high, 2) Characteristic trends are obtained by therapeutic drug classes and organs/tissues where adverse reactions occurred, and 3) Concordance of bio-pharmaceuticals is lower compared with that of small chemical pharmaceuticals. These evaluation results of concordance between clinical adverse events and toxicity findings in animals are considered useful to confirm the appropriateness of the nonclinical studies for pharmaceuticals as well as to know the extrapolation of nonclinical study data to clinical adverse events. More detailed investigation is needed to clarify the relatively low concordance of bio-pharmaceuticals found in our research. Reference: Olson, H., Betton, G., Robinson, D., Thomas, K., Monro, A., Kolaja, G., Lilly, P., Sanders, J., Sipes, G., Bracken, W., et al. (2000). Concordance of the toxicity of pharmaceuticals in humans and in animals. Regul. Toxicol. Pharmacol. 32, 56-67.

2279 IMAGING AND ASSESSMENT OF CORNEAL VESSELS IN A RAT SUTURE MODEL FOR NEOVASCULARIZATION USING A CONFOCAL SCANNING LASER OPHTHALMOSCOPE (CSLO).

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In response to ocular insult, corneal transplant, or disease, blood vessels can grow into the normally avascular cornea reducing visual acuity or even resulting in complete blindness. Several animal models of corneal neovascularization (NV) have been developed, including placement of silk sutures within the corneal stroma to generate an inflammatory response to a foreign protein. These models have been used to evaluate the efficacy of compounds with potential anti-angiogenic or anti-inflammatory properties. Traditionally, corneas are evaluated over a serial time-course using immunohistochemical (IHC) assays that look for blood vessel and/or lymphatic vessel development. In order to reduce the number of animals necessary to evaluate the time-course of vessel growth/regression, a CSLO was used to image the progression of corneal vessel growth in the same animals over 14 days. Three silk sutures were placed in the cornea of one eye at 120º intervals in 8 Sprague-Dawley rats to stimulate NV. Digital images of the treated and untreated corneas were captured using a CSLO, prior to and following intravenous injection of sodium fluorescein, 7 and 14 days after suture placement. Images were loaded into image processing software and the penetration of blood vessel growth quantified. Both the non-fluorescein and fluorescein methods resulted in high resolution images with clear delineation of vessel growth. The fluorescein images provided better contrast and therefore easier measurement. After 7 days, corneal vessels penetrated approximately 20-40% from the limbus to the stroma (1-2mm) and by Day 14, most had reached the sutures (4mm/100% of the distance). Occasionally a suture dislodged in the early phase of the study resulting in no vessel growth within the cornea. In conclusion, the CSLO generated clear quantifiable images of blood vessel growth and allowed follow-up in the same animal over time, reducing the number of animals required for the traditional time-course study.

2280 USE OF SPECTRAL-DOMAIN OPTICAL COHERENCE TOMOGRAPHY (SD-OCT) TO MONITOR SUBRETINAL ADMINISTRATION IN ANIMAL MODELS.

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Spectral-Domain Optical Coherence Tomography (SD-OCT) systems allow high speed and high resolution cross-sectional imaging of the retina and were developed as non-invasive diagnostic devices for imaging the human retina and to aid in assessment and management of posterior segment diseases (e.g. AMD, diabetic retinopathy, glaucoma). However, this instrument can also be used to monitor animal retinas in vivo both prior to and following subretinal administration on preclinical toxicity studies. The objective of this study was to monitor the transient physical separation of photoreceptors from the underlying RPE and choroid following subretinal administration. Left or right eyes of 15 male Göttingen minipigs received a single dose of 50 or 100 µL of a saline solution (PBS or BSS+) via transvitreal or transcleral subretinal administration. SD-OCT was performed immediately following dosing on Day 1, then on Days 7 and 14/15, as well as 1, 2 and 3 months following the dosing procedure. Retinal detachment at the dosing site (bleb) was noted during intervention but not all blebs immediately following administration on Day 1. These blebs had resolved in a majority of eyes by Day 7 and were no longer present in all eyes by the end of the 3-month observation period. Retinal folds/rosettes created during reattachment of the retina were observed in a few eyes. Additional observations included intraretinal multifocal punctate densities, subretinal material and focal retinal vasculature. These findings were generally noted early in the study (on Days 1 or 7) and remained stable until the end of the study. No retinal thinning was observed. These results demonstrate that SD-OCT is a useful in vivo tool for visually confirming subretinal administration in animal models, as well as monitoring changes in retinal structure over time.

2281 SAFETY PHARMACOLOGY IN THE GötTINGEN MINI-PIG: CARDIOVASCULAR, RESPIRATORY, AND NEUROLOGICAL INVESTIGATIONS.

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The Göttingen minipig has become a routinely used species in regulatory toxicology. Many similarities between humans and minipigs justify the use of this species for non-clinical safety assessments. In the context of safety pharmacology core battery, cardiovascular (telemetry), respiratory (spirometry) and neurological (functional observation battery) investigations were conducted in Göttingen minipigs with various positive control drugs. Cardiovascular modulators (dopamine, remifentanil, esmolol, medetomidine and xylazine) were administered to freely moving animals. Intravenous methacholine was used as a respiratory modulator. Amphetamine and xylazine were used as positive control drugs for neurological evaluations. A vehicle/control administration of saline was used with all 3 models to establish baseline. Dopamine was associated with a dose-dependent chronotropic and inotropic response. Remifentanil induced a dose-dependent QT shortening with positive chronotropic effects. A biphasic hypertensive followed by hypotensive response was observed with medetomidine while xylazine was associated with QT prolongation. Methacholine induced a dose-dependent increase in respiratory rate, tidal volume and minute volume (high dose +260%). FOB identified amphetamine-induced stimulatory effects and xylazine induced central depression. In conclusion, our results suggest that the Göttingen minipig is a suitable species to fulfill all aspect of the safety pharmacology core battery.

2282 DEVELOPMENT OF A GROUP-HOUSED, AMBULATORY, SURGICALLY CANNULATED, INTRAVENOUS INFUSION MODEL IN CYMONOLGUS MACAQUES (MACACA FASCICULARIS).

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The primate is often the appropriate animal model for safety testing of biopharmaceutical products and therapies, which are commonly administered by intermittent or continuous intravenous infusion. The aim of this study was the development of a regulatory acceptable ambulatory, surgically cannulated, intravenous infusion model in healthy naïve cynomolgus monkeys. The ambulatory model was chosen as this reduces restraint allowing group housing of animals, thereby significantly improving the wellbeing of the animals and reducing the effects of stress that could influence the interpretation of test article changes. The study was designed to reflect a regulatory compliant toxicology study of 28 days or longer and included the assessment of clinical signs, bodyweight, clinical pathology, organ weights and histopathology. Four cynomolgus macaques were trained to wear jackets and surgically prepared with a vascular access port (VAP) cannulated into the vena cava via the femoral vein. The animals were infused with 0.9% NaCl (up to 18 ml/animal/day) using either a continuous or intermittent administration regime. The minor port patency issues observed during the study were overcome by simple strategies. The data collected showed no adverse effects on clinical signs, body weight pattern, clinical pathology or organ weights. Histopathology consisted of minimal inflammation, fibrosis, thrombosis and thickening associated with the tissue surrounding the VAP and cannula tip. All findings were consistent with the histor-
pharmacological effects on sleep and arousal. Video-EEG monitoring appears to be a non-rodent model for studying these effects in conscious cynomolgus monkeys (Macaca fascicularis) with continuous video-EEG monitoring using telemetry. Circadian changes in EEG spectral band power (delta 0.5-4 Hz, theta 4-8 Hz, alpha 8-12 Hz, sigma 12-16 Hz and beta 16-24 Hz) were correlated with the sleep cycle in all animals. When compared with saline, caffeine significantly increased total and relative power of beta frequencies (16-24 Hz) which correlated with a decrease in sleep time. The effects of caffeine on absolute and relative power at frequencies ranging from 1 to 127 Hz (1 Hz increments) were evaluated to identify the most sensitive range to caffeine-induced effects. A decreased power in lower frequencies (1 to 13 Hz) was noted with progressively shorter effects for higher frequencies in this range and peak effect (relative and absolute) at 2 Hz. An increase of all frequencies above 15 Hz was also observed as expected for a CNS stimulant drug with a peak at 107 Hz observed 70 min after dosing. When non human primates are justified, video-EEG monitoring appears to be a useful non-rodent model to confirm pharmacological effects on sleep and arousal.

**2284 NOVEL TELEMETRIC APPROACHES TO MONITORING BLOOD PRESSURE IN A RODENT MODEL OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE.**

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Cardiovascular changes and disease are commonly associated with chronic obstructive pulmonary disease (COPD). A primary cause of both cardiovascular disease and COPD is smoking cigarettes. Cardiovascular symptoms may appear in the form of atherosclerosis, myocardial infarcts and/or systemic hypertension. We have found that chronic exposure of the spontaneously hypertensive rat to tobacco smoke results in COPD-like lung inflammation, airway obstruction, mucus hypersecretion and emphysematous changes, but very little is known regarding the cardiovascular impacts. This study compares changes in systemic blood pressure and heart rate in the spontaneously hypertensive (SH) and the normotensive Wistar Kyoto (WKY) rat models during exposure to tobacco smoke for up to 12 weeks. This comparison was done to determine if a hypertensive state would be aggravated by exposure to tobacco smoke. Rats were surgically implanted with a telemetry device and sensor into their abdominal aorta to continuously measure systemic blood pressure and heart rate. As expected, baseline systemic measures of blood pressure were significantly different in SH and WKY rats. However, repeated exposure to tobacco smoke by SH rats resulted in an initial elevation in systolic pressure that was attenuated with continued exposure, while WKY rats had significant elevation in systolic pressure during all smoke exposure days. Diastolic pressure was significantly and consistently elevated in SH rats with repeated exposure to tobacco smoke. Both heart rate and WKY rats had significantly reduced mean pulse pressure and significantly increased heart rate due to tobacco smoke exposure, suggestive of early signs of compromised heart function. These findings demonstrate telemetric approaches can be implemented to detect and document novel and progressive cardiovascular impacts of tobacco smoke under experimental settings.

**2285 COMPARATIVE ANALYSIS OF GENE EXPRESSION PROFILES FOR HEPATIC CYTOCHROMES P450 IN CYNONOMULUS MONKEYS BRED IN CAMBODIA, CHINA, AND INDONESIA.**


Cynomolgus monkeys (Macaca fascicularis) are widely used in preclinical research including drug-metabolism, toxicology, and pharmacology studies. Cynomolgus monkeys are bred in countries such as Cambodia (MacfaCAM), China (MacfaCHN), and Indonesia (MacfaIDN). However, expression profiles of hepatic genes have not been fully investigated in these monkeys. To compare hepatic gene expression profiles for MacfaCAM, MacfaCHN, and MacfaIDN, total gene expression profiles in liver samples (pooled from 5 animals) were analyzed by DNA microarray. Furthermore, gene expression levels of cytochrome P450 (CYP) 1-3 families, important for drug-metabolism, were confirmed by quantitative polymerase chain reaction using individual liver samples; MacfaCAM (n=20), MacfaCHN (n=17), and MacfaIDN (n=8). Hierarchical clustering analysis of total hepatic gene expression profiles showed that the expression profile of MacfaCAM was more closely related to that of MacfaCHN than to that of MacfaIDN. Mean expression levels of 14 CYP genes did not differ significantly (> 2.5 fold) between MacfaCAM, MacfaCHN, and MacfaIDN. In addition, substantial sex differences (> 2.0 fold) of CYP gene expressions were not observed in MacfaCAM. These results partly reflect the similarity in genetic background between MacfaCAM and MacfaCHN, as has been shown by mitochondrial DNA analysis. This could be accounted for by the notion that the colonies of cynomolgus monkeys bred in China were originally founded by monkeys from the same origin as MacfaCAM. Importantly, further analysis of metabolic activities measured using eight CYP substrates did not show substantial differences between MacfaCAM and MacfaCHN, partly reflecting similarities between MacfaCAM and MacfaCHN CYP genes. In conclusion, these data suggest that the expression profiles of hepatic genes, including CYP genes, in MacfaCAM and MacfaCHN are closely related.

**2286 HEMATOLOGY AND BLOOD BIOCHEMISTRY CHANGES RELATED TO AGE IN GÖTTINGEN MINIPIGS.**

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Minipigs are increasingly used as nonrodent models in nonclinical research. They are used in a broad range of studies and at a wide variety of ages and stages of development. In our laboratories, we use Göttingen minipigs of ages varying from neonatal up to around two years depending on the type of study. Early development of young Göttingen minipigs is rapid. From birth, their eyes are open and they are able to move around freely. By 4 weeks of age they are weaned and from around 4 to 6 months of age they reach sexual maturity. Despite this rapid growth and early maturity, they continue to grow during the first 2 years or so of their lives and an animal at the end of a chronic toxicity study will be significantly bigger and more developed than at the start. This prolonged growth period is also reflected by other changes in the animals other than just increase in size. All data generated during toxicology studies need to be interpreted, and findings need to be put into context using both concurrent control and historical data. We were interested to see how the routine parameters that we measure in toxicology studies change over time. We present a significant amount of control data from minipigs covering a range of ages from soon after birth up to nearly 18 months. These data have been reviewed to highlight changes that could be expected to be seen over time. In any study the control group provides a good comparison with the treated animals, but from time to time there will be results that need a deeper explanation, therefore background data is of value. Our study of the data indicates that for certain parameters, e.g. those relating to red blood cells, there are significant changes over short times, especially in the younger animals. However it is also the case that the values for other parameters (e.g. protein and creatinine) continue to change with age even in mature animals. These results demonstrate that a good database of age-matched results is a major requirement to support interpretation of findings when performing nonclinical toxicology studies in the Göttingen minipig.
2288 INTERSPECIES COMPARISON OF TOXICO-PATHOLOGICAL FINDINGS INDUCED BY 4-WEEK REPEATED EXPOSURE TO DICLOFENAC IN DOGS AND MICROPIGS.

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Dogs are most commonly used animal model for non-rodent studies in drug development. However, increased ethical concerns and drug over sensitivity have been critical issues. Micropigs have appeared as a new non-rodent model, but its weight could reach 30-35kg in long term studies causing difficulties in many aspects. We introduce PWG Micropig, much smaller than conventional minipigs, as a more plausible and efficient model for non-clinical studies. This study was to compare the toxicological responses of dogs and micropigs in a 4-week repeated exposure to diclofenac (DCF) known to cause hepato and renal toxicity as well as over sensitivity in dogs. Nine male beagle dogs and nine male micropigs were divided into 3 groups each and treated with 0 (vehicle control), low and high dose of DCF. Doses of 1 and 3 mg/kg/day were selected for dogs and 3 and 15 mg/kg/day for micropigs based on the preliminary 2-week study. In dogs, there were dose-related reduction of red cell parameters and elevation in total white blood cell counts. Significant reduction of alanine aminotransferase, alkaline phosphatase, total bilirubin, gamma-glutamyl transferase, total protein, albumin, and albumin/globulin ratio were noted at 3 mg/kg/day. In macroscopic examinations, perforation of stomach was observed in all animals treated orally.

2289 FEASIBILITY AND SAFETY EVALUATION OF AMBULATORY INFUSION IN SOCIALIZED CYNOMOLGUS MONKEYS FOR PRECLINICAL SAFETY STUDIES.


Increasing regulatory pressure for animal welfare improvement in safety studies has encouraged group housing of monkeys. Such requirements may become challenging in long term intravenous (IV) infusion studies which are usually conducted in tethered-restrained animals. The development of ambulatory pumps should allow socialization of monkeys, but it should be ensured that their natural exploratory behavior could lead to infusion setup dismantling, thus placing regulatory studies at risk.

We investigated the suitability of an ambulatory IV infusion method for use in a regulatory 4 week toxicological study in the monkey. We assessed standard toxicology endpoints and compared the results with background data from tether infused monkeys. Six female cynomolgus monkeys (Macaca fascicularis) were continuously infused (4.5 mL/h) by a peristaltic pump with physiological saline (0.9%) contained in a pouch, at a rate of 1 mL/kg/h through a catheter implanted in the vena cava. The distal tip exited at the neck level and was connected to a peristaltic pump (Pegasus Light, LAB). The pump and pouch assembly (approximately 200 g) was protected by a polysytrene case contained within a jacket worn by the monkey. Monkeys were socialized by group of three except when individual handling was needed. Usual toxicology in-life endpoints (growth, food intake, clinical pathology, cardiovascular and ophthalmological examinations) were assessed. Tissue sections of the catheter path, lungs, liver, spleen, heart and kidneys were histopathologically evaluated. All animals withstood their own infusion assembly and did not dismantle their connectors. There was no decrease in animal welfare as judged by exploratory behavior. The few histopathological lesions were considered to be those normally encountered with this route of administration and comparable with in house data generated from the tethered monkey model. We demonstrated that the use of an ambulatory pump allows socialization of cynomolgus monkeys in an intravenous infusion safety study, improves their welfare and does not compromise the outcome of the study.

2290 INVESTIGATIVE EMBRYOTOXICITY STUDY IN THE GOETTINGEN MINI-PIG BY INTRAVENOUS INFUSION USING AN AMBULATORY PUMP.

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The objectives were to test the feasibility of performing intravenous (IV) infusion using an ambulatory pump in pregnant Göttingen minipigs and to compare findings with our historical control data (HCD) from animals treated orally. Four female Göttingen Minipigs were received shortly after mating and then housed in pairs. A polyurethane catheter was implanted under isoflurane anesthesia into a femoral vein of each animal the day after arrival. The exteriorized catheter ran to a 150 mL plastic pouch containing physiological saline (PPS) and a programmable pump (Pegasus Light). Animals were infused at 1.0 mL/h with PPS following implantation. The pump and pouch (weighing about 200 g) were enclosed in a polysytrene case held in a jacket worn by the animals. Dosing was simulated by infusing the animals with PPS at 5 mL/kg/day from days 11 to 35 of gestation. Maternal clinical condition and body weight were monitored during the study. At necropsy, the females were examined macroscopically and litter parameters were recorded. The fetuses were weighed, sexed, examined for external and visceral abnormalities and processed for skeletal examination.

There were no notable infusion incidents or maternal observations. At C-section, all 4 females were pregnant with viable fetuses. The mean number of implantations was slightly lower compared with the HCD from oral studies. This could be indicative of a slightly increased pre-implantation loss due to the surgical procedure. There was no impact on subsequent embryo-fetal survival and no remarkable differences from the HCD amongst the fetal observations.

In conclusion, IV infusion via a surgically implanted catheter and ambulatory pump is possible in the pregnant minipig but slightly increased pre-implantation loss may be related to the surgery. At the dose volume used, the infusion was innocuous with respect to embryo-fetal survival and morphological development and is therefore suitable for use in embryotoxicity studies. Animal welfare is improved with respect to a tethered infusion system, since the animals can move freely and be group housed.

2291 SIX-WEEK EXPLORATORY STUDY ON VIGABATRIN-INDUCED RETINAL TOXICITY IN PIGMENTED (LONG EVANS) RATS.

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The study was performed to respond to a regulatory request to replicate retinal toxicity in the Long Evans (LE) pigmented strain using combinations of high intensity light (HIL) exposure, mydriasis and oral administration of Vigabatrin, a GABAergic agonist. The rats received combinations of light (normal or HIL at approximately 2,000 lux for 12 hours per day), Mydriasis (1 % atropine twice per week) and oral Vigabatrin (200/150 mg/kg/day). Retinal toxicity was assessed functionally by electro-retinography (ERG) elicited by different flash and flicker stimulations given under scotopic and photopic conditions and by histopathological examination after 1, 3 or 6 weeks. Functionally, in LE rats exposed to HIL and given mydriasis the changes were similar under both scotopic and photopic conditions and included altered ERG morphology, increased implicit time and decreased amplitude. These changes are consistent with retinal degenerative processes described in the literature. Microscopic lesions included degeneration of the photoreceptor layer and outer nuclear layer and, in some animals, degeneration in the outer plexiform layer and inner nuclear layer. Vigabatrin alone (without mydriasis) was not associated with functional or microscopic changes in the retina nor was HIL alone (without mydriasis) sufficient to induce retinal changes. LE rats exposed to HIL and given mydriasis had both functional and histopathological changes as above but in those animals also treated with Vigabatrin, the changes occurred earlier and were characterised microscopically by multifocal wavy appearance and rosette formation in the outer nuclear layer of the retina from week 3 of the study. Non-pigmented Sprague Dawley rats were also exposed to HIL and given mydriasis and had similar functional changes and less severe histological changes than in LE rats. In conclusion, the LE rat under the defined experimental conditions is a good model to demonstrate retinal changes induced by Vigabatrin.
The purpose of this project was to develop a model of glaucoma via surgical induction of increased intraocular pressure (IOP) in Yucatan miniature swine. Three pigs (two female, one male) had bilateral IOP measurements performed prior to surgical intervention to establish a baseline IOP for each animal from which future changes in IOP could be identified. IOP measurements (mm Hg) were taken with a Tonopen Vet Tonometer. In order to reduce venous drainage from the eyes, episcleral veins were scarified by cauteration in each eye. IOPs were periodically measured for several weeks post-cauterization surgery. Pharmacologic intervention was monitored for several weeks post-cauterization surgery. Pharmacologic intervention was then instituted with a commercially available synthetic prostamide analog with ocular hypotensive activity. Drops were applied once daily, and IOPs continued to be measured. After 7 weeks of daily treatment, eye drops were discontinued, and IOP measurements continued to be obtained. All animals presented with significant increases in IOP measurements post surgical intervention and significant decreases in IOP with pips. With this ocular hypertension model, the UVR exposure in the Yucatan miniature swine eye is also responsive to pharmacological therapy to reduce IOP and as such could be a potential model for future pharmacological research.

**2293 MINIATURE SWINE MODEL OF PHOTOTOXICITY TESTING.**

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Background/Purpose: This study determined the threshold doses for “solar erythema” and for phototoxic responses to 8-methoxypsoralen (8-MOP) in white skin Hanford and grey skin Yucatan miniature swine. Methods: For threshold erythema determinations, the UVR exposures included both UVA (315 – 400 nm) and UVB (290 – 315 nm) radiation by positioning one fluorescent ‘sunlamp’ among 10 piglets. For phototoxic toxicity responses, the UVB exposures included maximum UVB exposure (1.0-1.4 times the “instrumental MED” (MED) for Hanford and from 1.0-5.6 times the MEDi for Yucatan. For phototoxidrity determinations (i.e., with and without topically-applied graduated concentrations of 8-MOP), the UVB component was minimized by extinguishing the sunlamp, thus permitting higher UVA exposures. Results: The Hanford had the lower UV erythema dose threshold (1.0-1.4 times the MEDi) and the erythema that developed was readily observable. The exposure doses for the phototoxicity test were SJ/cm2 of UVA in 35 minutes or 10/fcm2 in 70 minutes. The phototoxic (vascular) response to 8-MOP was observed in the two highest concentrations (0.01% and 0.1%) in Hanford pigs, in a dose-related manner. Microscopic evidence of a dose-related response was also observed as the concentration of 8-MOP increased. Conclusion: This verified that the Hanford miniature swine is the preferable strain for phototoxic effects. In contrast, UVR exposure of the Yucatan pig skin produced tanning rather than erythema, confirming that the Yucatan is the more appropriate strain for studying the melanization response. For comparison, the Yucatan miniature swine provided nevi that remained after the UVR exposure in the our laboratory.

**2294 A PROOF OF CONCEPT STUDY FOR EVALUATING ANTIHYPERTENSIVE COMPOUNDS IN AWAKE, CHRONICALLY-INSTRUMENTED, PHARMACOLOGICALLY HYPERTENSIVE MONKEYS.**

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Systemic arterial hypertension is of epidemic proportions (~30% of the US population, ~1 billion, Worldwide) and results in significant morbidity and mortality. Approximately one-half of Americans have hypertension under control. Thus, there is enormous interest by, and value to, the pharmaceutical industry to develop safe and efficacious drugs to manage systemic arterial hypertension. Proof of safety and efficacy is relevant to the arena of Safety Pharmacology, and preclinical studies conducted using infrahuman mammalian surrogates for man are of obvious importance. Whereas most of these studies have been conducted on rodents and dogs, studies on non-human primates are appealing because of anatomical and physiologic similarities between them and man. This proof-of-concept study was conducted on awake, chronically-instrumented cynomolgus monkeys with systemic arterial hypertension produced by constant-rate infusion of angiotensin-II. After production and maintenance of systemic arterial hypertension, monkeys were given escalating doses of sodium nitroprusside to demonstrate the usefulness of the model for evaluating a known antihypertensive. Additionally, we established baroreceptor gain from the change in heart rate vs the change in blood pressure.
2297 POSSIBLE DETOXIFICATION MECHANISMS OF 2-CHLOROETHANOL INTOXICATION.

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2-Chloroethanol (2-CE, ethylene chlorohydrin, CAS 107-07-3) is used as an intermediate in the synthesis of ethylene oxide, and the metabolite of halogenated hydrocarbons. In Taiwan, some farmers apply 2-CE to grapevines to hasten sprouting. 2-CE poisonings and fatalities have been reported following oral exposure, inhalation or skin absorption. 2-CE exposure can result in severe metabolic acidosis, coma, respiratory failure, hypotension, and death after acute exposure. Chloroacetaldehyde (CA) is metabolized via the metabolism of 2-CE as alcohol dehydrogenase and catalyzes an increase in the intracellular CA levels, and is also found in blood. Recently, we found fomepizole, an alcohol dehydrogenase inhibitor can reduce 2-CE toxicity in rat. In this study, we found potential antidote fomepizole combined with N-acetylcysteine can protect 2-CE induced liver junctional separation in rat. And the liver cytochrome P450 2E1 protein level was significantly increased after fomepizole combined with N-acetylcysteine pre-treatment group in rat. Pre-treat human hepatocyte cell line with fomepizole and combined with N-acetylcysteine can increase cell viability in 2-CE (from 22.63% to 85.65%) and CAA (from 6.14% to 90.74%) intoxication cells. Otherwise, combination usage of two antidotes will be better than single treatment. 2-CE detoxification may mediate stopping CAA formation by alcohol dehydrogenase inhibition and CYP2E1 over-expression in liver.

2298 CONTINUOUS CARCINOGENIC SUSCEPTIBILITY MONITORING OF CB6F1 Tg RASH2 MICE.


CByB6F1-Tg(HRAS)2Jic (rasH2) mice are known to have a high susceptibility for human carcinogens. At present, this strain of mouse is produced by two breeding facilities, Taconic (Germantown, NY) and CLEA Japan, Inc. (Shizuoka, Japan), and supplied worldwide. We periodically compare the carcinogenic response of the mice produced by the two facilities to N-acetylneuraminic acid (NANA) and combined with N-acetylcysteine can increase cell viability in 2-CE (from 22.63% to 85.65%) and CA (from 6.14% to 90.74%) intoxication cells. Otherwise, combination usage of two antidotes will be better than single treatment. 2-CE detoxification may mediate stopping CAA formation by alcohol dehydrogenase inhibition and CYP2E1 over-expression in liver.

2299 IN VIVO EXPRESSION OF P16INK4A IN RESPONSE TO TOXICOLOGICAL EXPOSURES.

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In mammals, expression of p16INK4a is highly regulated. Excess expression can lead to cellular senescence and aging, while impaired activation is associated with cancer. The precise mechanism of p16INK4a regulation in vivo is poorly understood. In vitro systems have limited utility since proliferation in culture induces p16INK4a. Both extrinsic (chemotherapy and ionizing radiation) and intrinsic (telomere shortening and improper DNA damage repair) stimuli can induce p16INK4a, but the kinetics and cellular responses to these genotoxic insults have not been characterized in vivo. To address this question, we developed a murine strain of mice that expresses luciferase under the control of the endogenous p16INK4a locus and under control of the p16INK4a promoter (p16-LUC). We are exposing p16-LUC mice to arsenic (50 ppm), 42% fat diet (Western Diet), or cigarette smoke for a minimum of 6 months to mimic human exposures. Every other month, p16-LUC mice are imaged to measure luciferase induction. At 6 months of exposure, mice exposed to cigarette smoke display higher levels of whole body luciferase activity than no-smoke controls. We also detected higher levels of luciferase in mice on high fat diet (42% fat) compared to normal fat diet (4%) after 8 months. Experiments measuring the effects of p16INK4a expression exposure are ongoing. We are correlating luciferase expression in different organs with tissue damage as well as p16INK4a protein and mRNA expression. We are also using a similarly designed p16-GFP allele to further define the cellular expression of p16INK4a in response to toxicological stimuli. The data generated from these experiments will demonstrate how environmental exposures are linked to expression of p16INK4a, which is a mediator of tumor suppression and aging. (Supported by T32 ES07126, HHMI Translational Medicine Program, and U01 - CA141576)

2300 DEVELOPMENT OF AN ANIMAL MODEL OF ISONIAZID-INDUCED LIVER INJURY.

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Background: Isoniazid (INH) remains a mainstay in the treatment of tuberculosis despite the fact that it can cause liver failure. Studies from decades ago suggest that the toxicity is caused by the acetylated hydrazine (AcH2) metabolite of INH because covalent binding of AcH2 and not INH was detected in the liver of treated rats. However, this was based on an acute rat model using very high doses and with features very different from those observed in humans in which the onset is delayed, usually by more than a month. Recently we showed that INH itself can form covalent adducts with liver macromolecules. Methods and Results: Mice were treated with INH and screened for covalent binding using western blotting. We found that there is greater amount of covalent binding in mice than rats, but the amount of binding does not always correlate with hepatotoxicity. Attempts were made to develop a mouse model of INH hepatotoxicity by treating C57BL and Cbl-b (-/-) mice with INH for up to five weeks. We found that ALT was not a suitable measure of liver injury because INH interferes with the assay. Treatment of C57BL mice with INH did not result in an increase in serum glutamate dehydrogenase levels but treatment of Cbl-b (-/-) did. At the end of the treatment we found that in Cbl-b (-/-) mice there was a down regulation in IL-1 and IL-12 and in one mouse a dramatic increase in cells staining positive for F4/80. Attempts to break immune tolerance by co-treating animals with drug modified S9 protein orally were unsuccessful and in fact treatment with INH prevented the autoimmune hepatitis that was caused by injection of S9 protein along with Freund’s adjuvant. Conclusions: These studies demonstrate that bioactivation of INH itself leads to covalent binding. It seems that INH may actually lead to immune suppression which may prevent severe liver injury. Further work is being carried out to determine the mechanism of injury in this animal model and to see if it is consistent the liver injury in humans. This research was supported by grants from CIHR.
PS 2302 GENERAL TOXICITY STUDY OF F344 GPT DELTA TRANSGENIC RAT FOR ONE-YEAR FEEDING.

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Transgenic rodent gene mutation assays, recently adopted as OECD test guideline TG488, are useful methods to evaluate in vivo mutagenicity of chemicals in a target organ. The multiple copies of genetically neutral transgenes that contain reporter genes for mutation analysis are chromosomally integrated in the transgenic rodents. However, biological identities between the transgenic and non-transgenic animals are not well demonstrated. To evaluate the characteristics of F344 gpt delta transgenic (Tg) rat in general toxicity study, we investigated the clinical and pathological features of the gpt delta transgenic and non-transgenic F344 rats in one year feeding study. The six-week-old F344/N/Slc-Tg(gpt delta) and F344/N/Slc (60 animals each/sex/strain) were employed for this assessment. Twenty animals each in both sexes were subjected to necropsy at 13, 26 and 56 weeks after starting, then, clinical and pathological assessments were conducted. In F344/N/Slc-Tg(gpt delta), the values of several parameters in hematology, clinical biochemistry and organ weights were different from those in F344/N/Slc with statistical significance. These alterations, however, were within the range of historical control data of in housed F344/N/Slc, and thus were considered to be incidental. In histopathology, spontaneous lesions in F344/N such as adenoma in the pituitary, fibrosis in the heart, microgranuloma in the liver and so on were observed in both strains. These results suggested that there were no remarkable differences which indicate phenotypic modulation in Tg rats, and supported the conclusion that F344/N/Slc-Tg(gpt delta) has the similar characteristics to F344/N.

PS 2303 A COMPARISON OF SURVIVAL PATTERNS AND BACKGROUND PATHOLOGY IN SPRAGUE DAWLEY AND HAN WISTAR RATS.

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A survey was undertaken to compare survival patterns, major causes of mortality and incidences of neoplastic and nonneoplastic findings of these two rat strains in long-term studies. Differences in survival and tumor patterns were evident between the two. Data from 104-week studies conducted from 2001-2009 were compiled (8 studies using CRiCD(SD)Iajs BR (Sprague Dawley) rats, 12 studies using HsdBrHan Wistar (Han Wistar) rats). Marked differences were seen in body weight and survival between the two strains. SD rats were heavier at the end of 104 weeks with a higher mortality compared to Han Wistars. A number of differences in tumor data were evident between the strains. Benign phaeochromocytoma (SD 13.9%; HW 3.5%) and skin fibromas (SD 10.6%; HW 1.6%) were far more common in SD than Han Wistar males. Conversely, mesenteric lymph node heman-giomas (SD 1.9%; HW 8.6%), thyroid follicular cell adenoma (SD 1.6%; HW 9.1%) and thymic thymomas (SD 0.2%; HW 2.3%) were more common in Han Wistar than in SD males. Benign phaeochromocytoma (SD 4.9%; HW 0.3%), mammary fibroadenoma (SD 45.5%; HW 24.2%) and carcinoma (SD 22.3%; HW 6.4%), were more common in SD than in Han Wistar females. Mesenteric lymph node heman-giomas (SD 0.3%; HW 3.7%) and thymic thymomas (SD 0.2%; HW 6.6%) occurred more often in Han Wistar than in SD females. The commonest tumors occurring in each strain were pituitary tumors in both sexes (Male SD 38.0%; HW 35.0%; Female SD 65.4%; HW 64.2%) and mammary fibroadenomas in females (SD 45.5%; HW 24.2%).

PS 2304 PROFILE OF EARLY-OCcurring SPONTANEOUS TUMOURS IN CD-1 MICE.

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The profile of common background tumours in aged CD-1 mice is well-documented in the literature. However, in short-term studies, isolated occurrences of tumours in treated groups pose difficulties of interpretation. This survey was under-
taken to assess incidences and age of occurrence of tumours in mice on study up to 52 weeks. In addition, survival data were compared with a similar survey done on studies run at HLS between 1990 and 2002. Data from 17 studies of 78 to 104 weeks duration, run between March 2005 and July 2008, were used in this survey. These comprised 19 control groups; 1109 males and 1109 females in total. Overall, during the first 52 weeks of study 100 males died or were killed for welfare reasons, with neoplasia recorded as the cause of death in 20 animals. The earliest occurring tumour, seen in Week 18, was a fatal astrocy-toma of the central nervous system. The commonest tumour type was lymphoma; eight cases were recorded with seven as the cause of death and one as an incidental finding. The first lymphoma occurred between 26-30 weeks. Sarcomas of the skin were the next most common tumour type, followed by tumours of the lung (bron-chioalveolar adenoma and carcinoma. In the female groups, 67 animals died or were killed for welfare reasons during the first 52 weeks on study, with 19 deaths due to neoplasia. Again, lymphoma was the commonest tumour type and the earli-est occurring, being recorded in 15 females as the cause of death with the first one noted in Week 19. When compared with the data from an earlier survey (Early Oc-urrence of spontaneous tumours in CD-1 mice and Sprague-Dawley rats, 2004, Son and Gopinath), lymphoma was still the commonest tumour in both sexes. However, in the recent data incidence of lymphoma in males was lower than previ-ously (8 out of 100 decedents in the new data vs. 23 out of 101 decedents) and oc-curred later (26-30 weeks vs 6-10 weeks). Also, hepatocellular adenomas, previ-ously noted as incidental findings in three males which died before 50 weeks on study, were not recorded in the data presented here.

PS 2305 DIABETES AFFECTS BIODISTRIBUTION AND GENOTOXICITY OF CISPLATIN IN MICE.

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Introduction: Cisplatin is a highly effective anticancer drug that is nephrotoxic. Diabetes protects against cisplatin-induced nephrotoxicity but mechanisms and impacts on the anticancer activity of cisplatin remain unclear. This study evaluated the effects of diabetes on biodistribution and genotoxicity of cisplatin in Sarcoma-180-tumor-bearing mice. Methods: Swiss mice (25-30 g b.w.) were divided into four groups (n=8) as follows: Control (C), Cisplatin (CIS), Diabetes (DB) and Diabetes + Cisplatin (DB+CIS). Treatment: (i) a single dose of streptozotocin (150 mg/kg) in DB and DB+CIS; (ii) 3 days after, a subcutaneous injection of tumor cells in all four groups; and (iii) 8 days after, a single dose of cisplatin (20 mg/kg) in CIS and DB+CIS. Animals were killed 72 hours after cisplatin injection. Plasmatic urea and creatinine were determined by colorimetric assays (Labsystems®). Biodistribution of cisplatin was determined by Inductively Coupled Plasma Mass Spectrometry (ICPMS, 195Pt isotope). Genotoxicity was assessed by micronucleus test in peripheral blood 24 hours after cisplatin injection. Statistics: Mann–Whitney test for C x DB; C x CIS; and CIS x DB+CIS (p < 0.05, GraphPad Prism 5.0). Results: Compared to C, CIS presented significantly increased: (i) plas-matic urea; (ii) plasmatic creatinine; (iii) Pt concentration in kidney and tumor; and (iv) micronucleus frequency. All these effects were significantly reduced in DB+CIS. No significant difference was observed between DB and C. Discussion: Results showed that the protection of diabetes against cisplatin-induced nephrotoxicity is associated with modified biodistribution and decreased genotoxicity of the drug. Conclusion: Diabetes might impair the anticancer activity of cisplatin.

PS 2306 DEVELOPMENT OF A MOUSE MODEL OF DOXORUBICIN-INDUCED CHRONIC CARDIOTOXICITY.

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Doxorubicin (DOX) is a potent chemotherapeutic drug known to cause dose-re-lated cumulative and irreversible cardiotoxicity in cancer patients. Here we re-port the development of a mouse model of DOX-induced chronic cardiotoxicity for the identification of early predictive biomarkers of cardiotoxicity. Male B6C3F1 mice were administered a weekly dose of 3 mg/kg DOX, or an equivalent volume of
saline, intravenously via tail vein for 4, 6, 8, 10, 12, and 14 weeks, resulting in cumulative DOX doses of 12, 18, 24, 30, 36, and 42 mg/kg, respectively. During the course of the treatment, cardiac function was measured a day before dosing. At necropsy a week following the last dose, blood samples were collected for plasma measurements of cardiac troponin T (cTnT) levels. Also, hearts were collected for evaluations of cardiac lesions by light microscopy and mitochondrial morphology by transmission electron microscopy (TEM). Results show a significant increase in plasma cTnT levels in all mice exposed to cumulative DOX doses of 12 mg/kg and higher compared to saline-treated controls, indicating cardiac tissue injury. In addition, a dose-related increase in severity of cardiac lesions was noted in mice following cumulative DOX exposures to 24 mg/kg and higher. DOX-treated mice also showed a significant decline in heart rates at 24 mg/kg and higher cumulative exposures when compared to their saline-treated controls. Preliminary EM analysis revealed mitochondrial swelling with disorganization, fragmentation, and loss of cristae at 12 mg/kg DOX. Altogether, these findings demonstrate the development of mitochondrial damage following cumulative DOX exposures to 24 mg/kg and higher compared to saline-treated controls, indicating cardiac tissue injury. In this study, plasma cTnT levels in all mice exposed to cumulative DOX doses of 12 mg/kg and higher were measured at baseline and at 24 hours from dosing. Study 1: Moderate necrosis of the centrilobular hepatocytes was observed within 24 hours from dosing. Study 2: Mean serum AST, ALT, ALP, and TBL levels were increased markedly and reached maximum levels by 48 hours after dosing. These levels then decreased gradually and had almost returned to pre-dose levels by 12 days after dosing. These results are similar to those in previous studies using cynomolgus monkeys and rats. In addition, the lobular structure is distinct in the μMPs because interlobular connective tissue is abundant, whereas in the cynomolgus monkey and rat, it is unclear because interlobular connective tissue is scant. After single oral administration of CCl₄, the results from serum biochemical parameters (AST, ALT, ALP, and TBL) and histopathological examinations were similar to those in previous studies using cynomolgus monkeys and rats; however, the lobular structure of the liver is different. It is considered feasible to use μMPs for evaluating hepatotoxicity.

2307 EVALUATION OF HEPATOXIC EQUIVALENCY BY COMPARING CHANGES IN HEPATIC GENE EXPRESSION INDUCED BY TWO DIFFERENT FORMULATIONS OF ATORVASTATIN IN CHIMERIC PXB-MOUSE® WITH HIGHLY HUMANIZED LIVER.

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Bioequivalency of a generic drug and its brand name counterpart is judged by comparing pharmacokinetic properties of main component in each drug formulation. The pharmacokinetic equivalency is considered to ensure both formulations to be identical in intended use and efficacy. However, the contribution of the different impurities in excipients in safety characteristics are in question because clinical safety study is not required for the application of generic drugs. As a model study to evaluate the equivalency of risk of hepatotoxicity in humans, we examined changes in hepatic gene expression induced by two different formulations of atorvastatin which causes various hepatopathies. The examination was carried out using chimeric PXB-mice, in which more than 70% of hepatic parenchymal cells are replaced by human hepatocytes and has a potential to bridge the gap between rodent-type and human-type livers, and between in vivo and in vitro responses of human hepatocytes against hepatotoxicants. The Lipitor® Tablets 10mg (Pfizer Japan Inc.) or ATORVASTATIN TABLETS 10mg (Towa Pharmaceutical Co., Ltd.) was grated and suspended in 0.5% methylcellulose and orally administered to PXB-mice three-times daily at a dose of 100 mg/kg, followed by hepatic total RNA preparation and gene expression analyses using GeneChip® Human Genome U133 plus 2.0 Array (Affymetrix Inc.). The equivalency of changes in hepatic gene expression induced by two different atorvastatin formulations will be discussed in 18 biological pathways (such as NRF2-mediated oxidative stress pathway, fatty acid metabolism pathways) in addition to the global changes in hepatic gene expression.

2308 CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY IN MICROMINI-PIGS.

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Carbon tetrachloride (CCl₄) is known to induce hepatotoxicity. Recently, the Microminipigs (μMPs; Fuji Micra Inc., Japan) has emerged as a possible experimental animal model for non-clinical pharmacological/toxicological use. In this study, we compared hepatotoxicity following oral administration of CCl₄ in μMPs, cynomolgus monkeys, and rats in order to evaluate the feasibility of using μMPs for evaluating hepatotoxicity. Five females (Study 1: two females, Study 2: three females) were used. Study 1: Two female μMPs were euthanized 24 hours after CCl₄ oral administration and the liver was examined histopathologically. Study 2: CCl₄ was orally administered once at 0.4 ml/kg to three female μMPs, and the reversibility of the liver function up to Day 12 was examined by determining serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin (TBL) levels.
the drinking water and chow, respectively, at environmentally-relevant concentra-
tions for 56 weeks using a dose-ratio approach. At harvest, biotissues and tissues were
treated with 125 mg/kg/d AMG orally for 14 days. Using PCR arrays, signifi-
cantly changes in hepatic gene expression were found in apoptotic and mitochondrial
pathways that could contribute to the mechanisms of toxicity. In contrast, few
changes were related to inflammation and immunity except down-regulation of in-
flammatoty cytokines and chemokines such as IL-1 and CD40. No changes in pro-
tein levels of inflammatory cytokines were observed with a Luminex assay; however,
protein levels of innate immune markers such as Geo/KC, MCP-1, and IL-5 were
increased with AMG treatment at day 7 but returned to baseline by day 14. Liver
enzymes remained unchanged except for a subtle but noticeable increase in ALT and
GLO at day 3, a histogy revealed enlarged hepatocytes but no overt tox-
icity. A significant increase in peripheral blood neutrophils was found in the AMG-
treated throughout the study without changes in the total WBC count, suggesting
immune suppression. These results suggest that the primary response to AMG is
immune adaptation which may be the default response and why IDRs are generally
rare. Future studies focusing on innate immunity and direct activation of polymor-
phonuclear cells may be a better strategy to study the mechanisms of AMG-in-
duced IDRs. This research was funded by grants from the Canadian Institutes for
Health Research.

2311 IMMUNE INVOLVEMENT IN AMINOGLUTETHIMIDE-
INDUCED IDIOSYNCRATIC DRUG REACTIONS.

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As with most aromatic amine drugs, the aromatase inhibitor amino glutethimide
(AMG) is associated with a variety of idiosyncratic drug reactions (IDRs) ranging
from skin rash to serious haematological toxicities. AMG-induced IDRs are often
delayed and evidence suggests immune involvement; however, like most animal
models of IDRs, there has been limited reproducibility in rodents. A comprehen-
sive study in rats was performed to examine the early changes induced by AMG in-
cluding the effects on the immune and whether immune tolerance may contribute to
the difficulty of producing animal models of IDRs. Male Brown Norway rats were
treated with 125 mg/kg/d AMG orally for 14 days. Using PCR arrays, signifi-
cant changes in hepatic gene expression were found in apoptotic and mitochondrial
pathways that could contribute to the mechanisms of toxicity. In contrast, few
changes were related to inflammation and immunity except down-regulation of in-
flammatoty cytokines and chemokines such as IL-1 and CD40. No changes in pro-
tein levels of inflammatory cytokines were observed with a Luminex assay; however,
protein levels of innate immune markers such as Geo/KC, MCP-1, and IL-5 were
increased with AMG treatment at day 7 but returned to baseline by day 14. Liver
enzymes remained unchanged except for a subtle but noticeable increase in ALT and
GLO at day 3, a histogy revealed enlarged hepatocytes but no overt tox-
icity. A significant increase in peripheral blood neutrophils was found in the AMG-
treated throughout the study without changes in the total WBC count, suggesting
immune suppression. These results suggest that the primary response to AMG is
immune adaptation which may be the default response and why IDRs are generally
rare. Future studies focusing on innate immunity and direct activation of polymor-
phonuclear cells may be a better strategy to study the mechanisms of AMG-in-
duced IDRs. This research was funded by grants from the Canadian Institutes for
Health Research.

2312 COMPARATIVE EXPOSURE TO SOY BIODIESEL
EMISSIONS IN AN ALLERGIC MOUSE MODEL.

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We asessed the immunological effects following inhalation of emissions from 100% Soy biodiesel (S100) or a 20% mix with conventional petrodiesel (S20), in a house dust mite (HDM) allergic Balb/cj mouse model. Female mice (8/group) were exposed whole body (4 hr/d, 5 dwk, 4 wk) to emissions from S20 or S100 (0, 50, 150, or 500 μg/m3). On day 3 of exposure wk 1 and 2, mice were sensitized in-
tranally (i.n.) with HDM (0.70 antigen units of 1:1 Der f1: Der p1), while non-
allergic mice received saline vehicle only.All mice were challenged i.n. with HDM on
day 3 of wk 4 (2 d prior to necropsy). 1 to 5 h after the final exposure, allergic mice were hyperresponsive to MCh aerosol (flexiVent: enhanced total lung resist-
ance), but S20 had an allergen-sparing response that was assessed by allergen-recall responses in primary peribronchial lymph node cells (LNC) and splenocytes (SPN), and measurement of cellular proliferation by Brdu uptake, and allergic cytokine profiles. In non-allergic mice, 50 and 150 μg/m3 of S20 or S100 dampened proliferation of LNC (p<0.05) that was partially
restored by challenge with either Der f1 or Der p1. In vivo allergic sensitization was evidenced by enhanced proliferation of LNC in comparison with non-allergic counterparts. Exposures to all concentrations of S20 prevented allergen-driven pro-
liferation of LNC cultures in comparison with filtered air controls. LNC from al-
lergic mice exposed to 150 μg/m3 S100 had significantly augmented proliferation on challenge with Der f1 (p<0.0003) and Der p1 (p<0.0001). There were no sig-
nificant effects of either S20 or S100 on SPN proliferation. In conclusion, both S20 and S100 affected the immunological responses of primary LNC. Exposure to S20 not only dampened cellular proliferation but attenuated allergenic sensitization of LNC, indicating a more potent immunotoxic effect of S20 as compared with immu-
no-adjuvant promoting S100. (This abstract does not reflect EPA policy).

2313 ANTIINFLAMMATORY DRUG TESTING IN
MARMOSET MONKEY MODELS OF LPS-INDUCED
ACUTE LUNG INFLAMMATION.

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Current treatments for chronic obstructive pulmonary diseases (COPD) in humans are often insufficient and have only minimal impact on diseases progression despite world-wide rising incidence. It is thus important to develop reliable animal models that closely reflect anatomy and physiology of the human respiratory tract. Hence, to mimic pro-inflammatory aspects of COPD ex vivo and in vivo models based on acute lipo polysaccharide (LPS) provocation were established in the marmoset monkey (Callitris s jacchus).
Marmoset whole body (WB) blood samples as well as marmoset and human precision cut lung slices (PCLS) were stimulated with LPS. Dose response curves for roflu-
milast were determined in LPS stimulated marmoset and human PCLS. TNF-α
was measured in supernatants by ELISA and correlated with human data. In an in
vivo approach, marmosets were stimulated unilateral with 500 ng LPS intra-
bronchially after 5-day oral treatment with roflumilast (7 μg/kg/bw). Untreated
animals served as positive control. An ipsilateral bronchoalveolar lavage (BAL) was
performed 18 hrs post LPS provocation. BAL fluid was processed and analyzed for
neutrophilic influx and TNF-α secretion. Both, WB-assay and PCLS showed a dose-dependent TNF-α response to LPS with significant correlation (r²=0.9).
Further, TNF-α release in marmoset PCLS significantly correlates with human
PCLS (r²=0.9). Roflumilast treatment reduced TNF-α secretion significantly ex vivo in both species with comparable EC50 values (marmoset: 1.6 nM, human: 1.3
nM). LPS instillation in marmoset caused a strong respiratory inflammation as
shown by TNF-α increase and neutrophilic influx in BAL-fluid which in turn was
significantly suppressed by roflumilast. The correlation between marmoset and
human PCLS as well as the significant antiinflammatory effect of roflumilast in a
human relevant dose in vivo underlines the usefulness of the marmoset monkey as
an animal model for human inflammatory airway diseases.

2314 CONTINUOUS INTRAVENOUS INFUSION
IN GENETICALLY ALTERED MICE.

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Kirk.

The advance of biologics and the use of various mouse strains, now currently avail-
able, have increased the demand for a mouse continuous intravenous infusion mod-
els. This presentation looks at the feasibility of continuous intravenous infusion
techniques in genetically altered mice.

The mdx (C57BL/10 Sc Sn-Dmdmdx/J) mouse strain is commonly used to re-
search Duchenne muscular dystrophy as the mutant mouse does not express dys-
trophin. The mdx mutant has been used as a model for the human disease for over
twenty years. The mice, upon delivery (8 weeks old), have spontaneous necrosis of
the diaphragm, skeletal muscle and other muscle fibres. As the muscles are trying to
repair themselves, this leads to areas of fibrosis (hypertrophy). The mouse may there-
therefore have an unusual gait but will be mobile and capable of feeding and
grooming itself. Twenty four, 8 weeks old mice were used on this study. After an ac-
climation of 7 days, catheters were implanted into the vena cava via the femoral
vein. On completion of a recovery period of 7 days, infusion commenced for a pe-
riod up to 7 days. During this time no adverse effects on respiration during anaes-
thesia or study phase or other complications were observed. The nude mouse remains the experimental model of choice for testing the efficacy
anti-tumour agents. In the clinic, the anticancer agent, 5-FU, remains central to
terapeutic regimes, being used to treat numerous solid tumours, including
breast, colorectal, head and neck cancers. One disadvantage of 5-FU is its relative
short half-life (10-20 min) following intravenous bolus administration where
plasma concentrations rapidly fall below the cytotoxic threshold, thus limiting its
efficacy. This can be alleviated by administering a continuous infusion of 5-FU to
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maintain the plasma levels. We have previously established a method for adminis-
tering 5-FU by continuous intravenous infusion (by tail-cuff infusion) in the nude mouse (van Wijk et al (2006)).

In conclusion, from our work over the last several years, we have demonstrated the feasibility of drug administration, by continuous intravenous infusion, in at least 2 genetically altered mouse models.

Valproic acid (VPA) is an antiepileptic, anticonvulsant drug and a teratogen induced fetal valproate syndrome (FVS) in humans. FVS showed many phenotypic and genotypic features common to autism spectrum disorder (ASD). We are developing medaka as an animal model to study ASD. Fertilized eggs of medaka at three developmental stages were exposed to waterborne VPA (0-80 mM) in hatching solution for 48 h. The amount of VPA to cause 50% mortality (LC50) as observed 14 day post fertilization (dpf) is found to be developmental stage-specific. The em-
broeos of mice are extremely sensitive to VPA in early stages of development in the em-
broeos at late stages. VPA exposed embryos have malformed neurocranium and cardio-
vascular with delayed circulation, thrombus formation, and slow heart rate. The hatching efficiency is also reduced due to developmental delay. The mRNA analysis of several genes which are expressed during medaka embryogenesis, are found to be altered after VPA exposure. This study indicates that medaka can be used as a unique non-mammalian vertebrate model to study FVS.

Continuous or intermittent intravenous infusion via an indwelling catheter is used in preclinical studies to mimic the proposed clinical route or achieve higher blood drug levels than can be achieved by the defined clinical route. Awareness of back-
ground incidence of procedure-related complications plays an important role in the overall interpretation of the study. Historical incidence of procedure-related deaths, catheter repairs and rate of infection in rat, dog, mini-pig and pig IV infusion studies of up to 9 months duration conducted over the past 5 years was compiled. Post-operative procedure-related deaths have been maintained at a very low 1 to 2 % for rats and <1% for dogs, mini-pigs and primates. Cessation of dosing for a given animal due to experimental-related complications in saline infused animals is less or equal to 1%. Suboptimal delivery (catheter blockage or severing of the catheter) are events reported in infusion studies but are maintained at a low inci-
dence in our laboratories with good monitoring practices. Attriition rates due to procedure-related complications have been addressed in some laboratories by in-
creasing the study population. Our laboratories have instead maintained study pop-
ulations comparable to non-infusion studies, in-line with NC3Rs objectives, and perform surgical interventions for repair of a suboptimal delivery when needed. The use of sterile procedures during surgery and precautions taken during surgery conduct permit maintenance of very low infection rates (≤1%). These success rates are also feasible with other relatively innocuous vehicles (eg. D5W or PBS) and are similar for other infusion routes (subcutaneous, intrathecal, and other cerebrospinal infusions). Based on this background data, the low incidence of infusion-related adverse events encountered in our laboratory allows conduct of regulatory compliant studies with minimal experimental procedure-related interference for short or long-
term infusion regimens.

Valproic acid (VPA) is an antiepileptic, anticonvulsant drug and a teratogen induced fetal valproate syndrome (FVS) in humans. VFS showed many phenotypic and genotypic features common to autism spectrum disorder (ASD). We are developing medaka as an animal model to study ASD. Fertilized eggs of medaka at three developmental stages were exposed to waterborne VPA (0-80 mM) in hatching solution for 48 h. The amount of VPA to cause 50% mortality (LC50) as observed 14 day post fertilization (dpf) is found to be developmental stage-specific. The em-
broeos of mice are extremely sensitive to VPA in early stages of development in the em-
broeos at late stages. VPA exposed embryos have malformed neurocranium and cardio-
vascular with delayed circulation, thrombus formation, and slow heart rate. The hatching efficiency is also reduced due to developmental delay. The mRNA analysis of several genes which are expressed during medaka embryogenesis, are found to be altered after VPA exposure. This study indicates that medaka can be used as a unique non-mammalian vertebrate model to study FVS.
yield. The release of both tryptase and β-N-acetylglucosaminidase were compared as markers for mast cell degranulation and β-N-acetylglucosaminidase and cells from be more sensitive. Following optimization, Balb/c mice were treated intraperitonealy with OVA in combination with alum to maximize mast cells with surface bound anti-OVA IgE antibody. Peritoneal mast cells were isolated and subsequently challenged in vitro with and without OVA. Mast cells derived from OVA-treated mice and subsequently challenged in vitro with OVA were anticipated to release more β-N-acetylglucosaminidase compared with either mast cells not challenged with OVA or mast cells from untreated mice and challenged with OVA. A larger set of both allergic and non-allergic genes is being evaluated to further investi-gate the specificity of this approach.

2320 SURAMIN PROMOTES RECOVERY FROM GLYCEROL-INDUCED ACUTE KIDNEY INJURY.

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Acute kidney injury (AKI) is a common and potentially life-threatening complica-tion following ischemia/reperfusion and exposure to nephrotoxic agents. Suramin promotes recovery from ischemia/reperfusion-induced AKI in mice particularly after renal injury has already been established. In this study, we examined the effi-cacy and mechanism of suramin in glycerol-induced AKI, a model of rhabdomyo-lysis-induced AKI. Following intramuscular glycerol injection into male Sprague Dawley rats, serum creatinine levels maximally increased until 72 h and then decreased at 120 h. Suramin (1 mg/kg, iv) administered 24 h after glycerol injection, accelerated recovery from glycerol-induced AKI as evidenced by a decrease in serum creatinine and urinary glucose 72 h after glycerol injec-tion. These findings were corroborated by decreased renal kidney injury mole-cule-1 and improved histopathology. Quantitative PCR studies revealed increased tumor necrosis factor-α, interleukin-1β (IL-1β) and IL-18 mRNA after glycerol injec-tion, and suramin treatment decreased IL-1β and IL-18 at 48 h compared to glycerol alone. These cytokines along with transcription factor nuclear factor κB (NFκB) mediated increased expression of the intracellular adhesion molecule-1 (ICAM-1) in endothelial cells in AKI. NFκB and ICAM-1 protein, and leukocyte infiltration increased following glycerol injection, and suramin treatment decreased activated NFκB and ICAM-1, and leukocyte infiltration at 72 h compared to glycerol alone. Suramin also lowered renal TGFβ-1 expression, a key growth inhibitor, and increased renal cell proliferation, as evidenced by increased PCNA localization in proximal tubular epithelial cells, when compared to glycerol-alone. We suggest that suramin accelerates recovery from glycerol-induced AKI by decreasing renal inflamma-tion and increasing nephrogenic repair.

2321 EXTRAHEPATIC UGT1A1 EXPRESSION PLAYS AN IMPORTANT ROLE IN BILIRUBIN HOMEOSTASIS.

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UDP-glucuronosyltransferase 1A1 (UGT1A1) is the sole enzyme responsible for bilirubin glucuronidation, the rate-limiting step for bilirubin clearance. Deletion of the Ugt1 locus and all 9 Ugt1a genes, including Ugt1a1, leads to severe hyperbiliru-binemia in Ugt1a1 neonatal mice. The Ugt1α newborns can be easily identified by their unique orange skin color, resulting from the inability to clear total serum bilirubin (TSB). This mutation is neonatal lethal within 7 days, resulting from bilirubin induced neurological dysfunction (BIND) with TSB levels in the 15-20 mg/dl range. Expression of the human UGT1 locus in a Ugt1-null background (hUGT1 mice) rescues neonatal lethality, but hyperbilirubinemia with TSB levels in the 10-15 mg/dl range still develops in neonatal hUGT1 mice. The rescue is not associated with hepatic UGT1A1 expression; instead, UGT1A1 gene expression in the gastrointestinal tract plays an important role in preventing BIND. To examine specifically the role of hepatic bilirubin glucuronidation towards the prevention of BIND, we positioned Cre-recombinase specific lox P sites to flank exons 3 and 4 of the Ugt1 locus (Ugt1lox mice). When Ugt1lox mice were then crossed with albumin- Cre mice, the resulting offspring inherited deletion of the Ugt1 locus specifically in liver (Ugt1lox mice). Deletion of the Ugt1-β-N-acetylglucosaminidase (Ugt1a1 gene) in tissue from Ugt1lox mice was validated by lack of hepatic mRNA expression coupled with functional deletion by immunoblot and catalytic analysis. Interestingly, Ugt1lox mice show no visible abnormalities, mature to adulthood, with TSB levels in the 2-3 mg/dl range that develop shortly after birth and remain at this level as adults. The dramatic differences in TSB accumulation as noted between Ugt1α and Ugt1lox mice implicate an important role for extrahepatic bilirubin glucuronida-tion by UGT1A1 towards clearance and the development of hyperbilirubinemia. (Supported by USPHS grant GM086713 and P42ES010337)

2322 NEBULIZED THIOCYNATE INCREASES HOST DEFENSE IN A MOUSE MODEL OF P. AERUGINOSA LUNG INFECTION.

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Nebulized normal saline and hypertonic saline are used in the treatment of a num-ber of pulmonary diseases such as cystic fibrosis, asthma, and COPD. Benefits of these therapies include improved lung function and mucous clearance, and fewer lung infections in susceptible groups. Recent research has shown that thiocyanate (SCN) has both antihost and host defense effects and is a normal component of the lung epithelial lining fluid (ELF). Lung epithelia secrete SCN onto their apical surface as a buffer against oxidative and pathogenic stress. We demonstrated that SCN is an antihost agent by protecting cells from hypochlorous acid (HOCl), a major product of neutrophil myeloperoxidase, and cells deficient in SCN export are more vulnerable to HOCl. We exposed C57Bl6 mice to nebulized isoto-nic SCN (0.5%) and characterized its pharmacokinetics. Nebulized SCN in-creased ELF levels more than ten-fold immediately after treatment (Cmax=2.3 mM) and cleared with an apparent half-life of 4 hours. Plasma SCN concentrations were lower but mirrored the ELF at each time point. We treated mice infected with Pseudomonas aeruginosa using a chronic exposure fibrin plug model with either isotonic SCN or normal saline starting 24 hours after infection and then twice daily up to 72 hours. Mice given isotonic SCN rapidly regained bodyweight lost during the first 24 hours of infection and had significantly reduced neutrophil inflamma-tion and peroxidase activity after 72 hours compared to mice given normal saline. In addition, isotonic SCN promoted greater bacterial clearance than in untreated mice. SCN is a strong candidate for supplementation as adjunct therapy in patients suffering from lung infection or under chronic oxidative stress and inflamma-tion, as in cystic fibrosis and other infectious diseases of the lung. This study was funded by NIH grant HL084469.

2323 DEVELOPMENT AND EVALUATION OF AN LPS AIRWAY INDUCTION MODEL IN THE CYNOMOLGUS MACAQUE.


Pulmonary inflammation is associated with multiple human airway disease states including chronic obstructive pulmonary disease (COPD), asthma, emphysema, and cystic fibrosis. We developed a lipopolysaccharide (LPS)-induced airway inflamma-tion model in cynomolgus macaque that may be useful to mimic some as-pects of these conditions. Multiple LPS doses were administered via different routes including endotracheal (ET) and intravenous (IV). At various timepoints following dosing, blood and/or bronchoalveolar lavage fluid (BALF) samples were collected and analyzed for cellularity and expression of the cytokines IL-2, IL-4, IL-5, IL-6, IFN-γ, and TNF-α. The tracheal (ET) dosing of LPS resulted in a systemic acute inflammatory response, exemplified by an increase in concentration of monocytes and granulocytes in the BALF and the blood samples. ET treatment of LPS resulted in a sharp but transient increase in granulocyte and monocyte concentrations in the blood at T=6 hours that decreased to predose levels at T=24 hours. In contrast, a sharp influx of cells in BALF fluid at T=6 hours was sustained through T=24 hours in the BALF samples. Pretreatment with the anti-inflammatory agent dexametha-sone prior to LPS administration decreased the overall influx of granulocytes and monocytes into the lungs as seen in the BALF A flow cytometric panel containing CD3, CD16, CD20, CD15, CD34, CD14, CD32, CD25, and CD11b determined an influx of granulocytic cells and had reduced expression of CD32. In addition, ex vivo cellular assays were developed and validated to assess phagocytic ingestion and induction of the respiratory burst for future functional analysis of cell subsets within BALF. In summary, this model is able to investigate test article-related immuno-mological effects on the inflammation induced with inhaled LPS.

2324 PREWEANING OR LIFE-LONG ORAL MANGANESE EXPOSURE PRODUCES LASTING AND CHRONIC REDUCTIONS IN SKILLED FORELIMB USE IN ADULT RATS: A MONTOYA STAIRCASE EVALUATION.

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Motor impairment is a prominent feature of adult manganese (Mn) neurotoxicity in humans, but the extent and magnitude of motor deficits in adults following early life Mn exposure has not been characterized. Here we used the Montoya staircase
were orally administered to PXB-mice twice-daily at high doses (ca. 20% of re-porting weight of liver) followed by hepatic total RNA preparation and gene expression analyses using oligonucleotide microarray chips. These results were compared with corresponding results obtained from human hepatocyte studies in a database, Open TG-GATEs (http://toxico.nibio.go.jp) established by Toxicoegenomics Project and Toxicoegenomics Informatics Project conducted in Japan. The difference in hepatic gene expression in hepatocytes in vivo and in vitro will be discussed in terms of various biological pathways such as oxidative stress response, inflammation or fatty acid and steroid metabolism.

2327 A NOVEL SERIES OF ENDOTHELIN-A RECEPTOR ANTAGONISTS SHUTS DOWN PRETERM BIRTH IN THE ANIMAL MODEL: A LOOK AT MECHANISMS AND ALTERED GENE EXPRESSION.

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Defined as any birth occurring prior to 37 weeks of gestation, preterm birth (PTB) accounts for approximately 13% of all live births in the United States each year and is a leading cause of infant mortality. The single most common cause of spontaneous preterm birth is intrauterine infection in the mother (40%). Endothelin-1 (ET-1) is a potent vasoconstrictor peptide which is upregulated by inflammatory cytokines in the presence of infection, and is capable of causing an increase in myometrial tone, ultimately leading to premature delivery. In our previous work, we have shown that our novel series of 1-3-trisubstituted-2-carboxy-quinol-4-ones acts as high affinity Endothelin-A receptor antagonists (ET$_{A}$A). We have also shown that in C57Bl/6 mice, HJP-286, the n-propyl analogue and HJP-272, the prototype compound, successfully shut down PTB at doses in which we see no toxicity in the mother. Currently, we aim to elucidate the pathway by which these compounds act on the uterus, ultimately leading to the prevention of PTB. In order to simulate infection, mice were injected i.p. with LPS, a major component of the outer wall of Gram-negative bacteria, and were then injected with either an ET$_{A}$- RA, or PBS 10 hours later. Toll-like receptor 4 (TLR4) is a transmembrane protein capable of recognizing and responding to gram-negative bacteria, initiating the innate immune system in the host against non-self. Using QRT-PCR and examining a panel for 84 genes associated with infection and TLR4-mediated inflammation, we are able to identify the pathways affected by our novel compounds at the level of gene transcription. Using Bioplex, we are also able to measure inflammatory cytokines in treated vs control groups at the protein level. 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.

2328 USE OF 3-CHLOROPROPANEDIOL AS A POSITIVE CONTROL FOR INVESTIGATING CEREBRAL EDEMA IN MOUSE MODELS OF ALzheimer’S DISEASE USING MAGNETIC RESONANCE IMAGING.

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Magnetic resonance imaging (MRI) is used in Alzheimer’s disease (AD) clinical tri- als to monitor for microhemorrhage (MCH) and vasogenic edema (VE). Experimental AD therapies targeting removal of amyloid beta (Aβ) are associated with increased VE. Here is a mouse model system using CD-1, two transgenic mouse strains with AD-like pathology (PSAPP and Tg2576), and wild type littermates (WTL). Mice were given a single dose of 3-chloropropanediol (3-CPD) and monitored for VE using MRI. Doses of 3-CPD were titrated in CD-1 mice to an MTD of 70 mg/kg. Clinical signs were evaluated, and animals imaged after 1 week for edema and MCH. Tg2576, PSAPP, and WTL mice were similarly evaluated at 70 and 35 mg/kg, but MRI was at 24 hours and 1 week postdose. Brains were collected after the final imaging session, and evaluated histologically using hematoxylin & eosin, Perl’s iron stain, and immunohistochemical methods for Aβ and albumin. MRI hyperintensities (edema) were observed only at 70 mg/kg. MRI hypointensities (MCH) were observed in all PSAPP mice and in a few Tg2576. Albumin-negative vacuolations were seen in brain sections corresponding to MRI hyperintensities, suggesting non-proteinaceous edema. Perl’s iron stain confirmed iron in brain sections corresponding to MRI hypointensities. The pattern/location of edema after 3-CPD administration was specific and consistent within/between the mouse strains, suggesting that it is a useful positive control for evaluating

2325 IRREVERSIBILITY OF FIBROSIS AND PRENEOPLASTIC HEPATOCELLULAR FOCI BY OXIDATIVE STRESS AND HYPOXIA IN THE LIVER OF NONALCOHOLIC STEATOHEPATITIS RATS.


Nonalcoholic steatohepatitis (NASH), a disease with histological features resembling alcoholic hepatitis in the absence of alcohol abuse, is characterized by a combined pathology of steatosis, lobular inflammation, fibrosis, and hepatocellular degeneration, with systemic symptoms of diabetes or hyperlipidemia. This animal model has a potential to bridge the ADME &TOX. Research Institute, Sekiui Medical, Co., Ltd., Tokai-mura, Ibaraki, Japan, 2Phoenix Bio, Co., Ltd., Higashi-Hiroshima, 3Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Miyagi, Japan. Sponsor: T. Miyakea.

Chimeric PXB-mice, in which more than 70% of hepatic parenchymal cells are replaced by human hepatocytes, is a unique animal model that mimics human-type metabolism against various drugs. This animal model has a potential to bridge the gap between rodent-type and human-type livers and to explain the difference of in vivo and in vitro response of human hepatocytes against hepatotoxins. In order to compare the early response against hepatotoxicant treatment in human hepatocytes in vivo and in vitro, we have analyzed changes in hepatic gene expression in PXB-mice by using acetaminophen which causes hepatic necrosis, aspirin which causes steatosis and flutamide which causes steatohepatitis, in humans. These drugs

2326 DIFFERENCES IN HEPATOTOXICANT-INDUCED CHANGES IN HEPATIC GENE EXPRESSION IN HUMAN HEPATOCYTES IN VITRO AND IN THE LIVER OF CHIMERIC PXB-MOUSE.

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Magnetic resonance imaging (MRI) is used in Alzheimer’s disease (AD) clinical tri- als to monitor for microhemorrhage (MCH) and vasogenic edema (VE). Experimental AD therapies targeting removal of amyloid beta (Aβ) are associated with increased VE. Here is a mouse model system using CD-1, two transgenic mouse strains with AD-like pathology (PSAPP and Tg2576), and wild type littermates (WTL). Mice were given a single dose of 3-chloropropanediol (3-CPD) and monitored for VE using MRI. Doses of 3-CPD were titrated in CD-1 mice to an MTD of 70 mg/kg. Clinical signs were evaluated, and animals imaged after 1 week for edema and MCH. Tg2576, PSAPP, and WTL mice were similarly evaluated at 70 and 35 mg/kg, but MRI was at 24 hours and 1 week postdose. Brains were collected after the final imaging session, and evaluated histologically using hematoxylin & eosin, Perl’s iron stain, and immunohistochemical methods for Aβ and albumin. MRI hyperintensities (edema) were observed only at 70 mg/kg. MRI hypointensities (MCH) were observed in all PSAPP mice and in a few Tg2576. Albumin-negative vacuolations were seen in brain sections corresponding to MRI hyperintensities, suggesting non-proteinaceous edema. Perl’s iron stain confirmed iron in brain sections corresponding to MRI hypointensities. The pattern/location of edema after 3-CPD administration was specific and consistent within/between the mouse strains, suggesting that it is a useful positive control for evaluating

food pellet retrieval task to evaluate basal ganglia control of fine motor function. Fifty five male rats (n=11) were orally dosed with 0, 25, or 50 mg Mn/kg/d over PND 1-21 only, or lifelong from birth. Staircase evaluation began at ~4 months of age and proceeded daily for ~1 month; behavior also was rated during each daily 10 minute trial. Motor skill learning was normal in all treatment groups, though cooperative behavior was impaired across Mn exposure conditions, except in the pretreatment group which displayed more selective motor difficulties on only difficult to reach steps. Collectively, these results indicate that fine motor function in adulthood is impacted both by lifelong or early life Mn exposure. Forthcoming neurobehavioral evaluations of the studied cohort will further examine if non-motor functions such as attention, learning, and memory also are vulnerable to the neurotoxicity of lifelong or early life Mn exposure. Knowledge gained from detailed neurobehavioral assessments will contribute to understanding the neuronal and neurochemical specificity of resultant behavioral vulnerabilities due to neonatal Mn exposure.
edema. Also, the 3-CPD-induced edema can be monitored over time for resolution without sacrificing the animal, so that longitudinal studies are feasible. This model has the potential for evaluating the preclinical safety of all-lowering therapies with regard to detection of VE, and offers the advantages of longitudinal studies while holding to the principles of the 3 Rs for animal research – replace, refine, and reduce.

Conclusion. The feasibility of the unique model has been demonstrated; however, overwhelming phosgene injury immediately post exposure.

and gross pathology. The second phosgene-exposed animal died from an over-recovered and monitored to 24 h post-exposure. The phosgene-exposed animal had whilst under anaesthesia, then immediately recovered and monitored for 24 h.

Results. Two air-exposed animals and 1 phosgene-exposed animal were successfully induced using in a wide range of industrial processes. Exposure to high concentrations of CG results in an asymptomatic period prior to development of pulmonary edema. Currently no medical countermeasures exist for such poisoning, treatment being supportive in an intensive care setting. Evidence based treatment guidelines are needed to assist health care practitioners in the event of accident/deliberate release.

Small animal models have been used to screen candidate therapies, extrapolation of therapeutic benefit to man requires verification in a larger animal model e.g. the pig. There is therefore a requirement to develop a conscious large animal model of CG-induced lung injury in which to verify efficacious treatments. If successful this model will allow assessment of therapeutic interventions to TICs in a species closer to man.

Methods. Following a period of habituation, animals were surgically prepared to allow blood and physiological measurements to be taken (via exteriorized catheters attached to a tethering system). After surgical recovery (1 week) and baseline measurements (1 week) animals were exposed to either air (n=2) or phosgene (n=2) whilst under anaesthesia, then immediately recovered and monitored for 24 h.

Results. Two air-exposed animals and 1 phosgene-exposed animal were successfully recovered and monitored to 24 h post-exposure. The phosgene-exposed animal had a mild phosgene-induced lung injury at 24 h compared to air-exposed controls, demonstrated by reduced arterial blood oxygenation and oxyhemoglobin levels, and gross pathology. The second phosgene-exposed animal died from an overwhelming phosgene injury immediately post exposure.

Conclusion. The feasibility of the unique model has been demonstrated; however, further method development is required to achieve a dose of phosgene producing a more severe but non-lethal injury at 24 h in which to test therapeutic candidates.

The toxic industrial chemical (TIC) phosgene (CG) is a reactive intermediate used in the manufacture of various products including, inorganic chlorides and organic chlorides. It is also used as an intermediate in the production of various chemicals such as dichloromethane (CH2Cl2), chloroform (CHCl3), carbon tetrachloride (CCl4), and trichloroethylene (C2HCl3).

Currently no medical countermeasures exist for such poisoning, treatment being supportive in an intensive care setting. Evidence based treatment guidelines are needed to assist health care practitioners in the event of accident/deliberate release.

Supportive care is the mainstay of treatment until the end of the exposure period and until the 5th week of the recovery period. Therefore, while the phosgene-exposed animal died, it provides valuable information about the extent of injury and the effectiveness of supportive care. The results of this study suggest that the use of an ex vivo organ perfusion model may be a useful tool for evaluating the potential of new treatments for phosgene poisoning.

The study also highlights the importance of developing effective medical countermeasures for phosgene poisoning. While current treatments are supportive, more specific treatments are needed to prevent and treat complications such as pulmonary edema. Further research is required to develop effective medical countermeasures for phosgene poisoning, including the development of therapeutic interventions to TICs in species closer to humans.

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7. PS 2329 DEVELOPMENT OF A RECOVERY PIG MODEL OF PHOSGENE-INDUCED LUNG INJURY.

8. PS 2330 NEED FOR ACCLIMATIZATION OF RATS TO RESTRAINT FOR NOSE-ONLY INHALATION EXPOSURE.

9. PS 2331 ACETAMINOPHEN ABSORPTION TEST: A METHOD FOR THE EVALUATION OF GASTRIC EMPTYING IN CYNOМUS ГOКЕN КEY.

10. PS 2332 HUMAN FCGRIIA TRANSGENIC MICE AS A SUITABLE MODEL FOR STUDYING THROMBOSIS.

11. PS 2333 UTILITY AND TRANSLATABILITY OF RODENT TESTICULAR miRNAs AS POTENTIAL TESTS FOR INJURY BIOMARKERS.
class of potential biomarkers, when measured in blood, are non-invasive, accurately measurable and might translate between preclinical and clinical settings. In our search for testis-specific miRNAs, we found the literature confused and contradictory, so we profiled 74 miRNAs identified from literature in major tissues in the rat using ABI custom miRNA TaqMan low density array. Several X-linked miRNA clusters were identified as testis enriched. However, because of the rapid evolution of mammalian X-linked testis miRNAs, these X-linked miRNA clusters are only partially conserved in human. To refine our prediction, a computational analysis was performed to determine the functional conservation of testis miRNA clusters between rat and human. Target gene analysis of rat miRNAs and human miRNA orthologues was performed with TargetScan 5.2, and a biological network analysis was performed using Cytoscape-GoMetaCore. Comparison of networks between target gene sets of the non-conserved rat miR-463/human miR-888 cluster and the partially conserved rat/human miR-506 family showed the exclusive enrichment in Cell adhesion, FSH-beta, Development_Neurogenesis, and Cell cycle, which indicated the important roles of testis enriched miRNA cluster/family in Sertoli-germ cell interaction/function. thence, the delineation and enrichment of testis enriched biological networks of a conserved non-testicular-exclusive rat/human miR-424_450 cluster showed a broad functional involvement in DNA damage, Cytoskeleton, Cell cycle, and Inflammation. In summary, although with high evolutionary rates between rat and human orthologous testis miRNAs, our results demonstrate the functional conservation and potential translatability of testis miRNAs as testis injury biomarkers.

2334 SMALL-MOLECULE INHIBITION OF THE PI3K BETA ISOFORM CAUSES TESTICULAR TOXICITY IN THE RAT.

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Class 1A phosphoinositide 3-kinase (PI3K) alpha, beta, and delta isoforms are heterodimers composed of a catalytic subunit (p110 alpha, beta or delta isoforms) and an adaptor protein. Spermatogenesis is dependent on the stem cell factor (SCF)/c-kit/PI3K/AKT signaling pathway in spermatogonia which leads to spermatogonia proliferation and differentiation. Male mice expressing catalytically inactive p110 beta isoform have testicular atrophy and impaired spermatogenesis (Ciraolo et al. 2010). Furthermore, spermatogonia isolated from p110betta inactivated mice fail to phosphorylate AKT in response to SCF indicating that the beta isoform is important in this signaling pathway. We expanded further on these transgenic mouse data by using a small molecule inhibitor of PI3K beta isoform (28 day male rat toxicity studies with small molecule inhibitors of PI3K, we evaluated the in vivo pathogenesis of PI3K beta inhibition in the rat. In 4, 14 and/or 28 day male rat toxicity studies with small molecule inhibitors of PI3K, we observed Sertoli cell (cytoplasmic vacuolation) and germ cell (maturation arrest) toxicity when plasma exposure exceeded the PI3K beta isoform IC50. To further understand the role of the p110 beta isoform in rat spermatogenesis, we used in situ hybridization (ISH) and a functional spermatogonial assay using SCF-induced phosphorylation of AKT in c-Kit positive spermatogonia. Using ISH, p110 beta mRNA expression in the rat testes was shown to be associated with Sertoli cell proliferation. However, expression in Sertoli cells may have masked the expression of p110 beta in other cells at the base of tubules (e.g. spermatogonia). The results of the functional spermatogonial assay showed that PI3K beta IC50 significantly correlated with rat spermatogonial pAKT IC50. Taken together, these data suggests that PI3K beta inhibition leads to testicular toxicity in rats by two potential mechanisms: 1) direct Sertoli cell toxicity; and 2) disruption of c-kit/PI3K/AKT signaling in spermatogonia leading to maturation arrest.

2335 METAMIDOPHOSALTERSSPERMFUNCTION, FERTILIZATIONCAPACITY,ANDDNAINGERMLINECELLSATMITOSIS,MEOESIS,ANDEPIDIDYMIDMATURATIONINMICE.


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Methamidophos (MET) is a highly toxic organophosphorous pesticide (OP), its use in agriculture has increased in Mexico. MET decreased semen quality altering male reproductive function. We evaluated MET effects on sperm quality, function and fertilization capacity as well as on DNA, exploring the sensitive stage(s) of spermatogenesis. Adult male mice were administered MET (3.75 or 5 mg/kg bw/p.i/day/4 days) and euthanized 24 hours post-treatment (hpt) or 28 or 45 days (dpt). Spermatozoa were obtained from epididymides, evaluated for sperm quality, spontaneous and induced acrosome reactions (AR; Coomassie staining), mitochondrial membrane potential (JC-1), DNA damage (Comet assay), oxidative damage (malondialdehyde production-MDA) and in vitro fertilization; erythrocyte acetylcholinesterase (AChE) activity was also determined. At 24 hpt, MET inhibited AChE activity (43-57%) and slightly increased abnormal cells (6.2%). At 28 dpt, sperm motility (12-17% reduction) and morphology (3-8% abnormality) was affected at both doses, but not viability; at 45 dpt, dose-response decreases were observed in sperm quality: motility (5-14%), viability (9-14%) and morphology (6-15%). MDA production and mitochondrial activity were not affected at any dose or time. Sperm DNA damage was observed at 5 mg/kg/day at 24 hpt (7-fold), 28 (3-fold) and 45 dpt (2-fold); fertilization capacity decreased at 28 (13,2%) and 45 dpt (23.2%) but not at 24 hpt. Spontaneous and induced ARs were altered at 28 and 45 dpt at 5 mg/kg/day. These data suggest that sperm cell sensitivity is higher in spermatogonia and spermatocytes than in maturing spermatozoa. Study supported by CONACyT-Mexico (Grant #58213).

2336 BLACK COHOSH (ACTAEA RACEMOSA) AFFECTS REPRODUCTION IN THE HARLAN SPRAGUE DAWLEY RAT.

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Black cohosh (BC) is a perennial plant native to North America with long history of use to treat pain, especially from menstrual disorders and labor. Currently it is marketed as a supplement for perimenopausal women. The NTP performed toxicological studies using a BC formulation comparable to those currently available to the public. Results of uterotrophic (no estrogenic activity detected) and subchronic (minimal liver and hematological effects; positive micronucleus test) studies were reported previously. Here we report results of a Reproductive Assessment by Continuous Breeding study in rats. Parental generation (F0) adult male and female rats (N=23) were administered BC daily by gavage beginning a week prior to mating, through 3 mating and littering phases, until necropsy. Pups from the first two litters were euthanized on PND 4. The 3rd litter was allowed to reach adulthood. Offspring were administered BC after weaning at the same dose levels and frequency as the parents. Rats did not display any apparent BC-related toxicity or morbidity. BC did not affect F0 fertility after the first mating. However, administration of 250, 500 or 1000 mg/kg of BC was associated with fewer pregnancies after the 2nd and 3rd mating (94, 88, 49% vs. 91, 72, 72% compared to the respective concurrent controls). Pairs that did produce litters had less live pups per litter with each consecutive mating (88, 85, 85% and 94, 75, 55% of concurrent control for 2nd and 3rd litters). Both effects were time and dose-dependent. Crossover mating (high dose males mated to control females and vice versa) showed both sexes had decreased fertility; however the effect in males seemed to be more pronounced than females (74% and 49% less pregnancies than 3rd mating controls). These results suggest BC is a reproductive toxicant. Further studies will define mechanisms responsible for decreased fertility. These data are preliminary, have not been peer-reviewed and do not constitute the opinion or policy of the NTP.

2337 TOXICOGENOMIC INVESTIGATION ON RAT TESTICULAR TOXICITY ELICITED BY 1, 3-DINITROBENZENE.


A toxicogenomic analysis was performed on rat testis treated with a single oral dose of 1, 3-dinitrobenzene (DNB) at 10, 25 and 50 mg/kg. Affymetrix GeneChip analysis revealed a total of 186 and 304 gene probe sets which were up- and down-regulated, respectively by the DNB treatment, where spermatocyte death and Sertoli cell vaculation in the testis and increased debris of spermatogenic cell in the epididymis were noted. Up-regulated genes suggested oxidative stress (i.e., Hmox1, Pon2, Gstp1, Ak17a3 and Akr1b18) and apoptosis (i.e., Gadd45g, Ddit4 and Nos3), suggesting their contribution to DNB-induced testicular toxicity. In addition, Gene ontology analysis indicated that genes translating between preclinical and clinical studies. Among these up-regulated genes, DNB significantly enriched in the up-regulated genes following DNB treatment, and the major cell adhesion-related genes in the testis (i.e., Cdhl2, Cnn1a, Vcl, Zyx, Igfb1, Testin, Lamc3, Pdrl2 and Gin) were significantly up-regulated by DNB treatment. The up-regulation of cell adhesion-related genes which might be a consequence of cellular response for Sertoli-germ cell adhesion restructuring. Furthermore, we conducted a gene set-level expression analysis using four cell-type specific gene sets: pachytene spermatocytes (PS), Sertoli cells, spermatogonia plus early spermatocytes, and round spermatids. The microarray data demonstrated that the expression
level of PS-specific genes were explicitly down-regulated concomitantly, while those of the other 3 gene sets did not explicitly change their expression levels. The down-regulations of PS-specific genes and the up-regulations of cell adhesion-related genes were thought to reflect a decrease in the number of spermatocytes and dysfunction of Sertoli-germ cell adhesion junction, and therefore these genes would be potential genomic biomarkers for assessing DNB-type testicular toxicity, which may add value to the conventional histological toxicity evaluation by detecting spermatocyte injury and dysfunction of Sertoli-germ cell adhesion junction at the transcriptional level.

2338 EVALUATION OF GENOMIC BIOMARKERS AND RELATIVE POTENCY OF PHHALTATE-INDUCED MALE REPRODUCTIVE DEVELOPMENTAL TOXICITY USING A TARGETED RT-PCR ARRAY APPROACH.

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Exposure to certain phthalate esters (PEs) during sexual differentiation induces reproductive tract malformations in male rats due to reductions in fetal testicular testosterone (T) production and expression of steroidogenesis and insulin-like 3 (insl3)-related genes. In the current study, we used a 96-well rtPCR array containing key target genes representing sexual differentiation and development, steroidogenesis, gonadal development, and androgen signaling pathways to rank the relative potency of several PEs. We performed dose-response studies in which we dosed dams gestationally (GD) 14-18 with diisobutyl (DIBP), dipentyl (DPeP), dihexyl (DHP), diethyl (DHeP), diisononyl (DINP), or diisodecyl phthalate (DIDP) and serial dilutions of a mixture of 9 phthalates. All phthalates, with the exception of DIBP, reduced fetal testicular T production. Several genes involved in cholesterol transport, androgen synthesis and insl3 were down-regulated in a dose responsive manner by DIBP, DPeP, DHP, DHeP, DINP, and the 9 PEs mixture. Despite speculation about PPAR involvement in the effects of PEs on the fetuses, the potent PPARα agonist, WY-14643, did not induce PPAR-related genes or reduce fetal testicular T production or expression of related genes following GD 14-18 exposure, indicating that the anti-androgenic activity of PEs is not PPARα-mediated. The overall sensitivity of the fetal endpoints (gene expression or T production) for exposure, indicating that the anti-androgenic activity of PEs is not PPAR α-mediated.

2339 COMPARISON OF EX VIVO DBP AND IN VITRO MBP EXPOSURES ON FETAL TESTIS TESTOSTERONE PRODUCTION.

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In utero exposure to di-butyl phthalate (DBP) during sex differentiation reduces androgen production and produces a characteristic profile of gene expression changes in the fetal testes. The DBP metabolite mono-butyl phthalate (MBP) is hypothesized to produce these changes by a direct effect on the fetal testis. The current study investigated if direct in vitro exposure of the fetal testis to MBP would also reduce fetal androgen production as has been demonstrated ex vivo. Testes from gestation day (GD) 17 control male Sprague Dawley rat fetuses were incubated with either 0 (ethanol vehicle), 30, 100, 300 or 500 μM MBP in culture medium for 5 h, then the media were collected and replaced with fresh media of the same treatments and incubation continued an additional 3 h. Four control dams were used and testes from each litter were represented across all treatments (minimum n=4 per treatment). Concurrently as a positive control, 4 GD17 dams received a single oral dose of either 0 (corn oil vehicle) or 750 mg DBP/kg. Five hrs after dosing (coincident with the first 5 h in vitro incubation), testes from male offspring were removed and incubated ex vivo in untreated medium for 3 h (coincident with the 3 h in vitro incubation). Testosterone (T) production for the 5 h and 3 h in vitro and the concurrent 3 h ex vivo incubations were measured in medium by RIA. As expected, ex vivo T production from the fetal testes was reduced by about 40% compared to controls after a single oral dose of DBP to the dam. In vitro MBP treatment, however, did not significantly alter T production at either time point. These data support that the effects of DBP on fetal androgen production may not be due to the T direct effect on the T synthesis, and suggest that some extra-testicular factors may be required. Future studies will evaluate direct effects of MBP on the profile of phthalate-induced gene changes in this and other pathways. This abstract does not reflect EPA policy.

2340 A FETAL RAT TESTIS ENDORCINE AND GENOMIC “SIGNATURE” ACCURATELY PREDICTS THE PHHALTATE SYMNDROME OF MALFORMATIONS.

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Phthalate esters (PE) vary greatly in their potency to induce malformations during sexual differentiation in the male rat. Since in vitro assay batteries are currently unable to generate useful information on the potential of chemicals within this class to disrupt reproductive development, we evaluated these chemicals in a short term in vivo protocol (called the fetal phthalate screen: FPS) that relies upon testosterone production (T) and testes gene expression to discriminate ‘active’ from ‘inactive’ PEs. ‘Active’ PEs are further evaluated in full dose response studies using the FPS protocol and relative potency factors are calculated. To date, approximately twenty PEs and alternatives have been evaluated at a single dosage level and about ten of these have been examined in dose response studies. These results demonstrate that ‘active’ PEs produce a consistent ‘Signature’ including reduced T and cyp11b1, StaR, cyp17a1, SCARB, cyp11a1, Hsd-3b and Insl3 gene expression (Hannas et al. 2011). Comparison of the ED50s of the FPS Signatures, with the ED50s of the postnatal Phthalate Syndrome (pPS) reveals that this PPS Signature can be used to predict the potency of a PE to induce reproductive tract malformations. For example, in the FPS and in postnatal studies the relative potencies in mg/kg (ED50s for FPS:pPS) are dipentyl phthalate (50:132) > DBP (296:567) > DNP (864:1500) > DDEP (no effect: no effect). In addition, the relative potency factors for the FPS Signature can be used to predict the effects of mixtures of PEs in the fetal testes and on postnatal reproductive development. Although the current FPS protocol does not eliminate animal use it represents a considerable reduction in resources as it uses relatively small numbers of animals per dose group (3-5 litters) and only 5 days of dosing are required prior to necropy. This abstract does not necessarily reflect EPA or NTP policy. NTP, NIEHS/EA HHS Y1-ES-8014-01; EPA RW759322

2341 FASL REGULATES TESTICULAR GERM CELL C-FLIP LEVELS THROUGH GENE EXPRESSION AND UBQUITINATION.

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Fasl. (TNFSF6) is a ligand belonging to the tumor necrosis factor (TNF) superfamily of proteins. This ligand is expressed by testicular Sertoli cells and is implicated in the control of germ cell apoptosis. Our recent findings revealed that young peri-pubertal (28 day-old) FasL-/− mice show a dramatic increase in the basal germ cell apoptotic index (A.I., 20.58%) as compared to the C57BL/6J wild-type strain (5.16%). We have previously shown that exposure of young C57BL/6J mice to the prototypical Sertoli cell toxicant, mono-(2-ethylhexyl) phthalate (MEHP), causes a FasL/Flis-dependent increase in germ cell apoptosis. Surprisingly, exposure of young FasL-/− mice to MEH resulted in a decrease in germ cell apoptosis (~10% A.I. after 12 h of exposure). Western blot analysis showed that the expression of the c-FLIP protein, a well-described negative regulator of death receptor activation, was rapidly and strongly induced after MEH exposure in Fasl-/- mice, suggesting that alterations in the expression of c-FLIP in these mice accounts for their insensitivity of germ cells to toxicant-induced apoptosis. RT-PCR analysis showed that cflip mRNA levels were also strongly increased in MEH-treated Fasl-/- mice testes. Iitch, a HECT-type E3 ligase, has been shown to promote the ubiquitin-dependent degradation of c-FLIP. TG-Interacting factor (TGF) has been implicated in activating c-FLIP in these mice accounts for their insensitivity of germ cells to toxicant-induced apoptosis. RT-PCR analysis showed that cflib mRNA levels were also strongly increased in MEH-treated Fasl-/- mice testes. This abstract does not necessarily reflect EPA or NTP policy. NTP, NIEHS/EA HHS Y1-ES-8014-01; EPA RW759322
2342 THE STRUCTURAL VARIATIONS OF Y CHROMOSOME AZFc MULTICOPY GENES ON SPERMATOGENIC FAILURE.

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The male specific azosperma factor C (AZFc) region and its multi-copy genes have been identified to be implicated in spermatogenesis. The complex structure of AZFc predisposes to a series of genomic rearrangements mediated by non-allelic homologous recombination (NAHR) between amplicons, including the formation of deletions, duplications. However, the potential mechanism for the susceptibility of AZFc structural variations to spermatogenic failure is unclear, and which is one of the hot topics in the field. To further clarify the role of AZFc region in spermatogenesis and the possible molecular mechanism, we conducted comprehensive molecular analyses (deletion typing, copy number quantifying and Y chromosome haplogrouping) in 711 idiopathic infertile men and 390 healthy men. We con- 

irmed two previously reported fixations, the b2/b3 deletion in haplogroup N1 and the gr/gr deletion in haplogroup Q1. Remarkably, the frequency of the complete AZFc deletion in haplogroup N1 was significantly higher than that in the hap-

logroup Q1. These results suggest that the b2/b3 partial deletion was associated with a higher risk of complete AZFc deletion compared with the gr/gr partial dele-

tion. Compared to the gr/gr deletion, the b2/b3 deletion presents a shorter distance among recombination targets and longer recombination substrates, which may be responsible for the increased incidence of subsequent recombination events that can lead to the complete AZFc deletion in this Chinese study population. In addi-

tion, we found that additional AZFc duplications accompanying b2/b3 deletion, instead of b2/b3 deletion alone, led to the b2/b3 deletion-associated risk of sper-

matogenic failure previously reported in Han Chinese population. Our findings demonstrated that additional AZFc duplications did not compensate but convey the susceptibility of b2/b3 deletion to spermatogenic failure in the tested popula-

tion. Notably, genomic duplications and deletions in AZFc deserve comprehensive investigations to uncover spermatogenic roles of the AZFc region.

2343 WEIGHT-OF-EVIDENCE APPROACH IN EVALUATING POTENTIAL ANTIANDROGENIC SUBSTANCES.

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Potentially endocrine-related effects are investigated in mechanistic and regulatory toxicity studies of chemicals and agro-chemicals. One of the parameters considered to be able to detect antiandrogenicity in rats is the onset of male puberty, deter-

mined by the age of animals at preputial separation. Other parameters, like nip-

ple/area ratio; testicular weight; seminal vesicle wet weight and body weight, are also suitable endpoints for the assessment of antiandrogenicity. We have evalu-

ated 25 two-generation toxicity studies conducted in our lab over the last 12 years. The regulatory studies were conducted with pharmaceuticals (17) and agro-chemicals (8). A full set of toxicological studies (and for some compounds also mechanistic studies) are available for all compounds under investigation. Five out of 25 sub-

stances are suspected to act via an antiandrogenic mechanism, as evidenced by fur-

ther in vivo or in vitro studies. For some substances the entry into male puberty was delayed without any further evidence of antiandrogenicity or other endocrine effects. As there is evidence in the literature that entry into puberty is related to body weight, an evaluation has been conducted, whether offspring body weight corre-

lates with the day of preputial separation. The biological variation in terms of day of preputial separation of our untreated male animals at postnatal day 21 was 47 days (median at day 42), body weights at day of preputial separation: 142-215 g (me-

dian: 174 g). The evaluation of the data revealed that the age of untreated and doused male ani-

mals at the onset of puberty was inversely correlated with offspring body weight at postnatal day 21. Using general linear models it could be shown that lower body weights significantly delay entry into puberty. The isolated finding of delayed preputial separation is not sufficient to prove an antiandrogenic mode of action.

2344 PHENOLS EXPOSURE AND MALE INFERTILITY OF HAN CHINESE IN THE MIDDLE AND LOWER REACHES OF YANGTZE RIVER.

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Aim: Phenols are widely used from personal use to agricultural and industrial needs. The health risks of phenols exposure have become hot issues of public con-

cern recently. Due to various usage and living habits, phenols exposure varies

among different populations and regions. Methods: To explore the relationship be-

tween phenols exposure and male infertility of non-occupational Han Chinese in the middle and lower reaches of Yangtze River, we conducted a case-control study with 803 idiopathic infertile men and 604 fertile controls. By using UPLC-

MS/MS, individual exposures to phenols were evaluated by urinary concentrations of nine environmental phenols (bisphenol A; 2,3,4-trichlorophenol; 2,4,5-

trichlorophenol; pentachlorophenol; hexachlorophene; 4-tert-octylphenol; 4-n-

octylphenol; benzophenol-3). Results: The median adjusted concentra-

tions of phenols were higher or lower than those in the U.S reports, respectively. Among nine phenols, increased urinary concentrations of 4-tert-octylphenol were found to be highly associated with increased male idiopathic infertile risks. Conclusion: Our data provide a total insight of various kinds of phenols exposure and male infertility in eastern Chinese population. These findings should be of con-

cern because of the ubiquitous exposure of phenols.

2345 AGE-DEPENDENT ROLES OF ITCH DURING MOUSE TESTICULAR DEVELOPMENT.

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Ubiquitin is used as a signaling molecule to tag proteins for various purposes such as degradation or localization. The addition of ubiquitin to its target protein (ubiq-

uitination) occurs in three steps: 1) an E1 utilizes ATP to activate the ubiquitin molecule, 2) ubiquitin is transferred to an E2 conjugating enzyme, and 3) an E3 ligase facilitates transfer of the ubiquitin molecule to its substrate. Previous reports showed that ubiquitination of proteins increases in the testis following toxicant exposure, but little is known on the functional importance of changes in ubiquitination during testis development. Here we examine the role of the specific E3 ligase, Itch, in the postnatal development of the testis by evaluating Itch deficient mice (Itch-/-). A physiological analysis of these mice revealed that the knockout animals have smaller body and testis weights than their wild type counterparts, although they have similar body/testis ratios, suggesting that these mice are just smaller overall. Reproductively, Itch-/- mice have significantly smaller litter sizes (average number of pups 5 vs 8), but similar survival rates. Quantification of the total number of mature spermatids revealed that adult 56-day-old mice have significantly lower counts (9.06 ± 6.056) than wild type (12.56 ± 5.012), which corresponds to a higher apoptotic index (8.8% vs. 6.93%) at 28 days of age. Histological exami-

nation of the testis uncovered several age-dependent phenotypes, including an in-

crease in the number of tubules containing meiotic cells at 28 days and disorgani-

zation of spermatids at heads at 56 days. Protein expression profiles revealed changes in several junctional proteins, including Integrin γ3 and β1 Laminin, which may be responsible for the observed spermatid disorganization. A comprehensive develop-

mental analysis is currently in progress for each of these end points. Taken together, the data suggests that Itch plays an important and age-dependent functional role in testis development.

2346 EVALUATION AND VALIDATION OF HUMAN INHIBIN B ELISA (GEN II) ASSAY IN THE RAT AND CYMOLOGOUS MONKEY.


Inhibin is a heterodimeric glycoprotein consisting of an alpha and a beta subunit linked by disulphide bridges. Inhibin B is produced by the testes as well as the ovaries, and is responsible for the selective negative feedback control of FSH secre-

tion. In males, inhibin B is synthesized by the sertoli cells in the testis, and can be used as a direct marker of sertoli cell function and spermatogenesis in adult males. Hence, it is considered a biomarker for detecting testicular damage. Here, we report the evaluation and validation of the human inhibin B (INH-B) ELISA (Gen II) from Beckman Coulter, Inc. to measure serum inhibin B levels in male rats [Sprague Dawley (SD) or Wistar Han (WH)] and male cynomolgous (Cyno) monkey.

Validation criteria included linearity, reproducibility, and recovery evaluations. Results showed acceptable linearity for samples that were spiked with calibrators from the INH-B ELISA kit in castrated (SD rat, WH rat or Cyno monkey) serum or dilute different amounts of intact rat (SD or WH) or Cyno serum with its re-

spective castrated serum (R²>0.9 for all). Reproducibility was good (R²>0.9) among different populations and regions. Methods: To explore the relationship be-

tween phenols exposure and male infertility of non-occupational Han Chinese in the middle and lower reaches of Yangtze River, we conducted a case-control study with 803 idiopathic infertile men and 604 fertile controls. By using UPLC-

MS/MS, individual exposures to phenols were evaluated by urinary concentrations of nine environmental phenols (bisphenol A; 2,3,4-trichlorophenol; 2,4,5-

trichlorophenol; pentachlorophenol; hexachlorophene; 4-tert-octylphenol; 4-n-

octylphenol; benzophenol-3). Results: The median adjusted concentra-

tions of phenols were higher or lower than those in the U.S reports, respectively. Among nine phenols, increased urinary concentrations of 4-tert-octylphenol were found to be highly associated with increased male idiopathic infertile risks. Conclusion: Our data provide a total insight of various kinds of phenols exposure and male infertility in eastern Chinese population. These findings should be of con-

cern because of the ubiquitous exposure of phenols.
2347 ADVERSE EFFECT OF ALUMINUM ON SECRETORY PROTEINS AND ANTIOXIDANT ENZYMES IN THE EPIDIDYMIS OF RATS—PROTECTIVE EFFECTIVE OF VITAMIN E.

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Aluminium has several industrial uses and is widely used in the production of medicines like analgesics, antacids and anti-diarrheal besides finding use as a food additive and water purification agent. Considering the toxic nature of aluminium with less work on reproductive system, the present study was planned to investigate the effect of aluminium chloride on secretory products, enzymatic and non-enzymatic antioxidants and its possible recovery by vitamin E treatment in the epididymis of adult rats. Adult male rats were administered with aluminium chloride, 100 mg/kg body weight, orally, daily for 45 days. Second group of rats were treated with aluminium chloride along with vitamin E. Third group of rats treated with vitamin E alone and the fourth group served as withdrawal group. All the groups of rats were compared with the control group. At the end of the experimental period the animals were sacrificed and the epididymis was dissected out. Antioxidant enzymes like catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione-s-transferase were markedly diminished in the epididymis of aluminium chloride treated animals. The non-enzymatic antioxidants vitamin C and vitamin E were also decreased. The epididymal lipid peroxidation and hydrogen peroxide were significantly increased. Glycerine phospholipid, choline acid, carotene and acetyl carnitine contents were markedly decreased. Vitamin E treatment counteracted the effect of aluminium chloride. In the withdrawal group most of the parameters were brought back to near normalcy. The present study suggests the reproductive toxicity of aluminium by altering secretory products and inducing the oxidative stress in the epididymis and its possible recovery by vitamin E treatment.

2348 APOPTOSIS INDUCED BY 4-TERT-OCTYLPHENOL IN RAT TESTIS AND THE INTRINSIC PATHWAY ACTIVATION.

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Concerns regarding 4-tert-octylphenol (OP) have increased as this chemical displays estrogenic activity. The objective of this study is to elucidate the molecular mechanism(s) underlying the detrimental effects associated with OP as well as to explore whether intrinsic pathway of apoptosis induced by OP was activated. According to our previous in vivo study, the expression levels of some apoptotic related genes, i.e. Bcl-x and death effector domain-containing protein (sperrmatogenesis, apoptosis regulator activity) and sperm membrane protein (YWK-II) were decreased in adult rats treated by OP. Here, the Bcl-2 family proteins expression and intrinsic pathway activated signals were further observed by western blot and flow cytometric analysis (FCM).

The results showed increased apoptosis of testis cells occurred in a concentration-dependent manner by FCM assay (Fig 1). The expression of Bcl-xL was down-regulated, while the expression of active Bax up-regulated (Fig 2). OP also down-regulated the expression of 32 kDa procaspase-3, which was cleaved to generate active subunit (17 kDa) and could induce Cyt C release (Fig 2 & 3). Taken together, these results suggest that OP may induce rat testis apoptosis and could trigger apoptosis via mitochondria-dependant intrinsic pathway by regulating Bcl-xL/Bax, Cyt C release and caspase-3 activation. Moreover, these findings may provide useful indicators of the adverse effects of OP on male reproductive system and prove particularly important in elucidating that Bcl-2 family proteins may play a key role in testicular function change elicited by OP. and discovered that the severity of testicular damage is more dependent on the number of cycles of treatment than the cumulative dose. Theoretically, spermatogonial stem cells (SSCs) should be able to repopulate the testis after cisplatin exposure has ceased. We hypothesize that an increase in the mitotic activity of SSCs during the initial exposure to cisplatin renders them increasingly susceptible to cisplatin-induced injury during the next treatment cycle, underlying the mechanism of treatment-induced infertility. Here we investigate Sertoli cell (SC) factor(s) that stimulate SSCs to enter the cell cycle after cisplatin exposure; namely, glial cell line-derived neurotrophic factor (GDNF). Adult C57 mice were exposed to 5 daily intraperitoneal injections of 2.5 mg/kg cisplatin. Mice were sacrificed at days 1, 3, 7, and 16 of the recovery period and testes were removed for immunohistochemical (IHC) analysis. In order to measure mitotic activity, BrdU was administered intraperitoneally 1.5 hours prior to sacrifice. IHC was performed using antibodies against GDNF, incorporated BrdU, and PLZF (undifferentiated spermatogonia marker). IHC analysis of BrdU showed a 1.37-fold increase in the proliferative rate of early germ cells over controls. GDNF immunostaining in cisplatin-treated mice was particularly prominent along the basal membrane, the region where SSCs reside. PLZF labeling decreased immediately following cessation of treatment, increased by day 7 and returned to control levels by day 16. Future experiments are targeted to test the direct role of GDNF, as well as potential regulatory factors, in increasing the sensitivity of SSCs to cisplatin-induced injury.

2350 ZINC DEFICIENCY EXACERBATES DIABETIC TESTICULAR CELL DEATH BY DOWN-REGULATION OF AKT EXPRESSION AND FUNCTION: ESSENTIAL ROLES OF PTEN, PTP1B, AND TR63.

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Since zinc plays an important role in the spermatogenesis and diabetic patients are often with zinc deficiency, this study was to investigate the impact of zinc deficiency to diabetic effects on testicular cell death and possible mechanisms. Type 1 diabetic model was induced in FVB mice with multiple low dose of streptozotocin. Zinc deficiency was induced by zinc chelator, N, N, N', N'-tetrakis (2-pyridylmethyl) ethylenediamine (TPEN). After diabetes onset, both hyperglycemic and age-matched control mice were given TPEN intraperitoneally for four months. Testicular zinc levels were decreased in diabetes/TPEN group. Testicular cell apoptosis was increased in diabetes group and further increased in diabetes/TPEN group. For mechanistic study, Western blot assay revealed that Akt-mediated glucose metabolism signaling was down-regulated in the testis of diabetes group and further decreased in diabetes/TPEN group. These studies suggest that zinc deficiency significantly exacerbated diabetic induction of testicular cell death probably via down-regulation of Akt expression and function by up-regulation of Akt negative regulators. Therefore, prevention of zinc deficiency for diabetic patients is of vital importance in order to avoid the exacerbation of diabetic inhibition of glucose metabolism in the testis.

2351 EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS RELATED TO SPERM QUALITY AND DNA INTEGRITY.

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The objectives of this study were to explore i) whether exposure to polycyclic aromatic hydrocarbons (PAHs) contribute to the alteration of male sperm quality and DNA integrity of cokene workers, and ii) whether gene polymorphism can modulate the effect induced by PAHs. Our specific aims were to i) determine the correlation between 16 individual PAH species and their biomarkers and sperm quality and DNA integrity, ii) determine sperm oxidative damage by identifying specific DNA lesions, iii) assess the correlation between gene polymorphisms and sperm quality and DNA integrity. A longitudinal study included repeated measurements to account for PAH exposure as it relates to toxic effects on sperms during
spermatogenesis. Personal breathing-zone air samples and urine samples were collected to determine PAH external and internal exposure levels, respectively. Semen was collected to assess sperm quality, DNA adducts, and oxidative damage. Blood samples were collected for genotyping. The high exposed group had a significantly lower percentage of motile, viable and normal morphological sperms as compared to the control. The PAH exposed group experienced higher DNA fragmentation percentages, 8-oxodG concentrations, bulky DNA adducts, and DNA adducts. The concentration of 1-OHP levels negatively correlated with normal sperm morphology and motility. PAHs with higher molecular weights tend to correlate with sperm quality and DNA fragmentation. GSTM1 null and CYP1A1 Msp1 men had higher sperm DNA fragmentation and 8-oxodG concentration. Exposure to PAHs altered sperm quality and increased oxidative DNA damage. Genetic polymorphisms influence the susceptibility of men to sperm DNA damage related to exposure to PAHs. Research results laid a foundation for future studies related to the effects of PAHs on reproductive health and examine gene and environment interaction.

2352 EVALUATION OF REPRODUCTIVE FUNCTION IN MALE CYMOLGUS MONKEYS—TESTICULAR VOLUME AND SPERM ANALYSIS PARAMETERS.


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Nonhuman primates (NHPs) are frequently used for toxicity studies of biopharmaceuticals since they are often the only experimental animals to express pharmacologic responses similarly to humans. There is an associated need to evaluate NHP male fertility abilities. We investigated the correlations between testicular volume, and sperm analysis and serum testosterone parameters in untreated males. One hundred and eighty-four animals aged 57 to 98 months and weighing 4.2 to 8.7 kg were used. Testicular volume was calculated from the major and minor testicular axes. Sperms were collected by electroejaculation, and examined to determine sperm count and motility. Motile sperm, curvilinear velocity, total head displacement amplitude, head oscillation frequency, linearity, track velocity, straight line velocity, and track linearity; IVOS sperm analyzer) and the rate of morphological malformation. Blood was collected twice for serum testosterone measurement by ELISA. We also investigated the effects of (α)-3-chloro-1,2-propanediol (α-chlorohydrin) on sperm motility in vitro. A slightly positive correlation between sperm count and age was noted. Testicular volume was positively correlated with sperm count and serum testosterone level. Motile sperm was negatively correlated with malformation rate. No correlations were noted between serum testosterone concentration and sperm count, or sperm motility and malformation rate. α-chlorohydrin affected sperm velocity in vitro at a concentration of 1% with 5-minute incubation. We concluded that testicular volume and sperm analysis parameters are good indicators for selection of cynomolgus monkeys in toxicity studies.

2354 RESVERATROL RESTORES FERTILITY INDICES IN MALE RATS ORALLY ADMINISTERED WITH BENZO(A)PYRENE.

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Benzo(a)pyrene (BaP) is a semi-volatile, persistent environmental pollutant. Apart from inhalation of BaP through cigarette smoke and occupational settings, humans are also exposed to BaP through consumption of contaminated foods. The objective of this study was to assess the ability of resveratrol (RVT) to block the effect of orally administered BaP on male fertility indices. Adult male F-344 rats were randomly assigned to receive BaP only (5 mg/kg) or RVT (50 mg/kg) + BaP or vehicle (tricaprylin; VEH control) for 60 days. Thereafter, all animals were anesthetized to facilitate blood samples collection for serum testosterone measurement. Subsequently, testes and epididymides were harvested, weighed and stored spermatozoa recovered for the determination of progressive motility and sperm density. The right testes of rats in each group were subjected to H&E staining post fixation in buffered formalin. Tests weights did not differ among BaP-treated, RVT + BaP and VEH control rats. However, mean epididymal weight was reduced among BaP-treated rats, an effect that was blocked by RVT compared to VEH control treatment (BaP: 0.44 ± 0.01; RVT + BaP: 0.50 ± 0.01; VEH: 0.48 ± 0.01 [P<0.01]). Spermatogenesis was blocked the activity of BaP in reducing both stored sperm motility (RVT + BaP: 78.0 ± 3.3; BaP: 57.0 ± 4.02; 82.0 ± 2.1 [P<0.005]) and density (RVT + BaP: 84.0 ± 4.0; BaP: 36.0 ± 6.0; VEH: 82.0 ± 4.0 [P<0.05]). H&E staining showed a significant disruption in the integrity of the Leydig cell compartment of the testes of BaP-treated versus those of control rats, a condition that was blocked by RVT. Interestingly, serum testosterone concentrations were significantly reduced in the BaP-treated versus control rats. However, testosterone concentrations were not affected when BaP was co-administered with RVT. These data suggest that RVT blocked BaP-induced disruption in endocrine-regulated fertility indices in male rats by maintaining the secretion of testosterone (funded by NIH grants 1S11ES00014-05 and 1R01CA142845-01A1).

2355 TESTICULAR AND EPIDIDYMID HISTOLOGIC CHANGES AROUND THE PERIOD OF SEXUAL MATURATION IN YUCATAN BOARS.

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Preclinical guidelines often specify the use of prepubertal, pubertal, or sexually mature animals. However, “puberty” and “sexual maturity” can be defined in a number of different ways which reflect androgen production, including the onset of mounting behavior, penile erection and/or ejaculation ± sperm capabilities, or, histologically, by a “threshold” portion of seminiferous tubules engaged in spermatogenesis ± epididymal sperm. Furthermore, “sexual maturity” can also be interpreted slightly differently, depending on the type of toxicology study programs (e.g., DART versus repeat dosing studies). Since Yucatan boars have been reported to reach “puberty” as early as 12 weeks or as late as 16 to 20 weeks of age, it is critical, regardless of how the stages of sexual development are defined, to know what is happening histologically in the testes at these various ages. Modified Davidson’s-fixed and PAS-stained testicular and epidydimal sections were evaluated from 12-, 14-, 16-, 18-, 20-, 22-, and 24-week-old Yucatan boars (n-minimum of 4/group). Approximately 200 seminiferous tubules were evaluated per testis for immature/mature tubules. The proportion of the total number of seminiferous tubules represented by “mature tubules” was calculated. The presence of sperm in the cauda epididymides was also noted. Round spermatids began to appear at 12 weeks of age, and a majority of 14-week-old boars had seminiferous tubules containing both round and elongate spermatids. While sparse numbers of sperm appeared in the epididymides of one boar at 14 weeks of age, at least half of the 16- and 18-week-old boars exhibited some spermatiation with sperm in the excurrent duct system. By 20 weeks of age almost all seminiferous tubules were “mature”, with sperm present in the epididymides. These novel data can be taken into consideration, along with other indices of sexual development, when designing toxicology experiments of varying durations which require Yucatan boars at a given stage of sexual maturity.
Prostatic buds are derived from the urogenital sinus (UGS) and later form into the prostate ductal network in adult mammals. In utero TCDD exposure causes dorsal and lateral prostatic buds in mice to form in inappropriate positions and prevents formation of ventral and some dorsal prostatic buds leading to ventral prostate agenesis. We have discovered an imbalance in WNT signaling as a consequence of in utero TCDD exposure. To obtain a global view of WNT signaling defects, we are performing sectional ISH to look at differences in WNT signaling components between vehicle- and TCDD-treated UGSs on E16.5 from C57BL6/J pregnant dams dosed with TCDD (5 μg/kg, po) or vehicle on E15.5. We discovered that Rspo2 expression is decreased by TCDD exposure in the ventral prostatic budding region corresponding to the UGS area most sensitive to TCDD. R-spondins (RSPOs) are promoters of canonical WNT signaling and are thought to act by preventing the binding of the extracellular inhibitors, dikkopffs (DKKs), to LRP receptors thereby preventing internalization of the LRP receptors. To further examine the role of RSPOs in prostatic budding either alone, or combined with β-catenin, β-catenin mice died perinatally. To determine whether their UGSs were capable of developing into prostate, they were transplanted on E18.5 into the subcapsule space of adult male nude mice and evaluated for prostatic differentiation. β-catenin caused a disorganization of UGS buds that resembled compacted epithelial prostate buds formed as compared to control UGSs treated with DHT alone. Furthermore, addition of RSPOs to in vitro culture UGSs exposed to TCDD leads to a partial rescue of the budding defects. Preliminary analyses indicate that basal epithelial cells positive for SOX9 or LEF1, confirmed downstream targets of canonical WNT signaling, show a decrease in cells positive for SOX9 or LEF1 in UGSs exposed to TCDD, but this effect is reversed upon addition of RSPOs. These data illustrate an important role for RSPOs as key modulators of prostatic bud formation and that addition of RSPOs may reduce the effects of TCDD exposure on prostatic budding. (Grant support: NIH E051332, T32 ES007015)

Propylparaben, an antimicrobial excipient used in pharmaceutics and cosmetics, has been described as having effects on sperm parameters and plasma testosterone concentrations of male rats following juvenile exposure (Oishi 2002). A GLP study was undertaken to confirm and further characterize these effects. Propylparaben was given by oral gavage to 4 main groups of 20 male Wistar rats at nominal doses of 3, 10, 100 or 1000 mg/kg/day in 1% hydroxyethylcellulose (10 mL/kg) for 8 weeks starting from post natal day (PND) 21. A control group of 20 males received the vehicle. One sub-group of 10 animals per group was necropsied at the end of the dosing period and the other after a 26-week treatment-free period.

The following endpoints were assessed morbidity/mortality, clinical condition, body weight, sexual maturation, LH, FSH and testosterone levels, organ weights, gross and microscopic pathology and sperm quality. Blood samples were taken from additional satellite animals at specific time-points after dosing on PND 21 and 77 for TK. There were no unscheduled deaths and no remarkable clinical changes in any group throughout the study. Similarly, there were no compound-related organ weight, macroscopic or microscopic changes in the testes and epididymides, and no evidence of an effect on sexual maturity, sperm count or motility, in any group at the end of the treatment and treatment-free periods. In conclusion, once daily oral (gavage) administration of propylparaben to male Wistar rats at nominal doses of 3, 10, 100 and 1000 mg/kg/day between 3 and 11 weeks of age was without any effect on reproductive parameters in any group. The nominal dose of 1000 mg/kg/day was therefore the no observed effect level. It did not confirm the results reported by Oishi.

The French Medicines Agency (Afsaps) has initiated and led this project, in association with an industry consortium (BMS, Orphan Europe, Pfizer, Reckitt Benckiser, Sanofi-Aventis, Servier).

Environmental exposure to endocrine disrupting chemicals is hypothesized to negatively impact male reproductive health. Within the testis, Sertoli cells are critical for proper germ cell maturation and spermatogenesis, and exposure to Sertoli cell toxicants is associated with Sertoli cell dysfunction and germ cell loss. The phthalate metabolite mono-2-ethylhexyl-phthalate (MEHP) is a known Sertoli cell toxicant in rodents; however, the effects of MEHP on human Sertoli cell viability and function are unknown. We have developed and characterized an in vitro model of adult human Sertoli cells to study the mechanisms by which environmental toxicants induce Sertoli cell injury. Primary Sertoli cells from a 23-year old donor (S23Y) obtained from Lonza proliferated readily in culture, eventually undergoing replicative senescence. S23Y cells exhibited morphology typical of Sertoli cells, expressed markers of Sertoli cell origin, including Sox9 and GATA4, and stained positive for Oil Red O. The presence of cytoplasmic lipid vacuoles was confirmed by transmission electron microscopy. Exposure of S23Y cells to subcytotoxic doses of MEHP induced rapid Phosphorylation of Akt (Ser473) and GSK3β (Ser9), indicating activation of the Akt pathway. Prolonged (48hr) exposure to higher doses of MEHP (250-1000μM) resulted in a dose-dependent decrease in cell number, although cells remained viable up to 500μM MEHP, indicating both inhibition of proliferation and induction of cell death. Additionally, we immortalized S23Y cells through expression of the catalytic subunit of human telomerase (S23Y/HaTERT) to provide a continuously proliferating population of Sertoli cells for more extensive study. S23Y/HaTERT cells maintained Sertoli cell-like morphology and Oil Red O positivity. We are currently validating the use of immortalized human Sertoli cells to study the mechanisms by which endocrine disruptors induce human Sertoli cell injury.

Sulfur mustard (SM), commonly known as mustard gas, is a bifunctional alkylating agent that has been historically used in chemical warfare and is still a #1 stockpile of warfare agent in US. Acute exposure to SM has been reported to cause chronic testicular damage and consequently oligospermia/azoospermia in men years after a single exposure. We have investigated the male reproductive toxicity of SM in rats and cynomolgus macaques. Groups of adult male F344 rats were exposed to SM (400 μg/kg, po) or vehicle on E15.5. We discovered that Rspo2 of C57BL6/J pregnant dams dosed with SM resulted in a decrease in cell number, but this effect is reversed upon addition of RSPOs. These results suggest that stabilization of β-catenin mimics inhibitory effects of TCDD on VP development. (Supported by NIH ES01332)
vapor at 150 mg/m³ for 10 or 15 minutes, or to filtered air for 15 minutes via in- tratracheal inhalation. Weights and histopathology of male reproductive organs, as well as semen parameters (counts, motility, morphology, and sperm DNA fragmen- tation) were evaluated at 4, 7 and 10 weeks post exposure. There was a significant reduction in organ weights of testis, epididymis, seminal vesicle, and prostate in SM-exposed rats. Semen analysis showed that SM exposure led to reduced sperm count, decreased sperm motility, and increased morphologically abnormal sperm in cauda Epididymis. Sperm chromatin separation analysis also demonstrated there was a significant increase of sperm with DNA fragmentation in cauda epididymis of rats exposed to SM. Histopathological examination revealed atrophy of seminifer- ous tubules, degeneration/deletion of round spermatids and spermatocytes, de- pletion of elongated spermatids, and germ cell sloughing into tubular lumen in SM- exposed rat testes. Sloughed cells /cell debris and reduced sperm content were also observed in caput and corpus segments of Epididymides in SM-exposed rats. Moreover, similar adverse effects were observed in macaques following intratracheal inhalation exposure to SM vapor at 100 or 150 mg/m³ for 10 minutes. Our animal models will be useful for further investigation of the mechanism and development of therapeutics for SM-induced gonadal toxicity. Research funded by NIH/NINDS #5U54NS058185 and the 2010 supplement grant.

2361 INHIBITION OF CYCLOOXYGENASE 2 REDUCES DIPENTYL PHTHALATE TOXICITY IN A 3- DIMENSIONAL IN VITRO RAT TESTES COCULTURE MODEL: EVIDENCE FOR AN ALTERNATE MECHANISM OF ACTION.

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Phthalate exposure is associated with changes in steroidogenesis, leading to the hy- pothesis that this is a primary mechanism of phthalate toxicity. However, phthalate- induced male reproductive toxicity has been shown to occur in the absence of changes to testosterone production, suggesting alternative mechanisms. Microarray data from our lab suggests that inflammatory pathways play a role in phthalate tox- icity. Others have found a link between phthalate exposure and changes in expres- sion of the inflammatory enzyme cyclooxygenase 2 (cox2). Furthermore, inhibition of cox2 enhances expression of the steroidogenic acute regulatory protein (Star), the rate limiting step in steroidogenesis. We hypothesize that phthalate-induced toxicity and regulation of steroidogenesis are mediated by cox2. We employ a 3D in vitro rat testes co-culture to explore the role of cox2 in phthalate toxicity. Cells were treated with 0, 50, 100, or 200µM dipentyl phthalate (DPP). Additional plates were pre-treated with the cox2 inhibitor NS-398 prior to 100µM phthalate treat- ment. Cytotoxicity and protein expression were measured at 8, 24, and 72hrs. Phthalate treatment resulted in a dose-dependent increase in cytotoxicity. Cox in- hibitor pre-treatment significantly reduced the cytotoxicity of at 8 hrs (p=0.049) and 24hr (p=0.035). While DPP exposure resulted in a significant decrease in Star pro- tein expression after 8 hrs (p=0.001), pre-treatment with cox inhibitor significantly attenuated this effect (p=0.034). DPP exposure significantly increased cox2 expres- sion at 8hp=0.009) and 24 hrs (p=0.0017). Cox inhibition also greatly increased cox2 protein expression. These results indicate that DPP-induced changes in testos- terone synthesis may be mediated by an inflammatory response. Supported by T32 ES007032, 1U10FD04242-01, 1U10FD004242-01, Colgate-Palmolive Grants for Alternative Research, and the Johns Hopkins CAAT.

2362 OXIDATIVE AND GENETIC DAMAGE IN GERMAL AND MONONUCLEAR CELLS BY METHYL- PARATHION EXPOSURE IN MOUSE.

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Methyl-parathion (Me-Pa) is an organophosphorus pesticide highly neurotoxic, which is also associated with growth hormone and testicular damage with adverse effects in the progeny. Collection of sperm cells can be a hard task in epidemiological sur- veys; therefore the aim of this study was to compare the oxidative and genetic dam- age in mononuclear (MNC) and sperm cells by repeated exposure to Me-Pa, to evaluate the usefulness of MNC as surrogate indicator of genetic-oxidative damage in male germinal cells. CD1 mice (12-weeks old) were exposed to repeated doses of Me-Pa (6 and 9 mg/kg bw/day/5 days, ip). Spermatogenesis were extracted from epi- didymis-vas deferens and MNC from peripheral blood. DNA damage was evalu- ated by the Comet assay (OTM parameter) and the oxidative damage by means of 8-OHdG levels by flow cytometry. Exposure to Me-Pa caused a significant dose-de- pendent increase in OTM values in both cells lines, 45-50% increase in sperm cells and 27-39% increase in MNC, although the slopes were different: 0.85 and 1.68 in spermatozoa and MNC, respectively. Levels of 8-OHdG in sperm cells showed an increase only at 9 mg/kg, 10-fold compared to the control group, while a dose-de- pendent increase was observed in MNC (1.6 to 3.5-fold increase). These results suggest that the genetic damage observed in MNC may be a predictor of the dam- age caused in male germinal cells by OP pesticide exposure, while the oxidative damage in MNC may not, suggesting that the antioxidant capacity and/or oxida- tive repair are different in these two cell lines.

2363 COMPARATIVE ASSESSMENT OF SEXUAL MATURATION IN MALE SPRAGUE DAWLEY AND WISTAR HAN RATS.

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The use of sexually mature animals in toxicology studies is important to reduce the detection of “lesions” that may be related to immaturity rather than treatment. Anecdotal evidence suggests that the male Wistar Han (WH) rat attains sexual matura- tion a few weeks later than the Sprague Dawley (SD) rat. Given the increasing use of WH rats in regulatory toxicology studies, this study was performed to character- ize the onset of sexual maturation and adult sperm numbers and motility in matur- ing SD and WH rats. Beginning on postnatal day (PND) 38, and at multiple time points through PND 91 groups (n=8) of untreated SD and WH rats were evaluated for maturation of the male reproductive system. Body and organ (testes, epi- didymides, prostate) and seminal vesicles were collected. Ejaculate and epi- didymis were fixed for microscopic examination, and the right testes and epi- didymis were frozen for determination of epididymal sperm counts and testicular sperm head counts using a computer-assisted sperm analysis (CASA) system. Sperm motility was measured on animals 56 days or older by CASA. The WH rats were smaller in size than the SD rats, with an average body weight of 339 g com- pared to 507 g on PND 77. Steady increases in testis, epididymal, and accessory sex gland weights were observed through the end of the study, and they were compar- able between the 2 strains. No differences in testicular spermatic head counts were detected and kinetics of the population of the different regions of the epididymis with sperm was very similar between the 2 rat strains. In WH rats sperm motility was at adult levels by PND 63 (~90%), while in SD rats sperm motility was at adult levels (~85%) by PND 70. The morphology of the testis/epididymis of SD rats 59 days of age or older is consistent with qualitative sexual maturity. Seven of eight WH rats were qualitatively mature by day 56, and all were by day 63. Based on the lack of any differences in spermatic histology, head and sperm counts, and sperm motility, we found no detectable difference in the maturation trajectory of the two strains.

2364 COMPARISON OF TOXICOGENOMIC RESPONSES TO PHTHALATE ESTER EXPOSURE IN AN IN VITRO RAT TESTES COCULTURE (TCS) MODEL AND RESPONSES OBSERVED IN VIVO.

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There currently exists a demand for the development of new in vitro methods of screening chemicals for various toxic effects, due to increasing pressure to test a greater number of chemicals while reducing the number of animals used for such testing. Previously, we developed a 3D testicular culture system which mimics the in vivo microenvironment of developing testes in the rat. We have demonstrated that our culture system is able to distinguish between developmentally toxic phtha- late esters (DTPeas) from developmentally non-toxic phthalate esters (DNTPeas) based on observed changes in gene expression patterns. In the current study we sought to compare genomic changes observed in this culture system with those ob- served in vivo after a week-long exposure to the same phthalate esters (Liu et al., 2005). A trend in which DTPe exposure was associated with a greater number of gene expression changes than DNTPe exposure was observed both in vitro and in vivo. Furthermore, we observed that probes linking genes in the Gene Ontology category “steroid biosynthetic process” formed distinct clusters after exposure to DTPe when compared with DNTPe or controls, with 11% of the 1000 most significant gene changes (ranked based on ANOVA test) occurring under in vitro and in vivo conditions. These phthalate esters gene ontology analysis using DAVID software and pooled analysis revealed a number of terms associated with broad based cellular processes were significantly altered in vitro (cell cycle, cell dif-
INTRAUTERINE QUINACRINE (iuQ) USED FOR HUMAN BIRTH CONTROL: UTERINE HISTOPATHOLOGY AND PLASMA QUINACRINE (pQ) DURING 96 HOURS AFTER IUQ IN RATS.

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This experiment demonstrated early sequential changes in uterine histopathology and pQ, during 96hrs (hrs) after iuQ treatment of Sprague Dawley (SD) female rats. Doses were selected to estimate maximum tolerated dose (MTD). Thirty-six rats were randomly assigned to seven treatment groups (2mg/kg [9/10g]). Quinacrine dihydrochloride dihydrate (Q), suspended in 2.0% methylcellulose (MC) on 0.9% saline (S) at 10, 20, 70 and 250mg/kg body weight or as a solid pellet (P;10mg/kg) was inserted transcervically during diestrus. Controls were IUQ and IUQ (n=9/10g). Plasma samples, collected from the tail vein at 0.5, 1, 2 and 4hrs (n=9/10g and 1/10g) and at necropsy (6, 24 or 96hrs, n=3/10g/mouse), were analyzed for pQ. Uterine tissues were examined histopathologically. Dose dependent acute endometrial necrosis (focal ulceration, EN) accompanied by inflammation, hemorrhage and stromal edema occurred by 6hrs after iuQ. Lesions were more severe and multifocal by 24hrs. In some animals at 70mg/kg lesions extended into the myometrium (ulcer;MU) and were multifocal. Severe MU likely caused deaths of 3/13 animals at 250mg/kg. Significant endometrial regeneration was occurring by 96hrs in a dose dependent manner, moreso at lower doses. The pharmacokinetic (PK) profile of pQ was dose related and biphasic—maximal at 1hr after iuQ, with a second peak at 6hrs. The 10mg/kg QP caused higher pQ than 10 or 20mg/kg QMC (Ps0.05). pQ was 10-fold greater at 250mg/kg than at 70, mg/kg and increased 25,000 and 340mg/ml at 1 and 6hrs. Systemic exposure to iuQ was greater (Ps0.05) after 10mg/kg QP and at 70mg/kg QMC than other iQ as measured by pQ area under the PK curves for both 0-6hr and 0-4hr periods. The pQ cleared rapidly—between 24hrs at 10 to 70mg/kg and by 6hrs at 250mg/kg iuQ. Conclusion: data suggest irreparable diffuse ulceration occurs at doses 0/70mg/kg QP. EN and MU were increased in a dose dependent manner by iuQ. The MTD is 10mg/kg iuQ in MC for a single dose iuQ in rats.

TRANSFER OF TRICLOCARBAN ACROSS THE PLACENTAL BARRIER.


Triclocarban (TCC), an antimicrobial compound commonly added to bar soaps, has recently been linked to endocrine disruption in studies using in vitro assays. TCC is a compound of particular concern because it is detected at low levels in wastewater treatment plant effluent. Exposure to endocrine disruptors through placental transfer may cause harm to the developing fetus. We hypothesize that TCC is transported across the placental barrier. Combined with accelerator mass spectrometry (AMS), we used the BeWo b30 human placental choriocarcinoma cell line in a transwell cell culture model to investigate the transfer of TCC across the placental barrier at environmentally relevant concentrations. The presence of a confluent monolayer was verified by light microscopy. Trans-epithelial electrical resistance, and the transport of FITC-dye and FITC-Dextran. Cells were exposed to environmentally relevant concentrations (1, 10, and 63 nM) of TCC. Due to the extremely low concentrations of TCC in this assay, 14C-TCC was used along with AMS to detect the transfer of TCC. AMS was sensitive enough to detect transfer across the cells at the earliest time point examined, 10 minutes, for all three doses. The three concentrations show a similar percentage of dose transferring across the BeWo b30 and human placental collagen barrier over the 8-hour time course. The percentage of dose transferred is less than 0.05% at 10 minutes and steadily rises to around 3%.

This study demonstrates TCC transfers at environmentally relevant concentrations across an in vitro model of the human placental barrier. Establishment of this transfer demonstrates the necessity to investigate if these levels of TCC exert any harmful effects on the development of the fetus. In future studies, we will examine the ex vivo dose using the BeWo b30 transwell assay with AMS. We also plan on using AMS with 14C-TCC exposures in drinking water of mice at environmentally relevant concentrations to investigate transfer of the compound to the fetus and the potential developmental effects the exposure may have. Supported by LDRD 11-LW-018 and NCR 13461.

SPECIES SELECTION FOR DEVELOPMENTAL AND REPRODUCTIVE TOXICITY EVALUATION OF A NEW LIVE ATTENUATED VACCINE AGAINST FLAVIVIRUS INFECTIOUS DISEASE.


To support the licensure of a new live attenuated vaccine (LAV) against flavivirus infectious disease, a developmental and reproductive toxicity (DART) program was initiated to investigate the potential risk for women of childbearing potential and their offspring during vaccination. In designing a DART program for LAV, the selection of the appropriate species is considered. The species must be relevant being pathogenically sensitive to the virus and must develop a detectable viremia and humoral response post-vaccination with transfer to the fetus. For systemic toxicity, the non-human primate had been recognized as the relevant species. However, its use for DART studies carried technical as well as ethical issues and alternatives were investigated.

The rabbit and mice were selected, since rabbits are commonly used DART species for vaccines showing humoral response and transfer to fetuses, and some previous data in mice suggested that viremia might be detectable. Preclinical studies were conducted, and models were adapted by using the IV route and by pushing the dose to improve the response to the vaccine. Data in non-pregnant and pregnant animals showed that rabbit females given three IV injections of 5 to 8 log of infectious dose, elicited a robust antibody response with good antibody transfer to fetuses and no evidence of toxicity in dams and fetuses, but no detectable virus at any dose level. The female mice given a single IV injection at the same dose levels showed detectable viral level in dams with transfer to fetus at the high dose only and limited antibody response, with minimal maternal toxicity at high dose, but not in the fetuses.

Based on these data, both rabbit and mice were selected for the pivotal DART program. The rabbit model was selected to investigate the effects of the LAV and anti-body response through pre-implantation to lactation. The mouse model was selected to investigate the exposure to the virus through embryonic and fetal periods or during lactation.

ASSESSMENT OF POSTNATAL DEVELOPMENT IN THE COMMON MARMOSET (CALLITHRIX JACCHUS) IN THE CONTEXT OF DART EVALUATION.


Generally, for nonhuman primate developmental and reproductive toxicity (DART) studies, macaque monkeys are the preferred model. In some cases, antibody cross-reactivity or metabolic compound profiles require the use of marmosets for DART evaluation. Since, the reproductive physiology of marmosets differs from that of human and Old World Monkeys, species-specific reference data are essential. Pregnancy outcome in 44 female marmosets was assessed. Abortion rate in pair housed marmosets was 18.2% and infant mortality rate was 13.6% on Day 0 post-partum (p.p.). Marmosets had up to four infants per litter – on average 2.4 infants/litter with a sex ratio of female:male 1:0.9. Hence, marmoset pregnancy outcome (Day 0 81.8%; Day 7 p.p. 59.1%) was in the range of cynomolgus monkeys (Day 0 p.p. 77.2% and Day 7 p.p. 70.2%) (Jarvis et al. 2010; Birth Defects Res B 89:175-187). Marmosets have the advantage of a comparatively large litter size among nonhuman primates. Overall, 14 male and 15 female newborns were studied at birth (neonatal) and on 28 days of age. They comprised general information, such as sex determination and ratio, body weight and external abnormalities. On day 0 p.p. and 28 p.p. we also performed morphological examinations on the head (head length and width, distance between the eyes), the body (crown-rump length, crown-heal length, tail length, and chest circumference), the extremities (arm length and leg length), and the anatomical distance. The data panel demonstrates the growth rates of neonatal marmosets in the first month of life. For 28 days old marmoset infants, the generated data panel also includes the length of the femur and organ weights of the following organs: brain, thymus, heart, liver, thyroid with parathyroids, spleen, adrenals, kidneys, ovaries, uterus, and testes with epididymides. In conclusion, (limited) DART evaluation is feasible in marmosets if macaque models cannot be used. The differences, however, limit the use of common marmosets in unconventional research strategies targeted on human pathology.
2369 DECREASED REPRODUCTIVE INDICES IN H-NAG-1 (GDF-15) MICE CORRELATED TO POTENTIAL ALTERATIONS IN MAMMARY GLAND FUNCTION.

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The NAG-1 (Nonsteroidal anti-inflammatory drugs (NSAID)-activated gene-1) gene has been shown to induce anti-inflammatory activity in vitro and in vivo. Transgenic mice have been generated that ubiquitously overexpress human NAG-1 (h-NAG-1). C57BL/6-Tf(TAG-GDF15) female mice hemizygous for the transgene (h-NAG-1+/−) mated with wild type (WT) males of the same strain have a reduced number of offspring at weaning compared to WT dams mated to h-NAG-1+/− males. To determine the cause of the decreased reproductive fecundity in females, groups of 16 h-NAG-1+/- and WT females on a standard diet of 6% fat were continuously mated to proven WT males for approximately 22 weeks. Females were weighed weekly and pups were weighed at post natal day (PND) 0, 7, 14, and 21. Although h-NAG-1 mice weigh less than WT throughout their life spans, both groups gained a comparable percentage of weight during pregnancy; there were no significant clinical observations in either group. h-NAG-1 (94%) and WT (100%) females produced a similar incidence of live births and the number of litters was equal (3.0). At birth, the weight of transgenic pups was comparable, and there were no differences in male/female survival ratio. There were no gross abnormalities noted in pups that did not survive. In the 1st, 2nd, and 3rd litters, the mean number of pups/litter at birth was less in h-NAG-1 (6.0) relative to WT mice (8.3), by PND 21, only 22% of h-NAG-1 pups survived for a mean number of 1.2 pups/litter, while over 66% of WT lived to PND 21 with a mean of 5.1 pups/litter. In general, milk spots were less prominent in h-NAG-1 pups. Mammary glands were collected on mice after the fourth litter or after 22 weeks on study and stained with carmine alum. While there was no change in ductal development, the data presented herein suggest altered mammary gland function may contribute to the decreased number of weaned pups from h-NAG-1+/-.

2370 COMPARISON OF PLACENTAL HISTOPATHOLOGY AFTER ORAL ADMINISTRATION OF EPOXICONAZOLE TO PREGNANT RATS AND GUINEA PIGS.

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In previous prenatal developmental toxicity studies with epoxiconazole conducted in rats and guinea pigs, significant fetal toxicity was seen in rat but not in guinea pig fetuses. In pregnant rats administered 50 mg/kg bw/day daily from gestation day (GD) 7-21, a high incidence of late fetal resorptions was associated with depletion of estradiol in maternal serum and severe damage of the placenta. Daily gavage administration of epoxiconazole to pregnant guinea pigs at GD 6 to 63 at up to 90 mg/kg bw/day (half the lethal dose in guinea pigs) did not result in significant depletion of maternal serum estradiol and did not induce any significant placental damage despite of comparable maternal plasma concentrations of epoxiconazole in rats and guinea pigs at 50 mg/kg bw/day. Differences in sensitivity to aromatase inhibition and different locations of major estradiol productions of epoxiconazole in rats and guinea pigs at 50 mg/kg bw/day). Differences in sensitivity to aromatase inhibition and different locations of major estradiol productions of epoxiconazole in rats and guinea pigs at 50 mg/kg bw/day. Differences in sensitivity to aromatase inhibition and different locations of major estradiol productions of epoxiconazole in rats and guinea pigs at 50 mg/kg bw/day. Differences in sensitivity to aromatase inhibition and different locations of major estradiol productions of epoxiconazole in rats and guinea pigs at 50 mg/kg bw/day. Differences in sensitivity to aromatase inhibition and different locations of major estradiol productions of epoxiconazole in rats and guinea pigs at 50 mg/kg bw/day. Differences in sensitivity to aromatase inhibition and different locations of major estradiol productions of epoxiconazole in rats and guinea pigs at 50 mg/kg bw/day.
0) through PND 3. Females were sacrificed on PNDs 28 and 33, and unexposed male siblings were sacrificed on PND 53 to assess sex differences. RFRP3 fiber density in the rostral periventricular region of the third ventricle (RP3V), cell numbers in the dorsomedial nucleus (DMN) and appositions on preoptic GnRH neurons were quantified using immunohistochemistry and confocal microscopy. RFRP3 fiber density and cell numbers were decreased in the E2 and LOW BPA groups, the same groups that displayed early vaginal opening. Similar group differences were also observed for the percentage of GnRH neurons with RFRP3 appositions, but this result did not reach statistical significance. None of the endpoints observed were sexually dimorphic. Collectively, our results indicate a role for decreased inhibition of GnRH release as a potential underlying mechanism for advanced vaginal opening.

2374 ATRAZINE-MEDIATED DISRUPTION OF STEROIDOGENESIS IN BLTK1 MURINE LEYDIG CELLS.
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Atrazine (ATR), a widely used triazine herbicide, has been associated with impaired reproductive development and function. ATR effects on steroidalogenic activity were assessed in BLTK1 cells, a novel murine Leydig cell line (BLT-1 cells, clone K1) using commercially available enzyme immunonassays for testosterone (T) and progesterone (P). BLT1K cells maintain an intact steroidogenic pathway producing low basal levels of T, express all necessary steroidalogenic enzymes (Star, Cyp11a1, Cyp17a1, Hsd3b, Hsd17b3, Cyp19a1 and Srd5a1) and 11β-metabolisable human choriionic gonadotropin (hCG) induces CAMP (~100-fold), P (~10-fold) and T (~5-fold) levels. BLTK1 cells were stimulated with 1, 3, 10, 30, 100, 300, 600 nM ATR, 3 ng/ml rhCG, a combination of 300 nM ATR + 3 ng/ml rhCG or DMSO vehicle. Media were collected for hormone level determination and gene expression evaluation was conducted after 24 hrs. ATR elicited concentration-dependent induction of P (~5-fold) and T (~3.5-fold). Co-treatment of 300 μM ATR with 3 ng/ml rhCG, which mimics Leydig cells stimulated by luteinizing hormone exposed to ATR, resulted in similar levels of P (~6-fold) and T (~4-fold) as with ATR alone, but lower levels when compared to 3 ng/ml rhCG alone, suggesting ATR antagonizes rhCG-induced steroidalogenic. This is consistent with the induction of steroidalogenic acute regulatory protein (Star) mRNA by rhCG (~12-fold) which was antagonized with ATR co-treatment by ~ 50% (6-fold), comparable to the induction elicited by ATR treatment alone (~4-fold). Similarly, the expression of Sre-reductase (Srd5a1), which is induced 2-fold by rhCG alone, was not evident with co-treatment of rhCG with ATR (no significant fold change), whereas ATR treatment results in 2-fold down-regulation. These data suggest that ATR antagonizes rhCG-induced steroidalogenic in BLTK1 Leydig cells by altering steroidalogenic enzyme gene expression.

2375 REPRODUCTIVE TOXICITY EVALUATION OF MAJOR SUBMARINE ATMOSPHERE COMPONENTS IN RATS.
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Recent congressional approval allowing women to serve on submarines necessitates additional investigation into the suitability of existing submarine breathing air standards. This study evaluates the general, reproductive and developmental health effects upon male and female rats exposed to mixtures of three critical submarine atmospheric components (carbon monoxide, carbon dioxide and oxygen) at concentrations that represent the current submarine standards for normal operating conditions (90 days), the 24-hour short-term exposure limit, and the 1-hour emergency exposure limit. The complete study is divided into three phases designed to determine whether existing Navy standards for these gases are health protective of male and female submarine crew members. The results of the first phase, which was a range finding study to screen for overt toxicities after 14 days of exposure, are described here. Four groups of 16 male and 16 female CD® IGS rats were exposed via whole body inhalation to clean air (0.4 ppm CO, 0.1% CO2, 20.6% O2), a low-dose gas mixture of 0.4% CO, 4% (3.9% CO2, 16.1% O2) and a mid-dose gas mixture (4% CO, 0.2% CO2, 15.0% O2) for 24 hours per day for 14 consecutive days. The tissues examined post-exposure included the brain (basal ganglia; hippocampus; hypothalamus), heart, pancreas, liver, spleen, kidneys, adrenal glands, pituitary gland, male reproductive organs (testes; seminal vesicles; prostate), and female reproductive organs (ovaries; uterus; vagina). The pathological findings were unremarkable, or incidental to exposure, and blood analysis results were within normal clinical parameters. The gas mixture exposure concentrations were well tolerated by the rats and are currently being used in a 90-day subchronic, 2-generation developmental and reproductive toxicity evaluation designed to assess the adequacy of the current atmospheric standards.

2376 EFFECT OF DIET-INDUCED OBESITY ON XENOBIOTIC METABOLIZING GENE EXPRESSION IN THE OVARY.
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Obesity is associated with compromised reproductive function, aberrant insulin signaling and development of Type II diabetes. The ovary contains a finite number of primordial follicles, which once depleted, cannot be replaced, leading to premature ovarian failure. Chemical exposures can deplete primordial follicles making ovarian xenobiotic metabolism critical for protection of the female germ cell. Insulin regulates liver xenobiotic metabolizing enzymes, thus impairment of insulin signaling could alter chemical metabolism. This study was designed to investigate the impact of diet-induced obesity on mRNA and protein expression of the ovarian insulin receptor (Insr) and four ovarian expressed xenobiotic metabolizing enzymes: Microsomal epoxide hydrolase (mEH), Cytochrome P450 isomor 2E1 (Cyp2e1), Glutathione S-transferase (Gst) isoforms mu (Gstm) and pi (Gstp). C57Bl6/J mice fed either a standard chow or a high-fat diet (HFD: 60% kcal fat) from 6 weeks of age for approximately 7 months entered into a diabetic state as established by glucose tolerance testing. Total ovarian RNA and protein were isolated from these mice and RT-PCR or Western blotting used to quantify mRNA level of Insr, mEH, Cyp2e1, Gstm and Gstp or protein level of INSR, MEH, GSTM and GSTP. Statistical analyses were performed using the unpaired t-test function of GraphPad Prism software. Obese mouse ovaries had increased mEH (2.34-fold; P < 0.1), Gstm (1.46-fold; P < 0.1) and Gstp (1.61-fold; P < 0.05) mRNA, but had decreased (0.29-fold; P < 0.05) Cyp2e1 mRNA, relative to their lean littermates. There was no effect of obesity on MEH and GSTP protein level; however there was a trend (P ≤ 0.1) for decreased INSR and GSPT protein. Taken together these results support the hypothesis that high fat diet-induced obesity results in aberrant expression of ovarian xenobiotic metabolizing genes through impaired insulin signaling (Supported by ES016818).

2377 FREQUENCIES OF PRENATAL LOSS, NONVIVABLE BIRTHS, PREMATURITY/PRETERM BIRTHS, AND POSTNATAL DEATHS IN CYNOMOLGUS MONKEYS.
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Background: Historical background data of prenatal loss, nonviable birth (stillbirth), premature and preterm births, and postnatal neonate mortality are essential for interpretation of reproductive and developmental toxicity (DART) studies. In this presentation, these frequencies in Cynomolgus monkeys (Macaca fascicularis) under experimental conditions (e.g., timed mating, individually housed, administered vehicle/control article) are summarized. Methods: Frequencies of prenatal loss (PL, including abortion, resorption, and in utero embryo-fetal death before gestation day (GD) 140), stillbirth (SB, birth of a nonviable neonate or in utero fetal death on or after GD140), premature birth (PMB, birth of viable neonate before GD140) and preterm birth (PTB, birth of a viable neonate between GDs 140 and 154), normal, uncomplicated birth (NB, birth of viable neonate after GD154), and postnatal neonate death (ND) were reviewed in control groups from 31 DART studies (including 13 embryo-fetal development studies and 18 pre- and postnatal studies). This includes a total of 510 maternal animals, with an age range of 3 to 11 years of GD0. Results: Mean frequencies (mean ± SD) of all studies were 16.7 ± 7.6% in PL, 13.7 ± 8.2% in SB, 3.3 ± 4.3% in PMB, 18.7 ± 11.7% in PTB, and 67.3 ± 15.1% in NB. Mean gestation length was 159 ± 8 days (including SB, PMB, PTB and NB). The normal distribution of 216 live births died (6.0 ± 5.3%), and 10 of the 13 deaths occurred within 7 days after birth. Frequencies of ND were 71% in PMB, 10% in PTB, and 2% in NB. Conclusion: Frequency of ND clearly increased in PMB (71%) when compared with that of PTB (10%) and NB (2%). Since PTB was not uncommon (18.7%) and frequency of ND in PTB neonates (10%) was generally comparable with that of NB (2%), PTB is considered to be with normal biological variation in Cynomolgus monkeys. These data are important to assess effects of test compound on maintenance of pregnancy, parturition, and neonate survival.
In this study, we examined effects of low-dose exposure to estrogenic compounds during the critical period for the hypothalamic sexual differentiation on female puberty in the rat. [Experimental Design]: Female Sprague-Dawley neonates were orally administered 17β-estradiol at a daily dose of 0 (vehicle control), 0.4 or 2 μg/kg from postnatal day (PND) 1 to 5. Some of the 2 μg/kg-treated animals were confirmed to form corpora lutea (CL) 7 days after VO. The necropsy, the numbers of oocytes shed and CL, and weights of ovaries and uterus were determined. [Results & Discussion]: The treatment did not affect growth of the animals, and VO was confirmed in all animals. The first ovulation was observed in 100, 87.5 or 50% of them with 0, 0.4 or 2 μg/kg-treated group, respectively. The 2 μg/kg-treated group, these values and weights of ovaries and uterus in the ovulated animals were smaller than those in the control. At the necropsy 7 days after VO, CL were formed in the ovaries of 87.5% of the 2 μg/kg-treated animals, and the number of CL was approximately 1-2 times larger than that counted at the first ovulation. These results indicate that neonatal exposure to low-dose EE exerts little effects on the timing of vaginal opening but delays the first ovulation with reduction in the number of oocytes shed. Since the treatment at the dose level of 2 or 0.4 μg/kg has been found to cause persistent estrus until postnatal week 8 or 16 at the least, respectively [presented at the 38th Annual Meeting of JST, 2011], the disruptive effects of the neonatal exposure to low-dose EE on female puberty may concern with the delayed effects on estrous cycle.

Humans in industrialized countries have nearly ubiquitous exposure to phthalates, plasticizing agents with noted potential for endocrine disruption. Mono-2-ethylhexyl phthalate (MEHP) is the primary metabolite of the most widely used phthalate, Di-2-ethylhexyl phthalate (DEHP). DEHP has been widely recognized as a male reproductive toxicant; and implicated as a human female reproductive toxicant following prolonged exposure to high doses of phthalates. In this study, pregnant C57BL/6 mice were exposed via oral gavage to corn oil, 100, 500, or 1000 mg/kg MEHP at gestational days 17 through 19. No overt maternal toxicity was observed. No significant delays in vaginal opening were observed and body and ovarian weights were similar between vehicle- and MEHP-exposed adult F1 females. However, F1 females did exhibit a delayed onset of estrus in both the 500 and 1000 mg/kg MEHP exposure groups relative to controls with the average onset of estrus was approximately 39.0 ± 2.0 days of age. A prolonged estrus stage and decreased incidence of the diestrous stage was observed in all exposure groups. Serum FSH and estradiol levels at estrus were significantly higher in the 1000 mg/kg group. In addition, altered mRNA expression levels were observed in adult F1 females for genes involved in stroiogenesis, LHCSR, aromatase, and StAR. Collectively, these findings suggest that late gestational exposure to phthalates leads to altered ovarian function and steroidogenesis in the F1 MEHP-exposed generation.

Signaling by phosphatidylinositol-3-kinase (PI3K), a lipid kinase, regulates primordial follicle growth and ovulation. In pre-ovulatory follicles, PI3K signaling is activated by binding of granulosa cell-produced Kit ligand (KITL) to the oocyte-expressed receptor, c-KIT. Ovotoxicity caused by 4-vinylcyclohexene diepoxide (VCD) and 7,12-dimethylbenzo[a]anthracene (DMBA) is mediated through decreased PI3K signaling and PI3K inhibition prevents VCD-induced but accelerates DMBA-induced primordial follicle loss in cultured neonatal rat ovaries. Microsomal epoxide hydrolase (mEH) is known to detoxify VCD but bioactivate DMBA, thus a role for altered xenobiotic metabolism during PI3K inhibition is hypothesized. This study investigated the involvement of PI3K signaling in regulation of ovarian xenobiotic metabolizing gene expression by phosphatidylinositol-3-kinase signaling.
of the ovarian xenobiotic metabolism genes mEH and Glutathione S-transferase (GST) isoforms, pi (p) and mu (m). Also, a role for P450 signaling in activation of transcription factors NF-E2-related factor 2 (Nrf2) and Aryl hydrocarbon receptor (Ahr), known to regulate xenobiotic metabolism gene expression, was determined. c-kit (upstream of P13K) mRNA level during P13K inhibition was also evaluated. Post-natal day 4 (PND4) rat ovaries were cultured in medium containing vehicle control (1% DMSO; CT) or 1x294002 (P13K inhibitor, 20 μM) for 2, 4 or 6 d. mRNA and protein levels were measured by RT-PCR and Western blotting, respectively. Inhibition of P13K signaling reduced (P < 0.05) Gap43, meh and Ahr but increased (P < 0.05) Gigm mRNA after 2d. After 4d, P13K inhibition increased (P < 0.05) Gap43, Gigm, meh and Ahr mRNA levels. Neither Nyf2 nor c-kit mRNA was impacted by P13K inhibition. Increased (P < 0.05) GSTP, GSTM1, mEH, AHR and Nrf2 protein levels due to P13K inhibition were observed at d4 and d6. These data indicate that P13K signaling regulates ovarian xenobiotic metabolism genes. Also, these findings support that the differential ovo-toxic impact of P13K inhibition during VCD and DMBA exposure may due to altered metabolism (Supported by ES016818).

Di-2-ethylhexyl phthalate (DEHP) is an environmental contaminant used as a plasticizer in polyvinyl chloride products. Mono-ethylhexyl phthalate (MEHP), the active metabolite of DEHP, increases reactive oxygen species production and decreases antioxidants in liver, kidney and testicular cells. To investigate whether placenta is a potential target of MEHP, we evaluated MEHP effects on oxidative DNA adduct formation, apoptosis and expression of redox-sensitive genes in placental cells using the human extravillous trophoblast cell line HTR-8/ SVneo. To measure hydroxy thymine adduct formation, cells were treated with DMSO (solvent control), 100 μM MEHP, 200 μM MEHP, or 50 μM tert-butylylhydroperoxide (TBHP; positive control) for 24 h. DNA was extracted and concentration of hydroxy thymine were quantified using mass-spectrometry. Treatment with 200 μM MEHP increased hydroxy thymine adduct formation two-fold compared with solvent controls. To assess apoptosis, caspase 3/7 activity was measured using a luminescence-based assay following 24-h exposure to DMSO (solvent control), 100 μM MEHP, 200 μM MEHP, or 50 μM camptothecin (positive control). A 1.5-fold increase in caspase 3/7 activity (p<0.05) was observed with 200 μM MEHP treatment. RNA expression of 84 redox-sensitive genes was measured in cells exposed to MEHP compared to solvent control. GLRX2, PTGS2, PRBN1, TXNRD1, SCARARA3, MSCR1, DHC2R4, and AOX1. This study suggests that MEHP induces oxidative stress in human placental cells.

**2383 MONO-2-ETHYLHEXYL PHTHALATE-INDUCED OXIDATIVE STRESS IN HUMAN PLACENTAL CELLS.**

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**2384 OXIDATIVE INSULT ACTIVATES PARTURITION-ASSOCIATED PATHWAYS IN A HUMAN PLACENTAL CELL LINE.**

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Preterm birth is a significant public health problem with over one-third of infant deaths directly attributed to premature delivery. Although mechanisms of preterm birth are not fully understood, it is well-established that prostaglandins play a key role in parturition. Moreover, cells within gestational tissues, including the placenta and extra-placental membranes, undergo programmed cell death, or apoptosis, during labor. Although parturition is associated with markers of oxidative stress within gestational tissues, the impact of oxidative stress on labor-promoting pathways has received little attention. The present study examined the effect of oxidative insult on prostaglandin (PG) and apoptotic pathway activation in placental cells using the human extravillous trophoblast cell line HTR-8/SVneo and tert-butylylhydroperoxide (TBHP) as a model pro-oxidant. After 4, 8 or 24 h exposure to 12.5, 25 or 50 μM TBHP, caspase-3/7 activity, PGE2 release, and prostaglandin-endoperoxide synthase 2 (PTGS2) mRNA expression were assayed. At 4 and 24 h, 50 μM TBHP induced significant 6.4- and 7.0 respective fold increases in PGE2 release into the culture medium (p<0.05). This was accompanied by significant 9.6, 6.7, and 2.9-fold increases in PTGS2 mRNA expression at 4, 8 and 24 h (p<0.05). Treatment with 50 μM TBHP also significantly increased caspase-3/7 activity by 5.3- and 5.9-fold at 8 and 24 h, respectively (p<0.05). Furthermore, the increased caspase activity was inhibited by pretreatment with the antioxidant iron-chelating agent deferoxamine (1 mM), suggesting that oxidative insult induced apoptosis. In summary, these results show that oxidative insult stimulates responses in human placental cells linked to parturition. Because many environmental chemicals induce cellular oxidative stress, this suggests a plausible mechanism that may underlie associations between some environmental pollutant exposures and increased risk for preterm birth.

**2385 A NOVEL QUANTITATIVE METHOD FOR THE COMPARISON OF MAMMARY GLAND DEVELOPMENT IN MULTIPLE STRAINS OF RAT.**

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The rodent mammary gland (MG) has been utilized as an endpoint for assessing the developmental toxicity of endocrine disrupting chemicals in few studies. These studies employ either basic dimensional measurements or developmental scoring of morphological characteristics, such as branching density, as a means to quantify MG development. The Sholl analysis is a quantitative method used to measure morphological characteristics in neuronal dendritic patterns. Briefly, development is assessed by overlaying the image with concentric rings and measuring the number of branching intersections. The present study describes the use of the Sholl method to objectively quantify morphological MG branching density as a measure of development. We employed this method to compare natural MG development in Long Evans (LE), Charles River Sprague Dawley (CRSD), and Harlan Sprague Dawley (HSD) rats, three strains commonly utilized in developmental toxicity studies. Mammary glands were collected from female offspring on postnatal day (PND) 25, 33, and 45 for whole mount preparation. Image analysis software (1.44p, National Institute of Health, USA) was used to create binary images of the glands and MetaMorph® Image Analysis Software (6.2r4, Molecular Devices, Sunnydale, CA) was used to conduct the analyses. Mammary gland development was more advanced in female HSD rats on PND25 as exhibited by significantly more intersections (475.3 ± 18.9, p<0.001) than either LE (311.6 ± 35.8) or CRSD (315.2 ± 20.2) rats while no significant differences were observed in body weight between female offspring of the three strains. This advanced development on PND25 in HSD glands compared to LE and CRSD glands was also reflected in the subjective scoring of morphological characteristics suggesting that both methods are adequate and consistent for assessing MG development. Additional puberal data was collected for comparison with mammary gland development. This abstract does not necessarily reflect NIHES policy.

**2386 BROMINATED DIPHENYL ETHER-47 ENHANCED MRNA EXPRESSION OF PRO-INFLAMMATORY CYTOKINES AND PROSTAGLANDIN-ENDOPEROXIDE SYNTHASE 2 IN HUMAN PLACENTAL CELLS.**

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Preterm birth is associated with infant death and adverse outcomes that can persist into adulthood. Although the etiology of preterm birth is not fully determined, it is well-established that prostaglandin production is a key player in the parturition process. Production of prostaglandins is stimulated by inflammatory cytokines and the increased expression of prostaglandin-endoperoxide synthase-2 (PTGS-2). Polybrominated diphenyl ethers (PBDEs) are widely used as flame retardant compounds. Brominated diphenyl ether-47 (BDE-47) is a PBDE congener that bioaccumulates in human breast milk, serum and placenta. Despite the presence of PBDEs in human placenta and the importance of cytokine regulation in parturition, the effects of PBDEs on pregnancy are poorly understood. The present study examined the effect of BDE-47 on mRNA expression of inflammatory cytokines and PTGS-2 using a human extravillous trophoblast cell line, HTR-8/SVneo, which was derived from first trimester placenta. HTR-8/SVneo cells were treated with 5, 10, 20 or 50 μM BDE-47 for 24 h. mRNA expression of interleukin (IL)-6, IL-8, transforming growth factor (TGF)-β1 and PTGS-2 was quantified using quantitative real-time RT-PCR. Treatment with 50 μM BDE-47 increased mRNA expression of the pro-inflammatory cytokines IL-6 and IL-8, as well as the prostaglandin synthase enzyme PTGS-2 in HTR-8/SVneo cells. There were no significant changes in mRNA expression of TGF-β1. These data indicate that exposure to BDE-47 enhanced expression of proteins important for signaling onset of parturition. Further research is needed to ascertain potential relevance of these findings to pregnancy.
2387 MONO-(2-EThYLeXyL) PhThALATE INDuces OxidATive StRESS IN MoUSE ANTRAL FOLLICLES.

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Phthalates are synthetic plasticizers that are widely used in plastics and many other common consumer products. Exposure to these chemicals can cause developmental and reproductive toxicity. Our previous studies show that di-(2-ethylhexyl) phthalate (DEHP) inhibits follicle growth by inducing oxidative stress. In this study, we tested the hypothesis that mono-(2-ethylhexyl) phthalate (MEHP), the active metabolite of DEHP, also inhibits the growth of antral follicles through an oxidative stress pathway. Antral follicles were isolated from ovaries of adult CD-1 mice (age 30-35 days) and cultured with vehicle control (dimethylsulfoxide, DMSO) or MEHP (0.1-100ug/ml) at Na-acetyl cysteine (NAC, an antioxidant) (0.5mM) for 96h. During culture, follicle size was evaluated daily as a measurement of follicle growth. At the end of the culture, follicles were collected and processed for in vitro reactive oxygen species (ROS) assays, which measure the presence of free radicals in the samples, as an indicator of oxidative stress. The results indicate that MEHP (1-100ug/ml) suppresses the growth of follicles compared to DMSO controls and that NAC (0.5mM) blocks the ability of MEHP to inhibit follicle growth. Furthermore, MEHP (1 µg/ml) significantly increases ROS levels compared to DMSO controls, whereas NAC (0.5mM) co-treatment protects the follicles from MEHP induced high ROS levels. Collectively, these data suggest that MEHP suppresses the growth of antral follicles via an oxidative stress mechanism. Supported by: NIHES019178 (JAF), Billie A Field fellowships (WW and ZRC), and the Environmental Toxicology Scholar Program (MSB).

2389 ASSESSING REPRODUCTIVE AND DEVELOPMENTAL ALTERATIONS ASSOCIATED WITH BENZOA(PYRENE EXPOSURE IN FUNDULUS HETEROCLITUS.

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Benz(a)pyrene (BaP) is a ubiquitous pollutant that is considered both a model polycyclic aromatic hydrocarbon (PAH) and confirmed carcinogen. Our previous work has shown that BaP significantly reduced aromatase (CYP19) expression. We hypothesize that this additional mechanism carries the potential to deregulate normal functioning of the hypothalamus-pituitary-gonad feedback loop and ultimately affect reproductive success. Our goal is to utilize a marine fish model Fundulus heteroclitus to further develop BaP-mediated toxicological endpoints including phenotypic and molecular consequences (steroid concentrations, vitellogenin, and sex steroid (testosterone, 17β-estradiol)) and therefore our study has immediate environmental relevance. A short-term reproductive bioassay was done using Fundulus adults exposed to waterborne concentrations of BaP (0, 1 or 10 µg/L) for 28 days. Males and females were kept separate days 0-14 and were combined for days 14-28. Offspring were collected to measure steroid concentrations at 5 and 10 days post-fertilization (dpf) and 1 and 3 weeks post-hatch (wph) and aromatase expression at 10 dpf and 12 wph. Ovett reproducive indicators including gonad somatic index, liver somatic index, sperm counts, egg production or fertilization success (n = 5-6 tanks per treatment; 3 fish of each sex per tank) were not significantly altered by BaP exposure. Oocyte stage distribution was not significantly different between treatments (n=10-12/fish/treatment). Vitellogenin was significantly increased in 10 µg/L BaP-treated females compared to control and 1 µg/L BaP-treated females after 4 weeks exposure. In conclusion, BaP-mediated changes in aromatase expression have the potential to cause changes in the HPG axis, but reproductive success was not significantly compromised in the parental generation by up to 10 µg/L BaP exposure. (Supported by NIEHS R03 ES018962).
were also observed at 0.5 mg/kg and more in both sexes. As for reproductive and developmental toxicology, no one female at 2.5 mg/kg had a normal delivery. Abnormal estrus cycle was observed in all females of satellite (non-mating) group at 2.5 mg/kg. Decreased delivery index and live birth index were observed at 2.5 mg/kg. No reproductive and developmental parameters were changed at 0.1 and 0.5 mg/kg. Based on the above results, NOAELs for the repeated dose toxicity and reproductive/developmental toxicity are considered to be 0.1 mg/kg/day and 0.5 mg/kg/day, respectively.

2392 CHROMIUM-VI EXPOSURE TO POSTNATAL RATS THROUGH MOTHER’S MILK DEPLETES ANTIOXIDANTS, INCREASES FREE RADICALS AND INDUCES FOLLICULAR ATRESIA.

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Hexavalent chromium, CrVI, has been used in a wide-range of industries. Chromium levels in the drinking water are reported to be increased in California, New Jersey and several other places in the USA, and also in developing countries. Women working in the Cr industries experience abnormal menses and have high blood levels of Cr. But the actual mechanism behind Cr-induced ovotoxicity is still unknown. Our recent study has reported that lactational exposure to CrVI delayed folliculogenesis and altered ovarian steroidogenesis. CrVI is highly toxic and rapidly reduced to CrIII once enter into cells resulting in increased free radicals. The objective of our study is to test the hypothesis that lactational exposure to chromium impairs follicle development and induces follicular atresia in the F1 offspring through oxidative stress pathway. Study 1: Lactating rats (n=75) were treated with different doses of CrVI (potassium dichromate 50 – 200 ppm) through drinking water from postpartum days 1-21. During the postnatal days (PND) 1-21 pups received CrVI through mother’s milk. After PND21 female offspring (F1) were given regular drinking water and diet, and were killed on postnatal days (PND) 25, 45 and 65. CrVI decreased serum hormones and increased follicle. Study 2: Antioxidants such as GPx, GR, GST, SOD and CAT were significantly decreased. Lipid peroxidation and H2O2 levels increased significantly both in the serum and in the ovaries. Study 3: Granulosa cells were cultured and treated with CrVI. CrVI decreased many of these enzymes and the effect of CrVI was mitigated by vitamin C. Supported by grants from the NIH/NIEHS to S.K.B (ES016605-01A1 and 1R21ES020561-01).

2393 EFFECTS OF CHROMIUM-VI ON THECA CELL CYCLE REGULATION AND APOPTOSIS.

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Hexavalent chromium, GVI, is an environmental heavy metal toxicant that contaminates soil, air and drinking water. CrVI is released into the environment from mining, manufacturing, and A. M. Api2. Chromium impairs follicle development and induces follicular atresia in the F1 offspring through oxidative stress pathway. Study 1: Lactating rats (n=75) were treated with different doses of CrVI (potassium dichromate 50 – 200 ppm) through drinking water from postpartum days 1-21. During the postnatal days (PND) 1-21 pups received CrVI through mother’s milk. After PND21 female offspring (F1) were given regular drinking water and diet, and were killed on postnatal days (PND) 25, 45 and 65. CrVI decreased serum hormones and increased follicle. Study 2: Antioxidants such as GPx, GR, GST, SOD and CAT were significantly decreased. Lipid peroxidation and H2O2 levels increased significantly both in the serum and in the ovaries. Study 3: Granulosa cells were cultured and treated with CrVI. CrVI decreased many of these enzymes and the effect of CrVI was mitigated by vitamin C. Supported by grants from the NIH/NIEHS to S.K.B (ES016605-01A1 and 1R21ES020561-01).

2394 EVALUATION OF THE REPRODUCTIVE TOXICITY OF ALLYL CYCLOHEXANEPROPONATE.


An oral (gavage) dosage-range reproductive toxicity study was conducted with the fragrance allyl cyclohexanepropionate in rats. Rats (8/sex/group) were dosed daily with 0, 75, 125, 250, or 500 mg/kg/day in corn oil beginning 14 days before cohabitation, through cohabitation, and to the day before euthanasia (males) or day 4 postpartum (females). Parameters included viability, clinical observations, body weights, feed weights, mating and fertility, delivery, litter observations, organ weights, and histopathology. Eight rats (4/sex) from the 500 mg/kg group were found dead on day 2 or 3 of the study; the remaining rats from the 500 and 250 mg/kg dosage groups were euthanized early on days 5 and 17 of study, respectively, due to excessive toxicity and mortality. All rats from the 0, 75 and 125 mg/kg dosage groups survived to scheduled euthanasia. Clinical signs from survivors included slight and/or moderate excess salivation and a reduction in body weight gains. The toxicity of allyl cyclohexanepropionate was most evident in the liver, which resulted in periporal vacuolation that lead to necrosis, and the development of cholangiofibrosis in all rats. Mating and fertility parameters were unaffected by dosages as high as 125 mg/kg. Pregnancy occurred in 7, 6, and 8 rats from the 0, 75, and 125 mg/kg dosage groups. Only two female rats were confirmed as mated in the 250 mg/kg dosage group but there were no successful pregnancies since this group was terminated during the cohabitation period due to clinical signs of toxicity. There were no effects on natural delivery. The average pup weight was reduced by 12% (125 mg/kg) on postpartum day 1. This reduction was considered biologically relevant when compared to the controls, but resolved by postpartum day 5. No treatment-related clinical observations occurred and all pups appeared normal at necropsy examination. The no-observable-adverse-effect-level (NOAEL) for reproductive toxicity is 75 mg/kg/day.

2395 IRREGULAR MENSTRUAL CYCLING: A LOGISTICAL HURDLE TO SUCCESSFUL BREEDING OF CYNOMOLGUS MONKEYS.

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Establishment of an optimal mating time is an important prerequisite for successful mating of female macaques for use in developmental toxicity testing. In general, an optimal mating time is calculated on the basis of individual menstrual cycle recorded for at least 2-3 consecutive months. Assessment of vaginal smears is minimally invasive and provides a convenient and reliable approach for monitoring the menstrual cycle. We have monitored the menstrual cycle of 118 naïve, individually housed monkeys of Chinese origin for over 8 months. The females were sexually mature; 4.5 ± 0.4 years old and weighed 2.98 ± 0.31 kg at arrivals. Females were screened for the presence of ovarian cysts and endometriosis using ultrasound prior to the beginning of menstrual cycle observation. The animals were maintained in a controlled lighting schedule; with lights on from 0600–1800 h and a room temperature of 24 ± 2 °C. After one week of acclimation period, females had their vaginal area swabbed daily with a cotton-tipped applicator to detect menses. A total of 965 cycles were evaluated. Mean number of cycles per animal evaluated was 8.2 ± 2.7 cycles. The mean total cycle length for all females was 34.5 ± 15.2 Days with mean cycle length per animal of 36.5 ± 10.7 Days. Overall 89% of the animals had mean cycle length between 21–44 Days long. Only 3% had mean cycle length < 21–44 Days. Several females have interrupted cycles with 1–3 missing cycles. Based on the result of this study, irregular menstrual cycle is a logistic hurdle for achieving the desired number of pregnant monkeys necessary for the study in a timely manner.

2396 INHIBITORY MECHANISM OF CYP1A1 EXPRESSION BY CAFFEIC ACID PHENETHYL ESTER-MEDIATED HYPOXIA INDUCIBLE FACTOR-1α AND ARYL HYDROCARBON RECEPTOR IN MURINE HEPATOMA HEPA-1C1C7 CELLS.

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Caffeic acid phenethyl ester (CAPE), an active compound of the propolis, is known for antioxidant, antiviral, antiinflammatory, antiinflammatory and anticarcinogenic properties. The influence of CAPE on cytochrome P450 1A1 (CYP1A1) expression and their mechanisms remain unclear. In this study, we examined the effect of CAPE on 3-methylcholanthrene (3-MC)-induced CYP1A1 in mouse hepatoma Hepa-1c1c7 cells. CAPE induced HIF1α-inducible factor-1α (HIF-1α) protein level and HIF-1α responsible element (HRE) luciferase activity in Hepa-1c1c7 cells. Additionally, CAPE-mediated HIF-1α decreased 3-MC-inducible CYP1A1 protein and mRNA expression. Moreover, 3-MC-inducible xenobiotic-response element (XRE)-linked luciferase and aryl hydrocarbons receptor (AhR) nuclear translocation were decreased by CAPE treatment. These results provide evidence that CAPE suppresses 3-MC-induced CYP1A1 levels and HIF-1α activation by CAPE contributes to suppression of 3-MC-inducible AhR-mediated CYP1A1 expression.
2397 INHIBITION OF CYTOCHROME P450 1A OR 3A, BUT NOT 2B/2C, ENHANCES USNIC ACID CYTOTOXICITY IN PRIMARY CULTURED RAT HEPATOCYTES.


Usnic acid (UA) is consumed as a dietary supplement to promote weight loss; however, dietary supplements containing UA have been associated with clinical cases of severe and sometimes fatal liver injury. UA has been shown to be hepatotoxic in rats and is extensively metabolized by hepatic cytochrome P450s (CYPs); therefore, we examined if UA metabolism results in the formation of cytotoxic metabolites or if metabolism is a detoxification process. Primary rat hepatocytes were exposed to UA in the presence of various CYP inhibitors. When CYP activity was suppressed by the non-isozyme-selective inhibitor SKF-525A (20 μM), the CYP1A inhibitor alpha-naphthoflavone (10 μM), or the CYP3A inhibitor ketoconazole (25 μM), the cytotoxicity of UA at 4-6 μM after 20 h of exposure was significantly increased as measured by lactate dehydrogenase leakage. At 2 h after UA exposure, an earlier time point prior to cell cytotoxicity, these CYP inhibitors potentiated UA-induced inhibition and uncoupling of cellular respiration as determined by the Clark type oxygen electrode. Cellular adenosine triphosphate (ATP) depletion by UA was also enhanced by these CYP inhibitors. The CYP2B/2C inhibitor, ticlopidine at 20 μM, showed effects similar to those of SKF-525A and ketoconazole, suggesting that UA is bio-transformed to less toxic metabolites in rat primary hepatocytes, mainly by CYP1A and CYP3A.

2398 EFFECTS OF USNIC ACID EXPOSURE ON HUMAN LIVER HEPG2 CELLS.


Usnic acid, a natural botanical product, is a constituent of some dietary supplements used for weight loss. It has been associated with clinical hepatotoxicity leading to liver failure in humans. The present study was undertaken for metabolism and toxicity evaluation of usnic acid in human hepatoblastoma HepG2 cells in culture. The cells were treated with the vehicle control and usnic acid at concentrations of 0-100 μM for 24 h at 370 C in 5% CO2. Following the treatment period, the cells were evaluated by biochemical and toxicogenomic endpoints of toxicity that included cytochrome P450 activity, cytotoxicity, oxidative stress and mitochondrial dysfunction and changes in pathway focused gene expression profiles. Usnic acid exposure resulted in increased P450 activity, cytotoxicity, oxidative stress and mitochondrial dysfunction in HepG2 cells. The pathway-focused gene expression analysis resulted in significant altered expression of 6 genes out of a total of 84 genes examined. Of the 6 altered genes, 3 genes were up-regulated and 3 genes down-regulated. A marked up-regulation of one gene, CCL21, associated with inflammation, one gene, CCNC, associated with proliferation and carcinogenesis, and one gene, UGT1A4, associated with metabolism as well as DNA damage and repair were observed in the usnic acid-treated cells compared to the vehicle control. Also, a marked down-regulation of one gene (CSF2) associated with inflammation and two genes (CYP7A1 and CYP2E1) associated with oxidative metabolic stress were observed in the usnic acid-treated cells compared to the control. The biomarkers used in this study demonstrate toxicity of usnic acid in human hepatoblastoma HepG2 cells suggesting oxidative and inflammatory mechanisms of action.

2399 CHEMICAL-DISEASE CATEGORY LINKAGE (CDCL): COMPUTATIONAL METHODS LINKING TRADITIONAL CHINESE MEDICINE AND WESTERN THERAPEUTICS.

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In Traditional Chinese Medicine (TCM), herbs are categorized in therapeutic classes and TCM practitioners will use a mixture of herbs from different classes to exert therapeutic effect and modulate different bodily functions. Many patients use TCM as a complementary regimen to western therapeutics but many interactions between TCM phytochemicals and western drugs are not fully understood. The aim of this study was to utilize computational methods such as TCM-ID, KEGG Pathway database and GeneGo’s MetaDrug, to establish possible interactions between TCM phytochemicals and western drugs. Several patterns of correlation between TCM classes and disease modulation have been observed. For instance, phytochemicals in the class of heat clearance interact with genes and proteins responsible for cell proliferation such as the apoptotic pathway, while those in the class of tonifying weakness interact with those responsible for immune response and energy metabolism. Such results have shown possible connections between TCM therapeutic classes, western therapeutics, and a disease under treatment; a relationship termed: chemical-disease category linkage (CDCL). CDCL may help predict possible interactions and targets of modulation between the two treatment approaches and it will also be useful for identifying possible TCM herbs that would be beneficial to the treatment of other diseases in the future.

2400 INOPHYLLIN A INDUCES OXIDATIVE STRESS MEDIATED-APOPTOSIS IN JURKAT T LYMPHOBLASTIC CELLS.

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Inophyllin A (INO-A), a pyranoxanthone isolated from Calophyllum inophyllum represent a new class of xanthone as potential chemotherapeutic agent. In this study, the molecular mechanism of INO-A-induced cytotoxicity was elucidated in Jurkat T lymphoblastic cells. The mode of cell death as assessed based on the externalization of phosphatidylserine demonstrated that INO-A induced Jurkat cells apoptosis in a concentration-dependent manner. Upon 30 min of INO-A treatment, there was a significant increase of tail moment which suggests that DNA damage was an initial apoptotic signal. Further flow cytometric assessment of the superoxide anion level confirmed that INO-A-induced DNA damage was mediated with a concomitant upregulation of reactive oxygen species (ROS). Interestingly, the level of ROS in INO-A treatment (7 to 9 fold increase) was significantly higher than goniothalamin and menadione-treated cells which were used as positive control (2 fold increase). Investigation on the thiols revealed an early decrease of free thiols in 30 min 50 μM INO-A treatment. Using Tetramethylrhodamine ethyl ester (TMRE), a potentiometric dye, the loss of mitochondrial membrane potential (MMP) was observed in INO-A-treated Jurkat cells as early as 30 min. The INO-A-induced apoptosis progressed with the simultaneous activation of caspase-2 and -9 which subsequently leads to the processing of caspase-3. Taken together, these data demonstrate that INO-A induces early ROS and DNA damage which subsequently led to the activation of an intrinsic pathway of apoptosis in Jurkat cells.

2401 STIMULATIONS OF BREAST CANCER CELL GROWTH AND CYTOCHROME P4501B1 GENE EXPRESSION BY THE SOY ISOFLAVONE GENISTEIN.

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The soy isoflavone genisteen has been found to possess both weak estrogenic and anti-estrogenic effects. There have been some controversial findings over the years about its effect on breast cancer development. The cytochrome p4501B1 (CYP1B1) is believed to play an important role in breast cancer development by bio-activating endogenous estrogens and environmental carcinogens. In this study, we examined the effects of genistein on breast cancer cell proliferation and CYP1B1 gene expression in human breast cancer MCF-7 cells. MTt activity in the cells was measured for consecutive seven days by using Infinite M1000 multimode microplate reader (Tecan). We found that cell proliferation was stimulated by 5 μM of genistein starting from the second day of culture. Real-time RT-PCR analysis showed a dose-dependent induction of CYP1B1 gene expression in the cells treated with 1, 5, and 25 μM of genisteen. However, genisteen was less potent in the induction of CYP1B1 gene expression than the environmental carcinogen 7,12-di-methylbenz[a]anthracene (DMBA). Genisteen at 5 μM also significantly increased the CYP1B1 mRNA level that was induced by DMBA. This study reveals that the soy isoflavone genisteen could stimulate breast cancer cell growth by increasing the levels of CYP1B1 in the cells that can bio-activate endogenous estrogens and environmental carcinogens.
Osteoporosis is systemic bone disease characterized by a reduction in bone mass, disruption in bone microarchitecture, and a consequent increase in bone fragility. Ovariectomized animal model has been widely used to study preventive treatments for postmenopausal osteoporosis with estrogen insufficiency. This study investigated bone turnover in ovariectomy (OVX) mice by repeated administration of Plantocyanidin grandiflorum plant-derived saponins (CNS) and C. grandiflorum (CG). The effect of CNS on OVX-induced mice was measured by organ weights, serum levels and micro-computed tomography (μCT) for bone remodeling. Administration of CNS suppressed the OVX-induced bone loss in OVX mice. CNS reduced OVX-induced body and organ weights. CNS recovered OVX-reduced serum levels of alkaline phosphatase, calcium and phosphorus inorganic. Bone morphometric parameters of femur revealed that CNS inhibited OVX-induced trabecular architecture using μCT. These results suggest that CNS exerts beneficial effects in postmenopausal osteoporosis, suggesting that CNS might be a candidate for phytoestrogen-like actions in postmenopausal osteoporosis.

2 Pipericine down-regulates cyclooxygenase-2 expression by inhibiting the activities of NF-κB, C/EBP and AP-1 transcription factors.

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Piperine, a major component of black pepper, is used as spice and nutrient enhancer. In the present study, we investigated the effects of piperine on phorbol 12-myristate 13-acetate (PMA)-induced pro-inflammatory protein cyclooxygenase (COX-2) expression in macrophages. Treatment with piperine suppressed PMA-mediated induction of COX-2 mRNA and protein. PMA-inducible production of PGE2 was inhibited by piperine in a dose-dependent manner. Transient transfection assays indicated that the inhibitory effects of piperine were mediated via NF-κB, CCAAT/enhancer-binding protein (C/EBP) and AP-1. Furthermore, piperine significantly inhibited PMA-activated induction of the ERK MAP kinase. Thus, piperine is an effective agent to attenuate COX-2 production mediated by the transcription factors NF-κB, C/EBP and AP-1, and these results enhance our understanding of the anti-inflammatory and anti-cancer properties of piperine.

2 Anacardic acid, 6-pentadecylsalicylic acid, is more cytotoxic and genotoxic in transformed human cell lines than in human peripheral blood mononuclear cells.

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Infections by Helicobacter pylori are proposed as the key event in gastric atrophy, adenocarcinoma, and gastric cancer. On the other hand, it has recently been described that some compounds (anacardic acids, such as 6-pentadecylsalicylic acid, or AA) extracted from Ampelopsis adenodendron (Esho et al., an endemic plant from Mexico), present bactericidal effects against H. pylori, a gastroprotector, and an anti-tumoral effects. Due to this, we expected that exposure to AA produces increased cytotoxicity and genotoxicity, as mechanisms of action, on a human gastric cancer cell line (AGS) than in other type of tumor cell lines, such as K562, or in peripheral blood mononuclear cells (PBMCs) from healthy donors. To this end, we exposed AGS, K562 and PBMCs cultures to AA, from 0.15 to 150 μM, and for 24- to 120 h. Determination of annexin V and propidium iodide incorporation indicated that AA exposure did not affect viability in PBMCs, but induced apoptosis in AGS and K562 cell lines. Evaluation of the metabolism of 3-(4,5-dimethylthiazol-2-yl)-2,5-dibromide diphenyl tetrazoisol (MTT), as a measurement of mitochondrial activity, indicated that PBMCs exposed to AA increased their respiratory rate, whereas the increase of this activity, consistent with cell death, was observed in AGS and K562 cell lines. Instead of cell death, AA exposure in PBMCs induced a cytostatic effect statistically significant from 30 μM after 48 h of treatment in CFSE loaded cells. As a parameter of genotoxicity we evaluated the frequency of micronucleus (MN) in the three cell types. AA exposure induced a small but significant increase in MN frequency only with the higher concentration used (150 μM) in all cell types, indicating a low genotoxic potential.

2 Cytotoxic compounds from leaves and young twigs of Caesalpinia bonducella (L.) Roxb.

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Caesalpinia bonducella (CB) has been used in folklore for the treatment of a wide range of diseases. This work aims to justify the use of CB as an anticancer plant and determine its bio-active constituents. Fractionation of CB led to the isolation of two new cassane diterpenes: 17α,24-diacetoxy-15(14)-epoxy-16,12-olide (1) and 12e-ethoxyl-1,17β-diacetoxy-21,5β-dihydroseryl-tacc-13(15)-en-16,12-olide (2) and seven flavonoids: Bonducellin (3), 2'-methoxy-isoliquiritigenin (4), 7,3'-diacetoxy-3,11-dehydrohydrosolavolannine (5), Luteolin (6), queretin-3-methyl ether (7), Kaempferol-3-O-B-D-xylonlyranoside (8) and Kaempferol-3-O-A-L-rhamonopyranosyl(1→2)-B-D-xylonlyranoside (9). Their structures were elucidated by MS and NMR spectroscopic methods. Cytotoxic activity of these compounds against BGC-823 gastric carcinoma and HeLa cervical carcinoma were investigated using sulforhodamine B assay. Compounds 3-7 showed cytotoxic activity against HeLa cell with IC50 values in range of 8.69 to 0.81 μg/mL while compound 3 showed cytotoxic activity against BGC-823 cell with IC50 value of 6.45 μg/mL in comparison with Taxol, with IC50 value of 1.02 and 0.78 μg/mL against HeLa and BGC-823 cells respectively. The presence of hydroxyl group on 3 position of the B ring may have been responsible for cytotoxic activity. The results also validate the traditional use of C. bonducella as an antioxidant herb and established flavonoids as a major phytochemical responsible for cytotoxic activity.

2 Composition of multiple lots of Valerian (Valeriana officinalis) extracts, root powders, and oils.

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Valerian has been selected for testing on the National Toxicology Program due to widespread use as a dietary supplement and the lack of adequate toxicological data. Twelve lots of valerian (eight extracts, two root powders and two oils) were procured from different vendors and analyzed for valeric acids (the typically standardized component) and sugars by HPLC, total carbohydrates by the phenol-sulfuric acid assay, starches by enzymatic assay, organic extractables by GC with mass-selective detection, inorganics by PIXE and water by Karl Fischer. The root powders, seven of the extracts, and one oil contained ≤ 0.1% valericanic acids, well below their stated content. One extract contained 0.5% valericanic acids, and one oil contained 7.6%. The total carbohydrate analysis found the extracts to contain ≤ 74% carbohydrates, the root powders ≥ 94% and the oils ≤ 3%. Of the carbohydrates in the extracts and root powders, 12% to 40% were characterized as starch and 1% to 16% as sugars. The extracts and root powders contained 5% to 7% water and 1% to 6% inorganics (predominately potassium, sodium, chloride, sulfur, calcium, and magnesium). The valerian extracts and root powders contained ≤ 1% hexane extractables, but the oils were entirely hexane extractable (primarily terpenes, terpenoids and organic acids). Although the oils contained many of the same compounds, they differed significantly in amount. One extract contained 0.5% valerenic acids, and one oil contained 0.1% valerenic acids, well below their stated content. One extract contained 0.5% valericanic acids, and one oil contained 7.6%. The total carbohydrate analysis found the extracts to contain ≤ 74% carbohydrates, the root powders ≥ 94% and the oils ≤ 3%. Of the carbohydrates in the extracts and root powders, 12% to 40% were characterized as starch and 1% to 16% as sugars. The extracts and root powders contained 5% to 7% water and 1% to 6% inorganics (predominately potassium, sodium, chloride, sulfur, calcium, and magnesium). The valerian extracts and root powders contained ≤ 1% hexane extractables, but the oils were entirely hexane extractable (primarily terpenes, terpenoids and organic acids). Although the oils contained many of the same compounds, they differed significantly in amount. Compounds present at ≥ 2% in one oil lot were t-citrene, δ-pinene, camphene, bornyl acetate, myrtanyl acetate and longipinocarvone. Those present at ≥ 2% in the other lot were isovaleric acid, bornyl acetate, myrtanyl acetate, 5(1H)-azulenone,2,4,6,7,8a-hexahydro-3,8-dimethyl-1-methylene(1)-pentyliden), palmitic acid, 6-(1-dodecyloxymethyl)4,8-di-methyl-3,5,6,7,8,8a-hexahydro-1-h-naphthenal-2-one, ethyl linoleate, linoleic acid and linolenic acid. This work was supported by NTP Contract N01-ES-55551.
A range of medicinal plants with anti-diarrhea properties are widely used by traditional healers in developing countries in the management of diarrhea, however, the effectiveness and safety of many of these plants with anti-diarrhea activity is not yet been comprehensively evaluated. In Northern Nigeria, Cochlospermum tomentosum is traditionally used in the treatment of diarrhea, in this study therefore the effectiveness of the aqueous root extract of Cochlospermum tomentosum in the treatment of diarrhea was investigated. The aqueous extraction of the root of Cochlospermum tomentosum yielded 10% W/W and the Phytochemical studies of the extract revealed the presence of tannins, alkaloids, flavonoids, anthraquinones, saponins, cardiac glycosides and carbohydrates. The medicinal lethal dose (LD50) of the extract was found to be more than 3000 mg/kg to suggest the relative safety of the extract. The anti-diarrheal activity of the aqueous root extract of Cochlospermum tomentosum (Cochlospermum Niloticum Oliv) was evaluated in winter strain albino rats using the following models: castor oil induced diarrhea, gastrointestinal transit of activated charcoal and castor oil induced enteropooling in rats. The extract significantly (p<0.05) reduced the number of unformed faces, and also the intestinal transection of enteropooling in rats. The extract significantly inhibited PCE changes were associated with high dose A4+ indicating a lack of genotoxicity, under the conditions of these studies and a no adverse effect level (NOAEL) of 2000 mg/kg was assigned. From the suspended cells, smears were made and scored for polychromatophilic erythrocytes (PCE)/200 erythrocytes and micronucleate PCE/2000 PCE. For the 28-day study, no significant A4+-related effects were observed among clinical observations, body weights, food consumption, functional observational battery, urinalysis, clinical pathology parameters, blood coagulation, mortality, or macroscopic and histopathological findings. For the genotoxicity study, statistical analysis of the bone marrow scores revealed that the positive control compound cyclophosphamide reduced PCE scores similarly in both sexes, an indicator of bone marrow toxicity. No PCE changes were associated with high dose A4+, indicating a lack of toxicity. It was concluded that A4+ did not exhibit toxicity, including neurotoxicity and genotoxicity, under the conditions of these studies and a no adverse effect level (NOAEL) of 2000 mg/kg was assigned.

Ribosome-inactivating proteins (RIPs) are RNA-N-glycosidases, which deurate ribosomal RNA. With the ribosome destroyed, protein synthesis is ceased and cell death results. Pokeweed Antiviral Protein (PAP) is a Type-I RIP produced by the American Pokeweed plant. PAP has been recognized as an important anti-viral agent and is able to inhibit infection without loss of host cell translation. PAP is among the RIPs that have been used to treat several human cancers and viral diseases. This study was designed to determine how PAP protects the American Pokeweed plant against wheat leaf rust by elucidating this antiviral mechanism which would shed light into the use of PAP not only for clinical purposes, but also for the prevention of infections of agricultural crops. Numerous isoforms of PAP are present throughout the plant and vary depending upon tissue location and/or stage of growth. To determine how PAP protects the American Pokeweed plant, Pokeweed plants were infected with a bacteria: a plant pathogen, Pseudomonas syringae pv. syringoperici (DC3000) and a human pathogen, Pseudomonas aeruginosa 01 (PA01). Cell death was observed with DC3000 infection, but not after PA01 infection. RT-PCR was carried out on infected leaves collected at various time intervals. PCR mRNA expressions were upregulated at the 24 time hour point for both bacteria, with a greater upregulation evidenced for the PA01 bacteria. In vivo and in vitro antimicrobial studies were carried out to examine the growth rates of the different bacteria within the leaf tissue, as well as, attempt to mimic those results in a laboratory setting. In vivo antimicrobial studies displayed an inhibition in bacterial growth over time, for both bacteria tested. In vitro antimicrobial tests resulted in PAP significantly inhibiting the PA01 bacterial growth, however, it appeared to have no effect on DC3000 growth. Based on these observations, PAP inhibits the growth of plant and human pathogens within the Pokeweed plant by employing different defense mechanisms.
Resveratrol is a natural polyphenol that was widely found in grapes, peanuts and mulberries. Vincadon G and ε-viniferin are resveratrol oligomers which have many biological activity such as antioxidant, antimutagen, antibacterial and anticancer. In this study, antimutagenic effect was performed using Ames test with pre-incubation method, and with and without the presence of metabolic activation of S9 on Salmonella typhimurium TA98 and TA100 strain. Mutagenic effects was evaluated using two-fold value of the negative control as the cut-off point. In this test, both compound showed no mutagenicity effect on both Salmonella typhimurium strain. Inhibitio effect of mutagenic activity for both compound induced by 2-aminoan-thracene in both strain showed high antimutagenic activity. The best inhibitory effect of mutagenic activity was 91.22% obtained following treatment with ε-viniferin on TA98 and 76.1% on TA100. Additionally, mutations induced by 2-nitrofluorene and sodium azide in the presence of metabolic activation was also reversed. Both compound showed high antimutagenic activity on both strain. The best inhibitory effect was obtained in TA98 strain with 87.62% for vincadon G and 85.22% for ε-viniferin. These results suggested that both vincadon G and ε-viniferin has the ability to protect against mutagenicity effect induced by direct and indirect mutagens.

Material and Methods: Six-week old male Balb/c mice were divided into four groups (A-D) of six animals per group. Mice in groups A and C were fed a regular diet for three weeks, while those in groups B and D mice were supplemented with 1% NM during that period. After three weeks mice in groups C and D received daily injections of 50 mg Am/kg intraperitoneally for 4 days, and group A and B received saline alone. After 24 h, mice were sacrificed, blood was withdrawn by cardiac puncture, serum was collected for clinical chemistry, and livers, hearts, kidneys and lungs were excised and weighed.

Results: There were no differences in weight gain and food consumed in control and test groups (A-D). Liver, kidney, heart and lung weights were comparable in all groups. Administration of NM to group C resulted in significant increase in serum creatine phosphokinase (CPK), whereas in the NM fed group D the serum CPK was not affected and comparable to the saline injection groups A and B. Am administration also resulted in significant increase in serum marker for heart aspartate aminotransferase (AST) in group C animals, but not in group D mice, which exhibited similar levels to that in groups A and B.

Conclusions: There were no differences in weight gain and food consumed in control and test groups (A-D). Liver, kidney, heart and lung weights were comparable in all groups. Administration of NM to group C resulted in significant increase in serum creatine phosphokinase (CPK), whereas in the NM fed group D the serum CPK was not affected and comparable to the saline injection groups A and B. Administration of NM to group C resulted in significant increase in serum marker for heart aspartate aminotransferase (AST) in group C animals, but not in group D mice, which exhibited similar levels to that in groups A and B.

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Pharmacological pathways suggesting a potential target for the prevention of obesity. HFD-induced hepatic lipid accumulation are thus mediated through AMPK signaling pathway. The specific AMPK inhibitor, compound C, downregulated the levels of sterol regulatory element-binding protein-1 (SREBP-1) and its target genes including ACC and fatty acid synthase (FAS). The specific AMPK inhibitor; compound C, efficiently inhibited interleukin-6-induced production of vascular endothelial growth factor (VEGF), migration and tube formation in human umbilical vein endothelial cells. Our findings demonstrate that kahweol inhibits metastasis and angiogenesis, at least in part, through the disruption of STAT3-mediated transcription of the MMP and VEGF genes.

Purple sweet potato (PSP) is a functional food rich in anthocyanins that possess disease-preventive properties. This study, we evaluated body weight, liver histology, and hepatic lipid content in high-fat diet (HFD)-fed ICR mice treated with AF. In addition, we characterized the underlying mechanism of AF's effects in HepG2 hepatocytes through Western blot analysis. AF (200 mg/kg/day) reduced weight gain, and hepatic lipid content in high-fat diet (HFD)-fed ICR mice treated with AF. AF (200 mg/kg/day) reduced weight gain, and hepatic lipid content in high-fat diet (HFD)-fed ICR mice treated with AF. In addition, AF downregulated the levels of sterol regulatory element-binding protein-1 (SREBP-1) and its target genes including ACC and fatty acid synthase (FAS). The specific AMPK inhibitor; compound C, attenuated the effects of AF on the expression of lipid metabolism-related proteins such as SREBP-1 and FAS in HepG2 hepatocytes. The beneficial effects of AF on HFD-induced hepatic lipid accumulation are thus mediated through AMPK signaling pathways suggesting a potential target for the prevention of obesity.

Saponins from the roots of Platycodon grandiflorum inhibit UV irradiation-induced matrix metalloproteinase-1 and inflammatory cytokine production in human keratinocytes by suppressing NF-kB and AP-1 pathway. The excessive reactive oxygen species (ROS) induced by ultraviolet (UV) radiation cause skin aging via matrix degradation resulting from matrix metalloproteinases (MMPs). Recently, saponins from the roots of Platycodon grandiflorum (CKS) were demonstrated to attenuate the cell damage induced by oxidative stress by quenching reactive oxygen species (ROS) and inducing antioxidant systems. In this study, we explored the inhibitory effects of CKS on UVA-induced matrix metalloproteinase-1 (MMP)-1 and investigated the molecular mechanism underlying those effects. CKS increased the cell viability and inhibited ROS production in HaCaT cells exposed to UVA irradiation. Pre-treatment of HaCaT cells with CKS inhibited UVA-induced production of MMP-1 and MMP-9. In addition, CKS decreased UVA-induced expression of the inflammatory cytokines IL-1β and IL-6. Western blot analysis further revealed that CKS markedly suppressed the enhancement of collagen degradation in UVA-exposed HaCaT cells. CKS also suppressed UVA-induced activation of NF-kB or c-Jun and c-Fos, and the phosphorylation of MAPKs, which are upstream modulators of NF-kB and AP-1.

The coffee diterpene kahweol inhibits metastasis and angiogenesis. Kahweol, a coffee-specific diterpene, is reported to have anti-cancer properties. However, the precise mechanism of kahweol on chemopreventive effect is unclear. In this study, we examined the anti-angiogenic activities of kahweol by targeting STAT3. Kahweol inhibited the migration and invasion ability of various cancer cells in vitro and decreased the secretion of matrix metalloproteinase (MMP)-2, MMP-9, and VEGF in cancer cells. Kahweol inhibited the phosphorylation of signal transducer and activator of transcription 3 (STAT3), suggesting that STAT3-responsive regions in the VEGF and MMP promoters may underlie the inhibitory effects of kahweol. Kahweol also exhibited an inhibitory effect on lung metastasis in the experimental B16-F10 melanoma metastasis model. Kahweol effectively inhibited interleukin-6-induced production of vascular endothelial growth factor (VEGF), migration and tube formation in human umbilical vein endothelial cells. Our findings demonstrate that kahweol inhibits metastasis and angiogenesis, at least in part, through the disruption of STAT3-mediated transcription of the MMP and VEGF genes.

Hepatoprotective and in vivo antioxidant effects of ethanol extract of whole fruit of Lagenaria breviflora (LB) was investigated in experimental animals. Forty nine (49) male Wistar albino rats of 150-250g were randomly divided into seven groups of seven. Rats in group I were administered with 0.9% Physiological saline (10mL/kg b.w), groups II, III, IV received ethanol extract of LB at 100, 250, 500mg/kg body weight respectively, group VI received colfosceril as reference hepatoprotective agent orally for 9 days. Carbon tetrachloride (CCl4) was administered intraperitoneally at 1.5 mL/kg bw into the rats in group II-VI to precipitate hepatic injury on the 9th day, meaning that the rats in group V received CCl4 alone. Rats in group VII also received extract alone at 500 mg/kg b.w. All the rats in the study were anaesthetized with ether and sacrificed for serum collection and analysis on the 10th day. Liver samples were prepared for histopathology. Data were expressed as mean Standard Error of Mean and analyzed using one way ANOVA. Difference of means are considered statistically significant at p<0.05. There was significant (p<0.05) increased in malondialdehyde (MDA) and hydrogen peroxide (H2O2) generation in serum of CCl4 treated rats (group II) while the serum glutathione (GSH) level crashed significantly. Pre-treatment with LB led to a significant increase in serum GSH, reduction in MDA and H2O2 generation. The activities of marker enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), bilirubin, creatinine and blood urea nitrogen (BUN) increased (p<0.05) significantly in CCl4 treated rats (group V) compared with extract-treated groups. The present study suggested that the treatment with LB extract ameliorated CCl4-induced hepatic damage and oxidative stress via its antioxidant and hepatoprotective properties.

Possible role of metabolism by the intestinal bacteria in geniposide-induced cytotoxicity was investigated in human hepatoma HepG2 cells. Initially, toxic effects of geniposide and its metabolite genipin were compared. Genipin, a deglycosylated form of geniposide, was cytotoxic at the concentrations that geniposide was not. As metabolic activation systems for geniposide, human intestinal bacterial cultures, fecal preparation (fecalease) and intestinal microbial enzyme mix were employed in...
the present study. When geniposide was incubated with human intestinal bacteria, either Bifidobacterium longum HY001 or Bacteroides fragilis, for 24 h, the cultured media caused cytotoxicity in HepG2 cells. Fecalase and intestinal enzyme mix were also effective to metabolically activate geniposide to its cytotoxic metabolite. The present results indicated that genipin, a metabolite of geniposide, might be more toxic than geniposide, and that intestinal bacteria might have a role in bio-transformation of geniposide to toxic metabolite. Furthermore, among three activation systems tested, intestinal microbial enzyme mix would be convenient to use in detecting toxics requiring metabolic activation by intestinal bacteria. [Supported by a grant (09172KFDA996) from Korea Food & Drug Administration].

**2423** INHIBITORY EFFECT OF DIHYDROARTEMSIIN AGAINST PHORBOL ESTER-INDUCED CYCLOOXYGENASE-2 EXPRESSION IN RAW264.7 MACROPHAGES.

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Artesunate (AS) is a clinically viable artemisinin derivative utilized for the treatment of mild to severe malaria infection. Dihydroartemisinin has recently been shown to possess anti-tumor activity in various cancer cells. However, the effect of the anti-inflammatory potentials of dihydroartemisinin has not been studied. This study investigated the effect of cyclooxygenase-2 (COX-2) and molecular mechanisms by dihydroartemisinin in phorbol 12-myristate 13-acetate (PMA) stimulated Raw 264.7 cells. Dihydroartemisinin inhibited PMA-induced production of PGE2 in a dose-dependent manner. Dihydroartemisinin reduced PMA-enhanced COX-2 expression and activity in Raw 264.7 cells. Additionally, dihydroartemisinin decreased luciferase activity of COX-2-regulation-related transcription factors including nuclear factor κB (NF-κB), activator protein-1 (AP-1), CCAAT-enhancer binding protein (C/EBP) and cyclic adenosine monophosphate (cAMP)-response element (CRE). Dihydroartemisinin also remarkably reduced PMA-induced p65, C/EBPβ, c-jun and CREB expression. Furthermore, dihydroartemisinin evidently inhibited PMA-induced activation of the mitogen activated protein (MAP) kinases, such as extracellular signal-regulated kinase (ERK), jun n-terminal kinase (JNK) and p38. These observations suggested that the inhibition of PMA-induced COX-2 activation is one of the mechanisms by dihydroartemisinin that promotes the anti-inflammatory activity in Raw 264.7 cells.

**2424** PREVENTION OF HIGH GLUCOSE-INDUCED HEPATIC LIPOGENESIS BY PHILLYRIN THROUGH AMP-ACTIVATED PROTEIN KINASE SIGNALING PATHWAY.

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Phillyrin, a major effective constituent of Forsythia koreana, is reported to exert anti-obesity effect in purified obesity mice. However, the mechanism by which phillyrin inhibits lipogenesis remains unclear. The objective of the present study was to investigate how phillyrin prevents lipogenesis in human hepatoma (HepG2) cells induced by high glucose. Phillyrin suppressed high glucose-induced lipid accumulation in HepG2 cells. The expression of critical molecule involved in lipogenesis, sterol regulatory element binding protein-1c (SREBP-1c), was attenuated in phillyrin-treated cells. Moreover, treatment of phillyrin increased phosphorylation of liver kinase B1 (LKB1), 5′-AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC), and inhibited the fatty acid synthase (FAS) expression. These results indicate that phillyrin attenuates lipogenesis in HepG2 cells by blocking the expression of FAS through AMPK activation, suggesting that phillyrin has potential utility as a chemopreventive agent for anti-obesity.

**2425** INHIBITORY EFFECT OF WINTERGREEN OIL FROM GALLAHERIA FRAGRANTISSIMA IN ULTRAVIOLET B-IRRADIATED COLLAGENASE EXPRESSION.

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Ultraviolet B (UVB) irradiation from the sun reduces type I procollagen production and increases matrix metalloproteinases-1 (MMP-1) expression in human skin and plays a major role in the process of photoaging. Wintergreen oil from Gaultheria fragrantissima is reported to exert high anti-inflammatory properties and used to as a health care product. However, the mechanism of action still now is unknown. The objective of this study was to investigate how wintergreen oil prevents expression of MMP-1 induced by UVB irradiation in human foreskin fibroblasts (H668) cells. Pretreatment of wintergreen oil enhanced cell viability and type I procollagen production in the UVB-irradiated H668 cells. Wintergreen oil inhibited UVB-induced expression of MMP-1. The activation of critical regulatory-transcription factors was inhibited (NF-κB) and nuclear factor-κB (NF-κB) was significantly inhibited by wintergreen oil. Furthermore, wintergreen oil decreased the phosphorylation of Ca2+/calmodulin-dependent kinases (CaMKII and CaMKII δ). These results indicate that wintergreen oil inhibited UVB-induced MMP-1 expression via Ca2+-dependent, AP-1 and NF-κB signaling pathway, suggesting that wintergreen oil has potential utility in the prevention of skin photodamage.

**2426** TOXICOLOGICAL EVALUATIONS OF THE AQUEOUS LEAF EXTRACT OF OCIMUM GRATISSIMUM.

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Toxicological evaluation of Ocimum gratissimum was carried out using haematological and serum biochemical changes accompanying prolonged administration of the aqueous leaf extract of the plant in Wistar rats. Twenty rats were randomly divided equally into four groups. Rats in group I were administered with 0.9% physiological saline (10ml/kg, b.w) as the control animals, while rats in groups II, III and IV were administered with aqueous extract at 400, 800 and 1600 mg/kg body weight respectively once daily for 28 days according to the acute toxicity study carried out. Parameters evaluated were complete blood count, serum protein, metabolites and enzymes. Samples from liver, spleen, heart, kidney and testis were processed for histopathology. Data were expressed as mean± Standard Error of Mean and analyzed using one way ANOVA. Difference of means are considered statistically significant at p<0.05.

Rats in group III and IV exhibited significant decrease in total white blood cell and neutrophils count while the lymphocytes count increased significantly for rats in group IV when compared to the control rats. Administration of the extract elevated the serum levels of alkaline phosphatase, aspartate amino transaminase, total protein and urea in test rats especially in group IV. Histopathology revealed mild hepatic and perportal mononuclear cell infiltration, mild diffuse tubular nephrosis with evidence of regeneration and hyperplasia in the epididymis. It was concluded that ethnomedicinal application of Ocimum gratissimum is quite safe at lower doses but it is hepatotoxic and nephrotoxic at higher doses.

**2427** IMMUNOMODULATORY EFFECTS OF JACAREUBIN IN HUMAN PERIPHERAL BLOOD MONOCYTOLEUCED CELLS.

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Chemical compounds derived from plants have proved to be a rich source to develop new drugs. Jacobebin is a xanthone isolated from the heartwood of Callopitygium brasiliense. In biological studies, it has show antibacterial activity, induction of apoptosis, cytostaticity by arresting cells in G2/M, and cytotoxic effects on human tumor cell lines, with less toxicity in untransformed human cells. However, the effect of Jacobebin on normal cells has not been studied. As peripheral blood mononuclear cells (PBMCs) importantly participate in the cancer challenge, and they are an obvious target of antineoplastic drugs due to their administration route, we studied the potential immunomodulator effect of Jacobebin in a human macrophage cell line (THP-1), and in PBMCs. The macrophages and PBMC (with or without stimulus) were cultured in RPMI medium and were treated with Jacobebin (0.5 to 50 μM) for 24, 48 or 72 h. Cell survival was evaluated by the metabolism of 3-(4,5-dimethylthiazol-2-il)-2,5-bromide diphenyl tetrazolium (MTT), as a measurement of mitochondrial activity, and cell proliferation was evaluated by 3H-thymidine ([3H]T) incorporation. Levels of secretion of anti-inflammatory cytokines, such interleukin (IL)-4, IL-10, and tumor growth factor (TGF)-β, and pro-inflammatory cytokines such as interferon (IFN)-γ, IL-2, IL-6, IL-8, IL-12, and tumor necrosis factor (TNF)-α were evaluated by ELISA, nitric oxide (NO) production by THP-1 cells was measured by Griess assay. MTT, and [3H]T assays showed that Jacobebin reduces cell viability from the concentration of 5 μM, and cell proliferation from the concentration of 0.5 μM after 72 h of exposure. Data on cytokine secretion will be presented at the poster. Further experiments will be performed with the THP-1 human cell line.
2428 CARDIOTOXICITY OF TRIPERYGNUM WILFORDII HOOK. F.


Tripberygium wilfordii Hook. F. (TWH), a Chinese traditional medicine, is widely used in the treatment of autoimmune diseases in Asia. Clinical literatures regarding the adverse effects and diseases caused by TWH are increasing, especially its cardiac toxicity. Due to the unique clinical efficacy in autoimmune diseases, TWH is prescribed widely. There have been some clinical case reports and animal studies of TWH; however, studies on its cardiac toxicity mechanism(s) are limited. Glycoside of Tripberygium Wilfordii (GTW) is a refined extract from peeled roots of TWH. GTM is one of the most widely used TWH formulations, thus it is used for the current study. An acute (oral gavage, 100 or 200 mg/kg) and a repeat dose (5, 15, and 45/90 mg/kg/day for 28 days) study of GTW in SD rats were carried out. Both studies showed that GTM increased cardiac Troponin I, mortality rate, and focal myocardial necrosis. To study the possible mechanism(s) of TWH cardiac toxicity, a Real Time xCELLigence Analysis System (ACEA Biosciences, San Diego, CA, USA) was adopted for studying the effects of triptolide (>99.5%, Sigma, St. Louis, MO, USA) the major active ingredient of TWH. More specifically, the beating rates of primary cultured neonatal rat cardiac cells treated with triptolide were monitored for continuous 20 hours. The results showed that triptolide (>20 μM) inhibited beating rates approximately 10 minutes after the administration but the beating rates returned to the normal range 1 hour after the administrations. For triptolide at 140 μM or higher, the beating rates were normal up to 15 hours after the administration, and then became irregular till study end. The irregularity might be due to cell death. The effects of triptolide on Human Ether-a-go-go Related Gene (hERG) encoded potassium current (Ikr) were also studied. The results showed that triptolide inhibited Ikr in a concentration dependent manner. Hence, it is suggested that triptolide can induce QT prolongation. In conclusion, special attention should be paid to cardiac toxicity when using GTM, triptolide or other formulations of TWH in clinical settings.

2429 TOXICITY PROFILE OF ACONITUM HETEROPHYLLUM WALL.

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Aconitum heterophyllum Wall commonly known as “Atis” or “Paris” belongs to family Ranunculaceae and is reported for its medicinal and pharmaceutical values since long. Roots which have been used mostly as poison than as drug are now reported to possess significant anti-inflammatory and analgesic properties with a high therapeutic index. The plant was used for treatment of diseases of nervous system, digestive system, rheumatism and fever. It also exhibits anti-fungal and anti-bacterial activity. Investigation was carried out to evaluate safety of aqueous extract of Aconitum heterophyllum roots by determining its potential toxicity after acute and chronic administration in Wistar rats. Roots of Aconitum heterophyllum were dried, powdered and extracted by maceration with water for 7 days. Dried extract was dissolved in distilled water and administered in different doses. For acute toxicity study, administration was carried out in six female Wistar rats by oral gavage at doses 175, 550, 1750 and 5000 mg/kg body weight according to OECD 425. Changes in general behaviour, mortality and body weight were recorded for 14 days and necropsy was performed on day 14. No mortality was observed. Repeated dose oral toxicity study was performed for 28 and 90 days as per OECD 407 and 408 respectively with dose 1000 mg/kg p.o. Animals were observed closely on each day for signs of toxicity and scored as per Functional Observational Battery (FOB). No mortality or changes in clinical signs and FOB were noted. No significant changes were observed in biochemical, hematological parameters and organ weights. Histopathological studies of important organs showed normal structure, suggesting no morphological alterations. Lack of mortality or clinically significant changes in biochemical and haematological parameters in rats after 90 days of daily dosing circumvent toxicity of extract. Thus, aqueous extract of Aconitum heterophyllum roots can be considered safe for therapeutic use.

2430 CYTOTOXICITY OF RESVERATROL AND ITS ANALOGS PTEROSTILBENE AND PICEATANNOL IN MACROPHAGES.

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Resveratrol (RES) is a stilbene which possesses antioxidant, anti-inflammatory and anti-cancer activities. We previously reported that RES and the related compounds pterostilbene (TPS) and piceatannol (PIC) were cytotoxic to RAW 264.7 macrophages after a 24 h incubation. Here, the effect of RES, TPS and PIC was investigated in 8 h incubation in a series of macrophages using blue dye exclusion to assess cell viability found that TPS was more toxic than either PIC or RES (LC50 values of 3.6, 11.5 and 15.2 μM respectively). When the MTT assay was used to assess viability, RES was found to be less cytotoxic than either TPS or PIC after 48 h (LC50 values of 31.5, 20.1 and 20.7 μM respectively). TPS differed from RES by the presence of methoxy groups instead of hydroxyl groups on the A ring. This structural difference may explain why TPS was the most toxic to RAW 264.7 cells. Several published reports indicate that RES induces apoptosis in cancer cells. To determine if RES can activate apoptosis in our model, RAW 264.7 cells were treated with RES (30 μM, 24 h) and the activity of caspase-3 (CASP-3) was monitored. CASP-3 activity was found to increase in a time- and concentration dependent manner. To investigate if the results above could be generalized to primary cells, macrophages isolated from female C57BL/6J mice were incubated for 24 h with RES, TPS or PIC. Peritoneal macrophages were more resistant to RES (LC50 > 200 μM), PIC (LC50 > 200 μM) and PTS (LC50 = 70 μM) than the RAW 264.7 macrophages, which are transformed cells. TPS was the most toxic among the stilbenes tested in the primary cells. Taken together, the in vitro and ex vivo comparative data suggest that the transformed macrophages are more sensitive than the primary macrophages to TPS, PIC and RES.

2431 PRELIMINARY TOXICITY AND PHYTOCHEMICAL STUDIES OF THE AQUEOUS EXTRACT OF FICUS PLATYPHYLLA IN FEMALE WISTAR RATS.

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Ficus platypylla Del. Holl., commonly known as gutta percha tree, grows widely in the Northern part of Nigeria. It is called gamji in hausa language. The leaves, seeds and bark of the plant have been used together traditionally to promote fertility. Previous studies on the central nervous and anti-inflammatory effects have been conducted on the stem bark of the plant. The present study was aimed to investigate the safety of Ficus platypylla. Phytochemical, acute and repeat dose toxicity studies were conducted on aqueous extract of the leaves, seeds and bark of F. platypylla. A Limit dose of 3000mg/kg of the aqueous extract was administered orally to female wistar rats in sequence to test for acute toxic effect. A dose of 700mg/kg was administered orally daily for 28days to another group of female wistar rats to ascertain the repeat dose effect. The control group of female wistar rats received 5ml/kg of distilled water (diluent of the aq. extract) for 28days. Biochemical and histological analysis were conducted. Phytochemical studies revealed that the extract contains saponins (1%), tannins (16.75%), flavonoids (24.3%), volatile oils, glycosides (2.47%) and steroids. The acute toxicity results showed that the extract has LD50 above 3000mg/kg and repeat dose toxicity studies of the extract revealed no damage to the glomeruli of rat kidney. Though the extract is relatively safe, its prolonged use may carry risk of renal damage.

2432 CARDIOPROTECTIVE EFFECTS OF PIQUÍA PULP FRUIT (CARYOCAR VILLOSUM) AGAINST DOXORUBICIN-INDUCED LIPID PEROXIDATION AND DNA DAMAGE IN RATS.


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Doxorubicin (DXR) is a widely used chemotherapeutic agent; however, its clinical use is limited due to its cardiotoxicity associated with an oxidative stress induction. Piúí (Caryocar villosum) is a native tree from Amazon rainforest and its fruit, which contains antioxidants such as carotenoids and phenolic compounds, is commonly consumed by local residents. The aim of this study was to investigate the genotoxicity and also the protective effect of piúí pulp fruit against lipid peroxidation and DNA damage induced by DXR in heart of rats by determining the thio-
barbituric acid reactive substances (TBARS) and comet assay, respectively. Piquí pulp fruit (75, 150 or 300 mg/kg b.w.) was administered by gavage in Wistar rats, for 14 days, and 24 hours before euthanasia, the animals received an injection of saline or DXR (15mg/kg b.w., i.p.). DXR increased the concentrations of TBARS in heart tissue and the animals that received piquí at all tested doses associated with DXR presented a significant reduction in TBARS. Further, piquí at all tested doses showed no genotoxic effects and the dose of 300 mg/kg b.w. significantly reduced DXR-induced DNA damage in cardiac cells. Thus, piquí ameliorated lipid peroxidation and DNA damage induced by DXR in rats. The constituents of piquí pulp fruit carotenoids, flavonoids and phenolic compounds, may be the responsible for these protective effects since they had already demonstrated antioxidant activity when isolated. To elucidate the phytochemical composition, we are currently performing analyses of piquí pulp fruit. Financial support: FAPESP and CAPES.

2433 STUDY OF THE ANTIGENOTOXIC AND CYTOTOXIC ACTIVITIES OF THE FRUIT SOLANUM SESSILIFLORUM BY THE MICRONUCLEUS TEST IN RATS.


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Maná-cubú (MC) (Solanum sessiliflorum) is a native exotic plant from Amazonian forest. Its fruits pulp contains phenolic compounds and carotenoids, as well as antioxidant capacity. Due to its use in folk medicine to control blood glucose and cholesterol levels and the lack of scientific information, the aim of this study was to investigate the genotoxic and antigenotoxic potential of MC pulp by the micronucleus test in peripheral blood and bone marrow cells from Wistar rats. The tests were performed with water or MC pulp (250 or 575 mg/kg b.w.) for gavage for 14 days; followed by intraperitoneal injection (i.p.) of saline or doxorubicin (DXR, 16 mg/kg b.w.), just after the last gavage and 24 hours before euthanasia. The animals were divided into six groups: negative control (saline solution i.p.); positive control (DXR i.p.); and two doses of MC associated or not with DXR. The animals treated with MC in both doses showed no statistical differences on the PCE/NEC rate and on micronuclei frequency in bone marrow and peripheral blood, demonstrating lack of cytotoxicity and genotoxicity effects. The highest dose of MC (375 mg/kg b.w) significantly reduced the micronuclei frequency induced by DXR in bone marrow (60.22%) and peripheral blood (64.52%) cells. This protective effect against DXR-induced DNA damage could be explained due to the antioxidant activity of carotenoids and phenolic compounds present in MC pulp. To elucidate the phytochemical composition, we are currently performing analyses of the pigments in MC pulp fruit to identify the compounds involved in these protective effects. Financial Support: FAPESP and CAPES.

2434 MONITORING OF PIG-A MUTATION IN HUMAN PERIPHERAL RED BLOOD CELLS.

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We have developed a simple, robust and inexpensive assay for measuring the frequency of human peripheral red blood cells (RBCs) having mutation in the endogenous phosphatidylinositol glycan class-A gene (PIG-A). The PIG-A gene is involved in biosynthesis of glycosyl phosphatidyl inositol (GPI). In the assay, PIG-A mutants are identified by immunofluorescent staining with monoclonal antibodies against CD59, a cell surface marker anchored to the exterior of the plasma membrane by GPI. The frequency of CD59-deficient, presumably PIG-A mutant, RBCs is then determined by multiparameter flow cytometry, using technology available at most research and clinical facilities. We determined the frequency of PIG-A mutant RBCs in 97 healthy volunteers and in 10 newly diagnosed cancer patients receiving antineoplastic chemotherapy with several genotoxic drugs known to be clastogens. In the majority of self-identified healthy donors, PIG-A mutant frequency was low (mean ± standard deviation = 5 ± 9%, 10-6) and stable. In cancer patients the PIG-A mutant frequency was measured before the start of chemotherapy and at several time-points while on/after the therapy. A clear, approximate 3-fold increase in the frequency of CD59-deficient mutant RBCs was observed in one patient at 4 and 6 months after the start of chemotherapy with cisplatin and etoposide. The results suggest that the RBC PIG-A assay can be useful for detection of genotoxicity in humans, especially when the frequency of mutant RBCs can be determined before the exposure to potential hazards and at several time-points thereafter. The assay may be used for monitoring volunteers in clinical trials of new drugs and for diagnosis of endogenous genetic instability as a predictor of susceptibility to cancer. All procedures related to blood collection and processing were reviewed by FDAs Research Involving Human Subjects Committee and received a categorical exemption from oversight. The study was partially funded by the FDAs Critical Path Initiative national strategy.

2435 DETECTION OF IDIOSYNCRATIC HEPATOTOXICITY POTENTIAL WITH METABOLITE PROFILING OF RAT SERUM.

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Idiosyncratic drug-induced liver injury (iDILI), where hepatotoxicity occurs in a few individuals treated with a drug that shows no liver injury in routine animal toxicology studies, is a serious concern for both the pharmaceutical industry and regulatory agencies. The question remains whether the potential for iDILI might have been detected in such studies if new measures had been added to the usual complement of clinical and histopathology. One promising measure is metabolite profiling (metabonomics/metabolomics). To address the question of whether iDILI could be detected with this approach, 15 compounds were selected: Phenylalanine, Fluortamid, propyl-thiouracil, Lamivudine, Methotrexate, Captopril, Nefazodone, Nevirapine, Valproic acid, and Zidovudine as positive for iDILI potential, with Atropine, Mannitol, Neomycin, Streptomycin, and Vancomycin as negative. Classifications were taken from public sources. Metabolite profiles were taken from MetaMap®Tox, a unique database of metabolite profiles from rat plasma obtained during 28 day rat studies of over 500 pharmaceutical, chemicals and agrochemicals. This database, developed by metanomics GmbH and BASF SE, was also used to create “mechanism of action” (MOA) metabolite signatures (“toxicity pattern”) for a number of known toxicities, and the metabolite profiles of the chosen compounds were examined for matches to various MOAs. Eight of the ten compounds with iDILI potential had metabolite profiles that matched with patterns of various liver toxicities, including cholestasis, oxidative stress, acetaminophen-type toxicity and peroxisome proliferation. Only methotrexate and lamivudine showed no such matches. By contrast, only one of the five non-DILI compounds (Atropine) showed weak matches with liver enzyme induction and with general liver toxicity. These results suggest that metabolite profiling may indeed have promise to detect compounds with iDILI potential.

2436 ASSESSING HUMAN EXPOSURE TO TRICLOCARBAN USING URINARY TRICLOCARBAN AND ITS OXIDATIVE METABOLITES AS BIOMARKERS.


3, 4, 4′-Trichlorocarbanilide (triclocarban, TCC) is widely used as an antimicrobial agent in a variety of consumer and personal care products including soaps, detergents, toothpastes, deodorants, and cleansing lotions. TCC is classified as a high production volume chemical which has been detected in surface waters, municipal water treatment effluents, and in about 60% of the streams throughout the United States. Because of the widespread use of TCC, the potential for human exposure is high. TCC is considered a potential endocrine disruptor, but its potential toxic effects in humans are still largely unknown. Results from in vivo and in-vitro studies suggested that urinary TCC and its two oxidative metabolites, 2′-hydroxy-TCC (2′-OH-TCC) and 3′-hydroxy-TCC (3′-OH-TCC), might serve as the biomarkers for human exposure to TCC. There is limited data on the exposure to triclocarban in human populations. In the present study, we characterized the human exposure to TCC using conveniently sampled populations. The Human Subjects Institutional Review Board approved the study protocol. Between 2009 and 2011, 156 urine samples were collected from a diverse group of 79 male and 79 female adult volunteers with no documented occupational exposure to TCC. We also analyzed 16 commercially available adult serum samples collected between 1998 and 2003. The analytical method used to quantify TCC and its metabolites (2′-OH-TCC and 3′-OH-TCC) in urine and serum was an on-line solid phase extraction coupled to high performance liquid chromatography-tandem mass spectrometry. Among the three compounds measured, TCC was detected the most in urine, with the frequency of detection at 35.4%. A trace amount of TCC was
Mercury (Hg) is an ubiquitous environmental contaminant, causing both neurotoxicity and immunotoxicity. Given its ability to amalgamate gold, Hg is used in small-scale gold mining. The goal of this project was to identify novel serum biomarkers of Hg-induced immune dysregulation. A ralanysis of serum samples from an epidemiological study on miners working in Amazonian Brazil was completed on stratified samples (based on Hg level and antineural autoantibody titer) using a protein microarray to probe for the induction of elevated auto-antibodies. We found statistically relevant correlations between high levels of Hg exposure and certain auto-antibodies. The antibodies were then analyzed in terms of immune system pathways. Pathways examined include those involved in antigen presentation, oxidative stress and macrophage signaling/activation. Auto-antibodies identified as novel biomarkers include antibodies to the following proteins: interferon-induced transmembrane protein (IFITM-1), STAT-6, FKBPL, heat shock transcription factor (HSF-1), colony stimulating factor (CSF-1), glothelin/obestatin propeptide (GHRH), metaloplaspeptide inhibitor (TIP-1), peroxiredoxin (PRDX-2), glutathione S-transferase alpha (GST-A1), and chaperonin (HSPD-1). These proteins play a wide variety of roles, including as antioxidants, in the regulation of pro- and anti-inflammatory cytokines, as well as danger and oxidative stress signaling. Dysregulation of these proteins is believed to play a role in autoimmune diseases such as rheumatoid arthritis, Sjogren's syndrome, and multiple sclerosis. Our next step is to validate these findings by probing the entire sample cohort for titers of these auto-antibodies.

Association of environmental exposure to polycyclic aromatic hydrocarbons with body burden of estrogen quinones in breast cancer patients using hemoglobin adducts as biomarkers.

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Both 17β-estradiol-2,3-quinone (E2-2,3-Q) and 17β-estradiol-3,4-quinone (E2-3,4-Q) and 1,2-naphthoquinone (1,2-NPQ) and 1,4-naphthoquinone (1,4-NPQ) are reactive metabolites of estrogen and naphthalene that are thought to be responsible for the estrogen and naphthalene-induced genotoxicity. The aim of this study was to establish a methodology to simultaneously analyze both estrogen and naphthalene quinone-derived protein adducts and to measure the background levels of these adducts in human hemoglobin (Hb) derived from female breast cancer patients (n=85) in Taiwan. Results from in vitro experiments confirmed that the production of estrogen quinone-derived adducts on Hb increased with increased concentration of estrogen quinones. Time-course experiments suggested that both E2-2,3-Q and E2-3,4-Q adducts rapidly reached maximum values at 10 min mark and remained constant thereafter for up to 24 h. We measured the levels of estrogen and naphthoquinone quinone-derived adducts in human hemoglobin, cysteinyl adducts of E2-2,3-Q-4-S-Hb, E2-3,4-Q-2-S-Hb, 1,4-NPQ, and 1,4-NPQ were detected in breast cancer patients (n=85) with median levels at 497 (range 281–764), 961 (range 700–1500), 258 (33.0–709), and 32.0 (12.0–79.0) (pmol/g), respectively. Levels of E2-2,3-Q-4-S-Hb correlated significantly with those of E2-3,4-Q-2-S-Hb (correlation coefficient r=0.643, p<0.001). Additionally, levels of 1,4-NPQ-Hb correlated significantly with those of 1,4-NPQ-Hb (r=0.549, p<0.001). We noticed that levels of 1,2-NPQ-Hb positively correlated with those of E2-3,4-Q-2-S-Hb (r=0.505, p<0.001) and E2-2,3-Q-4-S-Hb (r=0.340, p<0.001). This evidence suggests that environmental exposure to polycyclic aromatic hydrocarbons may enhance bio-activation of estrogen to the reactive quinone species. Overall, we conclude that the methodology developed in this study may be applied to epidemiological studies as biomarkers of estrogen homeostasis.

Uranium is a radioactive heavy metal with a predominately chemical toxicity affecting especially the proximal tubular structure of the kidney. Few experimental studies have examined the effect of chronic low-dose exposure to uranium on kidney biomarkers. None of them has studied the effect of additional co-exposure to other potentially nephrotoxic environmental or therapeutic agent. The aim of the present study is to examine the effect of treating uranium-exposed rats with a nephrotoxic drug. Rats were contaminated through drinking water by uranyl nitrate for 9 months (40 mg/L) and then treated by gentamicin for 4 consecutive days (0, 5, 25, 100 and 150 mg/kg). Kidney and especially tubular injuries were estimated with physiological, biological, histological and functional biomarkers. Neither physiological indicators (diuresis and creatinine clearance) nor standard plasma and urine markers (creatinine, urea and total protein) levels were deteriorated when uranium exposure was combined with nephrotoxicity induced by gentamicin. A histological study of the kidney showed a small increase in tubular necrosis in uranium-exposed rats compared to non-exposed rats (+30%, p<0.05). Gentamicin treatment significantly increased the level of Kim-1 gene and protein expression in renal cortex and in urine (from 25 to 100 fold, p<0.001) but no significant difference was observed between uranium-exposed or non-exposed rats. Similarly, the use of other sensitive markers (osteopontin, kallikrein) indicated tubular structure damages due to gentamicin that was not aggravated by uranium exposure. Finally, the use of novel markers of kidney toxicity provides new knowledge about the nephrotoxicity threshold of gentamicin, and allows us to conclude that under our experimental conditions, low dose uranium exposure did not induce signs of nephrotoxicity or enhance renal sensitivity to another nephrotoxicant.

**2438** ASSOCIATION OF ENVIRONMENTAL EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS WITH BODY BURDEN OF ESTROGEN QUINONES IN BREAST CANCER PATIENTS USING HEMOGLOBIN ADDUCTS AS BIOMARKERS.

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**2439** THE USE OF NOVEL NEPHROTOXICITY BIOMARKERS AFTER CHRONIC URANIUM AND ACUTE GENTAMICIN COEXPOSURE.

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Uranium is a radioactive heavy metal with a predominantly chemical toxicity affecting especially the proximal tubular structure of the kidney. Few experimental studies have examined the effect of chronic low-dose exposure to uranium on kidney biomarkers. None of them has studied the effect of additional co-exposure to other potentially nephrotoxic environmental or therapeutic agent. The aim of the present study is to examine the effect of treating uranium-exposed rats with a nephrotoxic drug. Rats were contaminated through drinking water by uranyl nitrate for 9 months (40 mg/L) and then treated by gentamicin for 4 consecutive days (0, 5, 25, 100 and 150 mg/kg). Kidney and especially tubular injuries were estimated with physiological, biological, histological and functional biomarkers. Neither physiological indicators (diuresis and creatinine clearance) nor standard plasma and urine markers (creatinine, urea and total protein) levels were deteriorated when uranium exposure was combined with nephrotoxicity induced by gentamicin. A histological study of the kidney showed a small increase in tubular necrosis in uranium-exposed rats compared to non-exposed rats (+30%, p<0.05). Gentamicin treatment significantly increased the level of Kim-1 gene and protein expression in renal cortex and in urine (from 25 to 100 fold, p<0.001) but no significant difference was observed between uranium-exposed or non-exposed rats. Similarly, the use of other sensitive markers (osteopontin, kallikrein) indicated tubular structure damages due to gentamicin that was not aggravated by uranium exposure. Finally, the use of novel markers of kidney toxicity provides new knowledge about the nephrotoxicity threshold of gentamicin, and allows us to conclude that under our experimental conditions, low dose uranium exposure did not induce signs of nephrotoxicity or enhance renal sensitivity to another nephrotoxicant.


**Exposure to Organophosphate Insecticides Among Children Living Along the U.S.-Mexico Border.**

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An important tool for evaluating pesticide exposure is biomonitoring, since biomarkers reflect the dose that actually entered the body and represent an aggregate exposure received by all routes. In several exposure studies, spot urine concentrations of metabolites of several organophosphate (OPs) insecticides (including chlorpyrifos) were measured from children (ages 1 to 9) who lived in the U.S.-Mexico Border Region. The distributions of chlorpyrifos-specific metabolite, 3,5,6-trichloro-2-pyridinol (TCPY), measured in two exposure studies were comparable to the U.S. population as indicated by the National Health Nutrition Examination Survey (NHANES), except at the higher percentiles (> 95th percentile) these children had higher-than-NHANES urinary concentrations. On the other hand, the distributions of non-specific metabolites, dialkylphosphates (DAPs), measured in two exposure studies were much higher than the NHANES distribution. To further investigate the sources of exposures for children with high urinary metabolite concentrations, pharmacokinetic (PK) and physiologically based pharmacokinetic (PBPK) models were used to simulate the pharmacokinetics of OPs. For model inputs, questionnaire, environmental measurement data, and study protocols were used to generate possible exposure scenarios (e.g., routes of exposure) and specify biomarker measurements (e.g., time when urine samples were taken). Environmental (indoor air) concentrations were measured in one study. Some studies had time-activity diaries, and another one had questionnaire data such as parental occupation information. Using these data and model simulations, our analyses identified several potential sources of exposures that may lead to these higher urinary metabolite concentrations. Examples of these sources included higher exposures from multiple routes, aggregate exposures to multiple OPs, and co-exposure to both parent and degradates. (This abstract has been cleared by the US EPA but solely expresses the view of the authors)

**Exposure to Tri-o-Cresyl Phosphate as a Possible Explanation of “Aerotoxic Syndrome.”**

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The aircraft cabin ventilation is supplied from unfiltered bleed air directly from the engine. Defective engine seals can result in the release of engine oil into the cabin air supply. Aircrew and passengers have complained of illness following such “fume events.” Adverse health effects are hypothesized to result from exposure to tri-cresyl phosphate, mixed esters added to jet engine oil. Our goal was to develop a laboratory test for exposure to tricresyl phosphate. The assay was based on the fact that the active-site serine of butyrylcholinesterase reacts with the active metabolite of tri-o-cresyl phosphate, cresyl saligenin phosphate, to make a stable phosphorylated adduct with an added mass of 80 Da. No other organophosphorus agent makes this adduct in vivo on butyrylcholinesterase. Blood samples from jet airplane passengers were obtained 24-48 hours after completing a flight. Butyrylcholinesterase was partially purified from 25 ml serum or plasma, digested with pepsin, enriched for phosphorylated peptides by binding to titanium oxide, and analyzed by mass spectrometry. Of 12 jet airplane passengers tested, 6 were positive for exposure to tri-o-cresyl phosphate that is, they had detectable amounts of the phosphorylated peptide FGEpSAGAA. No more than 0.05% to 3% of plasma butyrylcholinesterase was modified. None of the subject's had toxic symptoms. Four of the positive subjects were retested 3 to 7 months following their last airplane trip and were found to be negative for phosphorylated butyrylcholinesterase. In conclusion, this is the first report of an assay that detects exposure to tri-o-cresyl phosphate in jet airplane travelers.

**Oxidative Stress, Inflammatory, and Immune Response After Inhalation Exposure to Biodiesel Exhaust.**

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Biodiesel (BD) is an advanced fuel produced from renewable domestic sources. The broad uses of BD in different industries including mining may lead to potential health effects. We hypothesized that the toxicity of biodiesel exhaust (BDE) is dependent at least on three major mechanisms; direct reactivity of BDE PM electrophiles towards critical biomolecules, induction of robust inflammatory response associated with the nano-sized components of BD PM, and triggering of oxidative stress via depletion of essential antioxidants and activation of oxidative burst in inflammatory cells. In the current study, we evaluated these pathways in BALB/cJ mice 24 hr after 4 weeks of inhalation exposure to BD (0, 50, and 500 μg/m3). Air supply. Aircrew and passengers have complained of illness following such “fume events”. [This abstract has been cleared by the US-EPA but solely expresses the view of the authors]

**Serum Proteomic Analysis of Chronic Arsenic Exposure Using 2-D DIGE Technology.**

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Environmental arsenic exposure has been a major public health concern in the world. Changes in serum proteins could be potential biomarkers of effect for arsenic exposure. This study was to investigate the changes of serum proteome among human subjects exposed to different levels of arsenic in drinking water, and in the subjects with and without arsenic-related skin lesions. Individuals living in endemic arsenicosis villages of Shanxi Province, China were selected and divided into arsenicosis group with skin lesions and non-arsenicosis group without skin lesions. The non-arsenicosis group was further divided into low, medium, and high arsenic exposure groups based on the arsenic levels in drinking water, < 10 μg/L, 10-50 μg/L, and > 50 μg/L, respectively. Since 2003, five years before the survey, an improved water supply with arsenic < 10 μg/L has been provided to individuals in the arsenicosis group who had used the water containing high levels of arsenic (> 50 μg/L). An equal amount of serum from thirty subjects in each group were pooled and analyzed by two-dimensional differential gel electrophoresis (2-D DIGE) coupled with mass spectrometry (MS). Twelve spots were found to be differentially expressed among medium, low and high arsenic exposure groups, with a positive or negative correlation with water arsenic levels. Twenty nine spots were found to be related to arsenicosis. Finally, twenty five spots with high abundance are selected for MS analysis, and 13 proteins were identified, including plasma retinal binding protein (RBP4), α1-microglobulin, keratin 1 (K1), pigment epithelium-differentiating factor (PEDF), beta-2-glycoprotein 1 (BG2P1), hemopexin, and others. This study identified the serum proteins that may be candidate biomarkers of effect for arsenic exposure and arsenicosis. However, further validation studies are required to prove these observations.

**Expression of Aldo-Keto Reductase mRNAs in Human Lymphocytes of Smokers.**

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Aldo-keto reductases (AKRs) are a group of oxidoreductases that metabolize a wide range of substrates, including polyaromatic hydrocarbons (PAHs), generating metabolites (o-quinones) capable of initiating and promoting carcinogenesis.
Studies in human cell lines exposed to PAHs have shown changes in mRNA expression of several AKR isozymes, and high levels of AKR mRNA have been observed in diseases associated with exposure to tobacco smoke. The aim of this study was to evaluate the expression levels of the AKR1A1, AKR1C1, AKR1C2 and AKR1C3 genes in lymphocytes of human smokers and non-smokers. cDNA samples from 140 men (70 non-smokers and 70 smokers) were analyzed. The expression of AKR was determined by real time PCR using a TaqMan system and cDNA levels in urine measured by ELISA. The increased of AKR1A1, AKR1C1 and AKR3C3 expression levels were associated with urine cotinine concentrations in smokers, and were also dependent on the body mass index of participants. Exposure to cigarette smoke increased the expression of AKRs. This increase may affect the metabolism of endogenous substrates (such as hormones and bile acids), as well as exogenous substrates (such as anticancer drugs and environmental pollutants).

2446 QUANTIFICATION OF SPECIFIC DNA ADDUCTS BY LC/MS/MS IN THE LIVERS OF MICE GIVEN ESTRAGOLE AT CARCINOGENIC DOSES.

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Estragole (ES), a natural constituent of several herbs and spices, is hepatocarcinogenic in mice. The proximal electrophilic form of ES possessing a reactive carboxyla
gen is generated by cytochrome P450 and the sulfotransferase metabolizing pathway. In fact, ES is metabolized to both DNA and protein adducts. However, the precise extent of formation in vivo remains uncertain. To clarify the mechanism underlying ES-hepatic carcinogenesis, we developed quantitative analytical method for ES-specific DNA adducts, and examined DNA adduct levels in the livers of mice given ES. The analytical method for assessing ES-3'-N6-dA, ES-3'-8-dG, ES-1'-N4-dG, and ES-3'-N4-dA in mouse liver as determined with radioisotope-labeled nucleosides. However, the precise extent of formation in vivo remains uncertain. To clarify the mechanism underlying ES-hepatic carcinogenesis, we developed quantitative analytical method for ES-specific DNA adducts, and examined DNA adduct levels in the livers of mice given ES. The analytical method for assessing ES-3'-N6-dA, ES-3'-8-dG and ES-3'-N4-dA using LC-ESI-MS/MS along with sample preparations such as enzymatic DNA digestion was developed for analysis of in vivo samples. The limits of quantifications (LOQs) were 0.2 fmol on column for ES-3'-N6-dA and ES-3'-8-dG, and 0.07 fmol on column for ES-3'-N4-dA. In order to confirm the availability of this analytical method in vivo, the levels of these adducts were measured in hepatic DNA of mice treated with ES at doses of 37.5, 75, 150 and 300 mg/kg b.w. by gavage 5 days per week for 13 weeks. ES-specific DNA adducts were detected in the range of 1.55 - 40.62 (adduct/106 dG or dA) in ES-treated mice. The levels of the DNA adducts in descending order were as follows: ES-3'-N4-dA, ES-3'-8-dG, and ES-3'-8-dG. Our newly developed analytical method was able to measure the precise amount of the DA adduct together with the two major DA adducts in hepatic DNA of mice treated with ES. The data showed that the DA adduct was the most abundant form. Overall, the present method and data should be useful for understanding ES carcinogenesis.

2447 METHYLGLYOXAL PRODUCTION AND METABOLISM IN SACCHAROMYCES CEREVISIAE AS A FUNCTION OF GLYCOLYTIC FLUX.

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Methylglyoxal, a toxic dicarbonyl, is generated by the spontaneous degradation of glycolytic intermediates. Methylglyoxal can form covalent adducts with cellular proteins, lipids, and nucleic acids, and has been implicated in tissue damage in various diseases including type 2 diabetes mellitus, and in the aging process. We performed experiments using the organism Saccharomyces cerevisiae grown in media containing low, normal, and high concentrations of glucose to determine the relationship between glycolytic flux and methylglyoxal generation and subsequent adduction throughout the glycolytic pathway. Growth in high-glucose media resulted in increased generation of methylglyoxal and overall lower efficiency of glucose utilization as measured by growth rates. Results also show that cells grown in high-glucose media maintain higher glucose flux than cells grown in normal-glucose or low-glucose media. These results demonstrate that cell growth in a hyperglycemic environment results in increased generation of the toxic glycolytic by-product methylglyoxal as a consequence of increased glycolytic flux. We suggest that this mechanism may be relevant to the development of type 2 diabetes mellitus in the hyperglycemic human.

2448 BIOMARKERS OF ACRYLONITRILE EXPOSURE: SECOND-ORDER RATE CONSTANTS FOR THE REACTION OF ACRYLONITRILE WITH THE MOST REACTIVE SITES IN HUMAN HEMOGLOBIN.

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Military and civilian personnel may encounter the challenge of dealing with an exposure to a toxic industrial chemical used in an act of chemical terrorism. Acrylonitrile (AN) is produced in large quantities by the chemical industry and is acutely toxic. Our objective is to define the chemical signatures of AN-adducts in human blood that will allow for the triage of exposed individuals. In this project we have measured the second order rate constants for the reaction of AN with the most reactive sites in human hemoglobin in vitro. Fresh human red blood cell lysates were incubated, under pseudo first-order conditions, with 100mM AN at 37°C. As the reaction progressed, 10μl aliquots of the reaction mixture were quenched at various times in acidified acetone to precipitate the protein, the solvent was removed and the protein was alkylated with iodoacetamide and digested with trypsin. The tryptic digests were analyzed using an Acclera LC System coupled to a LTQ-Orbitrap XL mass spectrometer. Both the appearance of AN-added tryptic peptides and the disappearance of the equivalent unreacted tryptic peptides were monitored. The eleven most reactive sites were monitored and the second-order rate constants for the initial AN-adduction were measured, although multiple adductions at the site are not decoupled. The second-order rate constant measured was 1.0E-3 M-Min-1 for εK16. The increasing order of reactivity at the other sites relative to this lowest site is as follows: εK16(1.0) < βK17(1.2) < εH2O(2.5) < εK11(4.6) < εK7(5.0) < βH9(5.1) < βK59(5.3) < βK86(6.0) < βV1(21.0) < εV123(25.0) < εC93(40.0). As can be seen, the most reactive site for AN-adduction in human hemoglobin was εC93. Its rate of adduction was approximately twice that of the N-terminal valines, which have often been used as a biomarker of AN exposure. Supported by DOD, U.S. Army Medical Research and Material Command, W81XWH-10-2-0143.

2449 POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) IN URINE FROM XUANWEI, CHINA, AS BIOMARKERS OF EXPOSURE TO FUEL SMOKE.

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Xuanwei County has up to 20 times the lung cancer mortality in women compared to the rest of China. Epidemiologic studies have associated indoor combustion of smoky (bituminous) coal with lung cancer risk in Xuanwei. The International Agency for Research on Cancer (IARC) classifies indoor emissions from the household combustion of coal as carcinogenic to humans. The high levels of PAH exposure from smoky coal emissions have been suggested to contribute to the etiology of the extraordinary lung cancer rate in Xuanwei, although a causative relationship has not been established. We hypothesize that PAH biomarkers might be surrogates of fuel PAH exposure in these women. Few studies have reported urinary PAH levels from Xuanwei residents. We used automated headspace solid phase microextraction (HS-SPME) coupled with gas chromatography mass spectrometry (GC-MS) to simultaneously measure 12 two-ring to four-ring urinary PAHs in 0.7-ml aliquots of urine from Xuanwei women. Personal and indoor-air samples were also collected from the participants for measurement of air concentrations of benzo(a)pyrene (BaP) as a surrogate for PAH exposures. A pilot study of 38 samples showed that Xuanwei women in the a priori high-exposure group had significantly higher levels of urinary PAHs than women in the low-exposure group, for 10 of the 12 PAHs. Airborne BaP levels were significantly correlated with those of urinary PAHs, indicating that urinary PAH measurements were indicative of airborne PAH exposures. These data suggest that urinary PAHs can serve as biomarkers of PAH exposure. Here, we report the relationships between urinary PAH levels and the corresponding air BaP levels in a cross-sectional study. The data suggest that PAHs represent a wide range of PAH exposures. Our results support the feasibility of using urinary PAHs to monitor PAH exposure in epidemiologic studies, such as those in Xuanwei.
**2450** ROYENONE-INDUCED OXIDIZED MOLECULAR SPECIES OF CARDIOLIPIN IN HUMAN LYMPHOCYTES: CAN THEY BE USED AS BIOMARKERS OF MITOCHONDRIAL DYSFUNCTION ASSOCIATED WITH PARKINSON’S DISEASE?

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During the last three decades, epidemiological and toxicological studies have provided data that pesticides, including rotenone, contribute to the development of Parkinson’s disease (PD). Circulating lymphocytes are often used to study the pathogenic mechanisms in PD. We reasoned that oxidized molecular species of CL in human lymphocytes will progressively accumulate in the course of exposure to rotenone. We isolated lymphocytes from human blood obtained from the Central Blood Bank. Using an oxidative lipodomics approach based on 2D-LC/ESI-MS, we found that CL in human lymphocytes was represented mainly by readily oxidizable species containing polyunsaturated fatty acid residues. The contents of CL molecular species with polyunsaturated fatty acid residues - C18:2, C20:4 and C22:6 - were as high as 67.7%, 24.7% and 5.4%, respectively. Only a small amount (2.2% of total CL) of non-oxidizable CL molecular species containing C18:1 was found in human lymphocytes. Detailed structural characterization of oxidized CL molecular species in lymphocytes treated with rotenone (250 μM, for 18h) revealed the presence of oxidized CL molecular species. Their characterization demonstrated that oxidized CL was represented by a profile containing 13 different hydroxy- and hydroperoxy-molecular species. Further studies of specificity of rotenone induced CL oxidation in rat midbrain and correlations with the unique stereo-specific molecular species of CL formed in mitochondria of human and rat lymphocytes in response to rotenone exposure will determine the extent to which these profiles of lymphocyte CL oxidation may lead to reliable biomarkers of mitochondrial dysfunction associated with neurodegenerative disorders. Supported by NIOSH OH008282; NIH U19 AI068021, HL70755, HL094488, ES020693.


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We examined associations between biomarkers of allergy and inflammation, indoor environment in dwellings and incidence and remission of symptoms included in the sick building syndrome (SBS) and changes of the home environment in 452 adults followed from 1992 to 2002 within the Uppsala part of the European Community Respiratory Health Survey (ECRHS). The 10-year incidence (onset) of general, mucosal, and dermal symptoms was 12.7%, 25%, and 7.7%, respectively. Damness or indoor moulds at baseline was a predictor of incidence of general (RR=1.98), mucosal (RR=2.28) and dermal symptoms (RR=1.91). Females had higher incidence of general (RR=1.74) and mucosal symptoms (RR=1.71). Indoor painting increased incidence of general symptoms (RR=1.62). Bronchial hyperresponsiveness (BHR), eosinophil counts in blood, total IgE and eosinophil cationic protein (ECP) in serum at baseline were predictors of incidence of SBS. At follow up, BHR, total IgE and C-reactive protein (CRP) were associated with increased incidence of SBS. Moreover, subjects with doctors' diagnosed asthma at baseline had a higher incidence of general symptoms (RR=1.65) and mucosal symptoms (RR=1.97). In conclusion, female gender, dampness or indoor moulds, indoor painting and biomarkers of allergy and inflammation were associated with a higher incidence of SBS symptoms, in particular mucosal symptoms. The association between incidence of SBS symptoms and clinical biomarkers of allergy and inflammation suggests a common aetiology between inflammatory diseases such as asthma and rhinitis and SBS.

**2451** DETERMINATION OF ENDOGENOUS AND EXOGENOUS ACETALDEHYDE-DERIVED DNA ADDUCTS.

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Acetaldehyde (AA) is a ubiquitous chemical that is produced endogenously during normal metabolic activity; it is ethanol's primary metabolite and is used in a wide variety of industrial activities. AA is a known animal carcinogen, causing squamous cell carcinomas and adenocarcinomas in nasal tissue of rats and laryngeal carcinoma in hamsters following inhalation exposures. AA is highly reactive and can adduct to both proteins and DNA forming DNA adducts and DNA-protein cross-links. N1-Ethylidene-dG is the primary DNA adduct formed following AA exposure to DNA; while stable in DNA, it is unstable following digestion to nucleosides. To address the instability, DNA was reduced using NaCNBH3 to convert N1-ethyldiene-dG to the more stable N1-ethyl-dG adduct. As AA exposures are a result of endogenous and exogenous AA, a sensitive and selective method combining HPLC fraction collection, followed by detection and quantitation using nano-ESI-MS, was developed and validated. DNA was extracted, reduced with NaCNBH3 and enzymatically digested prior to HPLC fractionation and nano-ESI-MS/MS analysis. The limit of detection was 10 amol on column and the limit of quantitation was 20 amol on column. The method was validated with accuracy, precision, recovery, and matrix effects assessed. Endogenous N1-ethyl-dG was quantitated at 3.5 ± 0.1 adducts per 107 dG in calf thymus DNA. This method will be used to determine endogenous and exogenous N1-ethyl-dG adducts following [13C2]-AA exposure in cell culture. TK6 human lymphoblast cells (~2 x 107 cells/exposure) were exposed to [13C2]-AA for 12 hours at concentrations ranging from 0.00005 – 2.0 mM AA, along with negative controls. Following completion of the exposure, the media was removed, cells washed and frozen at -80°C prior to DNA extraction. The method will be used to determine the exposure-response relationships between endogenous and exogenous AA-DNA adducts following [13C2]-AA exposure.

**2453** VALIDATION OF A NONANTIBODY METHOD FOR DETECTION OF SERUM AFLATOXIN B1-LYSINE ADDUCT IN FISCHER 344 RATS.

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Serum aflatoxins B1-lysine adduct (AFB-lys) is a validated biomarker for studying human AF exposure. Recently we have developed a non-antibody based method for rapid detection of serum AFB-lys adducts in human populations in developing world. To further validate this method, male Fischer 344 rats were orally administered AFB via different dosing regimens and serum AFB-lys levels were assessed using this method. The single dosing regimen (SDR) included a control, 50, 250, and 1000μg AFB/kg body weight groups and blood was collected at 2h, 1, 3, 5, and 7 days post treatment. The repeated dosing regimen (RDR) included a control, 5, 10, 25, and 75μg AFB/kg body weight groups and blood was collected at 1, 2, 3, 4, and 5 weeks. Serum was prepared and AFB-lys was analyzed. In the SDR, AFB-lys adduct was detectable at the maximal level at 2h post treatment with 1.68 ± 0.22 ng AFB-lys/mg albumin in 50μg AFB/kg group, followed by 61% (0.66 ± 0.03), 65% (0.58±0.12), 83% (0.28±0.02), and 92% (0.14 ± 0.02) decreases in 1, 3, 5, and 7 days, respectively. Similar pattern was found for 250 and 1000μg AFB/kg groups. In the RDR, the prolonged exposure for 5-week increased serum AFB-lys levels to 0.54 ± 0.05, 1.06 ± 0.08, and 3.00 ± 0.20 ng AFB-lys/mg albumin at 5, 10, and 25μg AFB/kg groups, respectively. However, the maximal level of AFB-lys (9.06 ± 0.84 ng AFB-lys/mg albumin) was found at 2-week after repeated exposure to 75μg AFB/kg, followed by a 4% (8.67 ± 0.90), 12% (7.96 ± 0.31), and 22% (7.06 ± 0.43) decreases at 3-, 4-, and 5-week, respectively. These results indicated either saturation of the adduct level with exposure to this dose or impaired liver function of enzymatic activation of AFB in test animals. Excellent correlations between AFB administered and AFB-lys adduct level were found. Taken together, results of this study demonstrate the validity of our method for detecting serum AFB-lys adduct.

**2454** IDENTIFICATION AND CHARACTERIZATION OF INTRACELLULAR PROTEINS IN LNCAP CELLS EXPOSED TO BIZ-2.

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Prostate cancer is the leading cause of death in men in the United States. Currently, there are no known cures for this health problem. Kola Nut (Cula acuminate) or Bizzy Nut is a plant that contains androgenic-type hormonal activity. Work from
our lab has shown that Biz-2, an ether extract of Bizzy nut, causes increases in se-
ccretion of prostatic proteins such as PSA from LNCaP prostate cancer cell. In addi-
tion, Biz-2 has been shown to inhibit the growth of different type of prostate
cells. An understanding of protein secretion by LNCaP cells in the presence and ab-
sence of Biz-2 will allow us to determine the role of Biz-2 in the modulation of
prostate cancer cells. Our hypothesis is that Biz-2 changes the type of and amount
of proteins that are secreted by LNCaP cells. To test our hypothesis, we analyzed in-
tercellular proteins obtained from LNCaP cells by ion exchange chromatography
followed by MALDI-TOF-MS. Our analysis shows different population of pro-
teins in each ionic exchange fraction. We were able to show that other proteins in
addition to PSA were affected by Biz-2 exposure. Our results suggest that ion-ex-
change chromatography coupled to MALDI-TOF-MS is a good technique to de-
termine quantitative difference between the amounts of protein being produced in
response to Biz-2 in prostate cells.

Biomarkers can be used to study metabolic responses to environmental exposures,
general health, and disease progression. However, "biobanking" or biological sample
storage associated with large-scale studies can be expensive in terms of facility
space, equipment, sample tracking and financial cost. As part of a National
Children’s Study sub-study, we are interested in monitoring metabolic profiles to
detect environmental exposures and potential health impacts, as well as to study
inter- and intra-individual variability. To develop cost-effective analytical methods
for qualitative and quantitative measurements of endogenous and exogenous sub-
stances in biospecimens, we proposed creating a "virtual biobank". By generating
multiple metabolic profiles, we could capture the global profiles for each
biospecimen and store the data electronically for future queries. A smaller aliquot
of the sample could be stored or when the method is completely refined, the samples
could be destroyed. We used a liquid chromatography-quadrupole time-of-flight
(LC-QTOF) metabolomics approach to enable biomarker discovery and screening
for pesticides and/or xenobiotics exposure. Initial evaluation of our real-time ana-
lytics approach used urine collected from 3 adult-child pairs over 3 agricultural sea-
sons on 2 sample collection days. In our preliminary data for metabolic profil-
ing, we found differences between child and parent samples and to exposures
during the three agricultural seasons. Development of metabolomic databases as
well as a comprehensive screening methodology will provide rapid sample analysis
and the potential to move from physical storage of biospecimens to creating a
metabolite data repository or a "virtual biobank". Acknowledgements: NICHD HHSHN267200700023C, NIEHS 5P01ES009601 and USEPA RD83451401.

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PS 2455 CREATING VIRTUAL BIOBANKING USING A GLOBAL
METABOLOMICS APPROACH.

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One of the most important independent detoxification pathways of cyanide in bio-
logical systems is the reaction with cystine to produce 2-aminothiazoline-4-car-
boxylic acid (ATCA). Sub-lethal dose (4 mg/kg body weight) of potassium cyanide
(KCN) was injected subcutaneously into rat, and the plasma and organ distribu-
tions of the formed ATCA were studied. At this sub-lethal dose of KCN, ATCA
concentration was not significantly increased in the plasma samples, however, it
was found significantly increased in liver samples. These results suggested that ATCA
might not be a good diagnostic biomarker for cyanide exposure in plasma; however,
liver could serve as the right organ for the detection of ATCA in post mortem ex-
adominations involving cyanide exposure. For inter-laboratory and forensic settings.
To determine the residence time in the circulation and the phar-
macokinetics for this cyanide metabolite, it was intravenously injected to rats.
ATCA was extracted from plasma samples by solid phase extraction (SPE) and mol-
ecularly imprinted polymers stir bar sorption extraction (MIP-SBSE). Detection
and quantification of ATCA were achieved by using liquid chromatography-tan-
dem mass spectrometry (LC-MS/MS). It was found that intravenously injected
ATCA concentration was quickly decreased to half within 2.5 hours in the rat sys-
ram. However, after 2.5 hours, the concentration of ATCA in plasma stayed con-
stant at least 5 folds above endogenous level for more than 48 hours. This finding
of the ATCA residence time in plasma can be used for the evaluation of ATCA di-
agnostic and forensic value as a biomarker for cyanide exposure.

PS 2457 PLASMA RESIDENCE TIME AND ORGAN
DISTRIBUTION DETERMINATION OF THE CYANIDE
METABOLITE 2-AMINOTHIAZOLINE-4-CARBOXYLIC
ACID IN RAT SYSTEM.


It is reported that aluminum is a relatively safe metal to humans. However, there are
also some reports that aluminum has the toxicity to lung, nerve, bone, and kidney.
There are few reports that the associations between aluminum concentration in
blood or urine and clinical findings were studied, except for the association with
nervous findings. In this study, we measure the aluminum concentration in urine in
casting workers and investigate the relationships between the urinary aluminum
concentration and the preclinical findings. Nineteen healthy casting workers, who
did not take any medications, participated in this study. They did not show any sig-
nificant findings in the medical examination, in relation to aluminum. We col-
lected urine and blood samples from them. Aluminum in urine was analyzed using
inductively coupled plasma analysis. In addition, in order to check the preclinical
findings, we measured KL-6, SP-D, TRCP-5b, IL-6, and IL-8 in blood and δ-ALA
and β2-microglobulin in urine. These are intended to be lung, bone, kidney and in-
flammation markers. The aluminum concentration in urine ranges from 0.64μg/dl to
0.97μg/dl, and mean concentration is 0.81±0.14μg/dl. Preclinical findings are almost within reference values, except for a few data. There are no sig-
nificant relationships between aluminum concentration and preclinical findings.
There is a report that the mean concentration of aluminum in a non-exposed pop-
ulation, who did not use antacid drugs, was 0.33μg/mL in urine and the upper ref-
ference limit for aluminum in a healthy, non-exposed population was estimated to
be 0.6mg/mole in urine. Our results showed much higher urinary aluminum concen-
tration than that in non-exposed population. On the contrary, there is no signifi-
cant difference between the urinary aluminum concentration and the preclinical

PS 2458 A STUDY OF EXPOSURES AND PRECLINICAL
FINDINGS AMONG THE ALUMINUM CASTING
WORKERS.

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Shimotsuke, Japan and 2Division of Environmental Toxicology, Jichi Medical
University, Shimotsuke, Japan.

It is reported that aluminum is a relatively safe metal to humans. However, there are
also some reports that aluminum has the toxicity to lung, nerve, bone, and kidney.
There are few reports that the associations between aluminum concentration in
blood or urine and clinical findings were studied, except for the association with
nervous findings. In this study, we measure the aluminum concentration in urine in
casting workers and investigate the relationships between the urinary aluminum
concentration and the preclinical findings. Nineteen healthy casting workers, who
did not take any medications, participated in this study. They did not show any sig-
nificant findings in the medical examination, in relation to aluminum. We col-
lected urine and blood samples from them. Aluminum in urine was analyzed using
inductively coupled plasma analysis. In addition, in order to check the preclinical
findings, we measured KL-6, SP-D, TRCP-5b, IL-6, and IL-8 in blood and δ-ALA
and β2-microglobulin in urine. These are intended to be lung, bone, kidney and in-
flammation markers. The aluminum concentration in urine ranges from 0.64μg/dl to
0.97μg/dl, and mean concentration is 0.81±0.14μg/dl. Preclinical findings are almost within reference values, except for a few data. There are no sig-
nificant relationships between aluminum concentration and preclinical findings.
There is a report that the mean concentration of aluminum in a non-exposed pop-
ulation, who did not use antacid drugs, was 0.33μg/mL in urine and the upper ref-
ference limit for aluminum in a healthy, non-exposed population was estimated to
be 0.6mg/mole in urine. Our results showed much higher urinary aluminum concen-
tration than that in non-exposed population. On the contrary, there is no signifi-
cant difference between the urinary aluminum concentration and the preclinical
markers. Therefore, although the excretion of aluminum in urine increased in cast-
ing workers, it is demonstrated that aluminum is not harmful to humans, at least in case that aluminum in urine is below 4μmol/L.

**2459 STRAIN-DEPENDENT RESPONSES OF SUBACUTE EXPOSURE TO TRICHLOROETHYLENE IN MULTIPANEL STRAIN OF INBRED MOUSE MODEL.**

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Trichloroethylene (TCE), a useful industrial solvent and well-known environmental pollutant, is classified as “probably carcinogenic” in humans since chronic exposure to TCE can cause tumors at the liver and kidney in rodents and their metabolites are qualitatively similar to one in humans; however, there are many knowledge gaps about the determining factors in susceptibility of health outcomes and carcinogenesis after exposure to TCE. The objective of the present study was to explore the different responses after the exposure to TCE for 4-week, and consequently to identify more susceptible strains. In order to do so, we determined those TCE metabolites in the animal tissues, clinical chemistry, histopathological assay and weight of target organs. TCE administration was performed with corn oil as vehicle by gavage to male mice of seven strains. Hepatocellular injury and nephrotoxicity were quantified by alanine transferase (ALT) and beta-2-microglobulin (b2-MG). The necrosis of solid tissues was observed in hematoyxin and cosin staining. The cell proliferation and apoptosis in solid tissues were investigated by proliferating cell nuclear antigen staining and terminal deoxynucleotidytransferase-mediated dUTP nick end labeling assay. Considerable dose-dependent increase of TCE metabolites in the serum were found in most of strains after TCE exposure for 4 weeks. Regarding the biological effect, we found significant increase in the ratio of liver to body weight for 4 strains, and the ratio of kidney to body weight significantly increase in A/J strain (p<0.05). To the contrary there were neither significant increase of ALT and b2-MG in most of strains, nor noticeable signs in the pathological examination for target organs. While confirmation are required by further studies, our finding suggests that some clues in dose-response and mechanisms of TCE-induced toxicity associated with metabolism and genetic factors that may define susceptibility and may provide useful information for TCE risk assessment in humans.

**2460 APPLICABILITY OF HIGH-THROUGHPUT ‘OMIC TECHNOLOGIES IN EPIDEMIOLOGICAL STUDIES USING BIOBANK SAMPLES.**

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The combined use of more traditional biomarkers and the new ‘omics’ technologies in human population studies, can prove to be a powerful tool for the discovery of a new generation of biomarkers of exposure and/or effect that can help to elucidate disease causation. However, the application of ‘omics technologies in epidemiological studies raises practical questions in relation to sample suitability, as many biological samples have been collected decades ago without anticipation of the demands of these techniques. In the context of the European FP7 project EnviroGenomarkers (www.envirogenomarkers.net), a range of ‘omics technologies is being applied for the analysis of human blood samples from two biobanks (EPIC-Italy and the Swedish NSHDS). A validation study was conducted to identify minimum criteria for sample collection, for microarray-based transcriptomics, CpG island methylation, LC-MS/MS based metabolomics and wide-target proteomics. Fresh blood samples from volunteers were used to evaluate the influence of different anti-coagulants, bench times prior to processing, and storage temperatures employed in the two participating biobanks. Application of a newly developed approach for the isolation of RNA demonstrated that RNA yield and quality were adequate for gene expression analysis. However, principle component analysis on transcriptomics, proteomics and metabolomics data showed that samples with longer bench times differed from samples directly processed and that a maximum bench time of 4 to 8 hours is preferred. Furthermore, proteomics analysis showed that the use of anticoagulants should be standardized as much as possible within the biobanks, whereas for technical reasons EDTA should not be used for GC-MS. Applying such selection criteria and validated analytical procedures applied in this pilot study, we are able to obtain good quality ‘omics data from biobank samples.

**2461 PRESENCE OF AFLATOXIN B1 IN DIETS OF DAIRY CATTLE AND AFLATOXIN M1 IN RAW MILK.**


The demographic explosion has triggered a high food demand for human and animal populations, changing the traditional production systems into intensive production systems. This makes the massive production of grain and cereal to be stored hastily, resulting in conditions that could favor fungi growth which might produce mycotoxins. According to numerous reports, aflatoxin M1 (AFM1) is the most frequently detected aflatoxin in milk and dairy products in several countries. AFM1 is a hydroxylated metabolite of aflatoxin B1 which is considered a powerful carcinogenic to humans (IARC, group 1). However, the possibility of AFM1 producing cancer in humans is also considered (IARC, group 2B). AFM1 remains intact even after boiling or pasteurization processes. The aim of our study was to assess AFM1 contamination in raw milk during the winter season. The milk and silage samples were obtained from small dairy producers. Samples were analyzed for AFM1 and AFB1 via ELISA and The ROSA® Aflatoxin (Quantitative) tests respectively. The results show that 27% of the milk samples had detectable levels of AFM1 (> 5 ppb). In some cases, samples exceeded the AFM1 approved limit according to the Codex Alimentari and Norma Oficial Mexicana. Moreover, all milk samples positive to AFM1 came from cattle that fed on silage with AFB1 detectable levels. These results confirm that the source of AFM1 milk contamination was the AFB1 contaminated silage. Also, results suggest an early and unnoticed exposure to AFM1 which could affect public health, especially children.

**2462 BLOOD MERCURY SPECIATION IN BRAZILIAN RIPARIAN POPULATIONS.**

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Mercury (Hg) is one of the most toxic environmental pollutants. It exists primarily in three forms: elemental mercury (Hg0), or metallic mercury, inorganic mercury (Hg-i), particularly mercuric chloride and organic mercury (Hg-o), mainly represented by methylmercury (MeHg) and ethylmercury (Et-Hg), and the organic forms of mercury are more toxic than the inorganic ones. Then, it is very important the development of simple and fast methods for Hg speciation (Hg-i, MeHg, EtHg) in biological samples. Then, the aims of this work were to evaluate analytical method for Hg speciation in blood by using LC coupled to ICP-MS and comparison with the use of GC coupled to ICP-MS. For the by using HPLC-ICP-MS the extraction of Hg species was carried out with the use of ultrasonic energy. For the speciation methodology with GC-ICP-MS the extraction was carried out with the use of microwave-assisted extraction. Validation of the proposed methods were evaluated based on the analysis of the SRM NIST 906 and ordinary blood samples collected from Brazilian living in the Brazilian Amazon exposed to mercury. The proportion of Hg species (MeHg:Hg) in blood samples was 94.6:5.4 MeHg:56.3μg/L, Hg-i 3.9μg/L, Et-Hg not detected, n=26). The Hg species concentrations confirm the Hg exposition route from Brazilian Riparian populations (by eating fishes which have high MeHg concentration). No statistical difference was observed when compared the obtained results of Hg species by using HPLC-ICP-MS and GC-ICP-MS.

**2463 ASSOCIATED FACTORS WITH MERCURY EXPOSURE IN KOREAN ADULTS.**

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Mercury (Hg) is a non-essential toxic metal which is widely distributed in the environment. This study was performed to evaluate the factors associated with Hg exposure in Korean adults who have not been exposed to Hg occupationally. Exposure to Hg was evaluated with total Hg concentration in blood. The study subjects consisted of 1,024 healthy adults older than 19 years of age. We collected information about demographic characteristics, dietary habits, occupational history, smoking habit, alcohol consumption, duration of residence, and medical history.
We conducted medical examination for all subjects, and analyzed the concentrations of blood Hg for 987 subjects. The geometric mean of blood Hg was 4.35 μg/L, it was higher in old age, men, current or ex-smokers, drinkers, and low education than in young age, women, non-smokers, non-drinkers, and high education, respectively. Individuals who consumed seafood within 3 days prior to blood collection showed a higher mean blood Hg level than those who did not. After covariate adjustment, age, gender, alcohol drinking, size of residence area, and seafood intake of within 3 days of sampling were statistically significant contributing factors to the blood Hg. These findings suggest that blood Hg are associated with various socio-demographic factors, lifestyles and diet habit such as fish consumption. The results of this study could be useful as a fundamental data for the identification of a possible high exposure group to Hg, risk assessment and risk communication with them.

### 2464 THE BLOOD MERCURY CONCENTRATION AND THE PROPORTION OF TOTAL/METHYLMERCURY AND RELATED FACTORS IN KOREAN ADULTS.

Y. S. Hong¹, B. G. Kim¹, C. H. You¹, E. M. Jo¹, G. Y. Kim¹, B. C. Yu¹ and D. S. Kim². ¹Preventive Medicine, Dong-A University, Busan, Republic of Korea, ²Environmental Epidemiology, National Institute of Environmental Research, Incheon, Republic of Korea. Sponsor: J. Jung.

The purpose of this study is to evaluate the relationship between the fish consumption and the blood methylmercury/total mercury concentration in Korean adults by measuring the mercury concentration in blood, directly. The study population consisted of 400 adults aged 20 or older from 30 subareas in Busan, Ulsan and Gyeongbuknam-do in Korea from August to October, 2010. We recruited the allocation of regions, age groups, gender, alcohol drinking, size of residence area, and seafood intake groups by sex, male, female, and age groups (20s, 30s, 40s, 50s, and 60s). The geometric means of the total mercury and methylmercury concentration in blood were 5.27 μg/L and 4.06 μg/L. The proportion of methylmercury/total mercury is 78.53%. The methylmercuryconcentrination coastal areas (4.26 μg/L) was less than that in inland areas (5.32 μg/L). The methylmercuryconcentration in males (4.68 μg/L) was significantly higher than that in females (3.52 μg/L) and the proportion of methylmercury/total mercury in males had the increasing trends with the rises of total mercury level in blood, significantly. Multiple regression analysis showed that the blood methylmercury level was associated with fish consumption (p < 0.001) and drinkingstatus (p = 0.002), but the proportion of methylmercury/total mercury had significant variables. It was first report about the relationship between the blood total / methylmercury concentration and related factors in Korea. Our findings suggested that fish consumption, as well as gender difference, alcohol consumption affect the methylmercury level. Yet, the mechanism of pathology has not been clarified. Thus, Additional studies are needed of explaining the biological and lifestyle differences in the risk of mercury.

### 2465 EFFECT OF MERCURY ON CYTOCHROME P450A1, 1A2, AND 1B1 IN C57BL/6 MICE AT DIFFERENT EXTRAPEPTHELIAL TISSUES.


We have previously demonstrated that Hg²⁺ induces the constitutive and inducible expression of Cyp1a1 in murine hepatoma Hepa1c1c7 at the mRNA, protein, and catalytic activity levels. However, our efforts and along with other published studies were mainly conducted at the in vitro level and were mainly concerned with hepatic effects of Hg²⁺. Thus, the objective of the current study was to investigate the effect of Hg²⁺ in the absence and presence of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the AhR-regulated cytochrome P450 (Cyp) genes in the kidney, lung and heart in C57BL/6 mice. For this purpose C57BL/6 mice were treated with a single intraperitoneal injection of Hg²⁺ (2.5 mg/kg) in the absence and presence of TCDD (15μg/kg). Our results demonstrate that, at the mRNA levels, Hg²⁺ alone significantly induced the constitutive expression of Cyp1a1 mRNA in the lung and heart but not in the kidney. Hg²⁺ alone was also able to induce the Cyp1a2 and Cyp1b1 mRNA in both kidney and heart whereas; it decreased their expression in the lung. Interestingly, Hg²⁺ decreased the TCDD-mediated induction of Cyp1a1 and Cyp1b1 in the kidney while it increased Cyp1a2 in the lung. At the protein levels, Hg²⁺ significantly increased the constitutive Cyp1a1 and Cyp1b1 proteins. However, Hg²⁺ decreased the TCDD-mediated induction of both enzymes in the kidney, while potentiating theirs levels in the lung. At the catalytic activity levels, Hg²⁺ alone significantly induced the Cyp1a2 and Cyp1b1 activities in the kidney while inhibited the Cyp1a1 in the lung. On the contrary, Hg²⁺ inhibited the TCDD-mediated induction of both enzymes in the kidney. These results demonstrate that Hg²⁺ modulates the constitutive and inducible AhR-regulated Cyp genes in a tissue- and Cyp-specific manner. Furthermore, our results indicate more complex regulation of these genes at the in vivo level that warrants further investigation.

### 2466 INCREASED EXPRESSION OF AQUAPORIN-4 BY METHYLMERCURY IN THE BRAIN OF THE COMMON MARMOSET.

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In an acute model of methylmercury (MeHg) exposure using common marmosets, the gene expression of aquaporin (AQP1, AQP4 and AQP11) was examined to understand the relationship between MeHg exposure and AQP expression in the brain. MeHg (1.5 mg Hg/kg body weight/day; p.o.) was given to three marmosets for 14 days, followed by no administration for 14 days. All marmosets in the MeHg-administered group showed slight akinesia immediately before sacrifice. In the frontal lobe, occipital lobe, and cerebellum, the mean total mercury concentration in the MeHg-administered group was 26.7, 31.4, and 22.6 μg/g, respectively. Slight apoptosis was observed in the occipital lobe of the MeHg-administered group. Glial fibrillary acidic protein (GFAP) expression increased in the brains of the MeHg-administered group measured by immunohistochemistry (HIC) and mRNA expression, and the highest activation of GFAP was observed in the occipital lobe. IHC revealed the increase of AQP1 expression in the occipital lobe of the MeHg-administered group, indicating microglial activation by MeHg. Quantitative real-time RT-PCR analysis showed up-regulation of the AQP4 mRNA in the sampled areas of the frontal lobe, occipital lobe, and cerebellum of MeHg-treated group. The AQP4 protein expression of marmosets with western blot analysis in the occipital lobe and the cerebellum of the MeHg-administered group increased significantly, but no obvious up-regulation of AQP4 was observed in the frontal lobe. A significant difference in the expression of AQP1 and AQP11 in the frontal lobe, occipital lobe, and cerebellum was observed between control and MeHg-administered groups from these results, AQP4 expression in occipital lobe and cerebellum was stimulated by MeHg-exposure, and it would be correlated with increase of activated astrocytes.

### 2467 DIFFERENCES IN METHYLMERCURY-INDUCED DOPAMINERGIC NEUROTOXICITY IN WILDTYPE, SKN-1 KO, AND MUTANT PARKIN CAEVRHABITIS ELEGANS.

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Mercury is a persistent environmental contaminant that exerts its toxic effects on the nervous system through molecular mechanisms that remain unknown. Epidemiological studies have pointed to the contribution of methylmercury (MeHg) to dopamine neuron vulnerability and the predisposition to Parkinson’s disease (PD). Nr2f2, a phase II antioxidant transcription factor, has been shown to be involved in MeHg neurotoxicity. Overexpression of Nrf2 inhibits MeHg-mediated cell death and deficits in Nrf2 leave cells vulnerable to MeHg. We hypothesize that skn-1, the Caenorhabditis elegans (C. elegans) orthologue of mammalian Nr2f2, is an important factor in MeHg-induced dopaminergic neurodegeneration. Additionally, we tested the vulnerability of nematodes with mutant parkin, a juvenile onset PD gene, to MeHg to delineate the contribution of MeHg to PD. We knocked down skn-1 (skn-1 KO) in C. elegans and exposed N2 (control), skn-1 KO, and parkin mutant worms to 0, 10, 20 and 30μM MeHgCl for 30 minutes following synchronization. Our data suggest that skn-1KO (LD50=17μM) and parkin mutant worms (LD50=15μM) are more sensitive to MeHg than controls (LD50=24μM). Dopaminergic neuronal morphology was observed via fluorescent analysis. Presence of puncta was observed at 20μM MeHg. Decreased longevity was observed at high doses in all strains. The amount of MeHg, as measured by ICP-MS, correlated with expression of anti-α and anti-β (C. elegans LAT1 homologues) transporters and was significantly different across strains. Transcripts of skn-1 and GST-4 were elevated in the N2 and parkin mutant strains following exposure and reactive oxygen species were produced at the 20μM dose in all strains. Dopamine content, measured via HPLC, was not significantly different between strains at the 20μM dose immediately following treatment but strain differences were apparent 48hrs post treatment. Our data suggest that genetic background is an important determinant of mechanism of toxicity. ES R01 07331.
Methylmercury is an environmental pollutant that causes central nervous system disorders. However, the details of the mechanism underlying methylmercury toxicity are unclear. In order to elucidate the molecular mechanism involved in methylmercury-induced disorders, we performed DNA microarray analysis of the cerebellum of methylmercury-treated mice. Methylmercury chloride was administered subcutaneously to male C57BL/6 mice at 10 mg/kg/day for 7 consecutive days, and cerebellum was excised 24 hr after the last injection for DNA microarray analysis. The expression levels of 21 genes increased two-fold or more in response to methylmercury. The genes with methylmercury-induced elevated expression levels included many genes encoding proteins involved in inflammatory reactions, including those encoding the chemokines CCL2, CCL4, CCL7, CCL9 and CCL12. We next examined effects of methylmercury on expressions of CCL2, CCL4, CCL7, CCL9 and CCL12 in various organs of methylmercury-treated mouse by using the quantitative PCR. Not only in cerebellum but also in cerebrum, expression levels of CCL2, CCL4, CCL7, CCL9 and CCL12 were increased significantly by methylmercury treatment. In kidney of methylmercury-treated mouse, expression levels of four chemokines except CCL4 were markedly increased compared to control group. However, in liver and spleen, expression levels of CCL2, CCL4, CCL7, CCL9 and CCL12 were not changed by methylmercury treatment. Therefore, methylmercury is thought to enhance expressions of some chemokines in the brain and the kidney in mouse. However, expression of CCL4 might be increased by methylmercury specifically in the brain. These results indicate that CCL4 might be a specific target protein responsive to methylmercury in the brain.

Clinical studies show that patients with CoCr implants have higher levels of Co and Cr ions in their blood and urine up to 3 years post-surgery and recent studies indicate that CoCr implant patients exhibit a higher incidence of bladder cancer. However, the potential carcinogenic effects of cobalt and chromium in bladder cells remain unknown. Cr(VI) is well known human lung carcinogen and its mechanism involves the generation of aneuploid cells. Consistent with this mechanism, clinical studies show that exfoliated urine cells in CoCr implant patients exhibit high levels of aneuploidy. The purpose of this study is to investigate the effects of Co and Cr on chromosome stability in primary human urothelial cells. We found that chronic exposure to Cr(VI) induced aneuploidy in human urothelial cells. Exposure to 5 μM Cr(VI) for 120 h induced aneuploidy in 38% of metaphases with 23% hyperdiploid metaphases, 6% hypodiploid metaphases and 9% tetraploid metaphases. Chronic exposure to Cr(VI) also induced genotoxicity with 5 μM Cr(VI) for 120 h inducing 15% metaphases with damage and 17 total aberrations in 100 metaphases. These data suggest that Cr(VI)-induced genotoxicity and aneuploidy may be involved in the increased risk of bladder cancer in patients with CoCr orthopedic implants. Future work is aimed at investigating the effects of Co alone and Co and Cr together in human urothelial cells. This work is supported by NIEHS grant ES016893 (J.P.W.) and ARO grant # 911NF-09-1-0296 (J.P.W.)
Vanadium compounds are used in industrial applications including production of high-strength steel and catalysts. Vanadium can exist in many different oxidation states with (0), (+2), (+3), (+4) and (+5) being the most common. Vanadium compounds also vary widely in their reactivity and solubility in water and simulated lung fluid. A series of 4-hour acute nose-only inhalation toxicity studies were conducted in mice and rats as first steps to fulfill toxicity data gaps for selected vanadium compounds. Three vanadium compounds (powders) of various valence states (V2O3, V2O4, and V2O5 and vanadyl sulfate (VOSO4)) were tested. Groups of male and female animals (rats and mice) were sequentially exposed to a series of aerosols (MMA: 1.81 to 2.77 μm) concentrations beginning at 2.0 mg/L, followed by 1.0, 0.5 and/or 0.05 mg/L, based on mortality results of preceding exposures. Body weights were obtained periodically and all animals were observed for mortality and signs of toxicity during the exposure and throughout the 14-day post-exposure observation period. All animals were subjected to a necropsy. The lung was the target organ for all vanadium compounds tested. In addition, this series of acute studies established the relationship between vanadium valence state and acute inhalation toxicity in male and female rats and mice; higher valence states were more acutely toxic. Although repeated-inhalation exposure data are available for V2O5, such data are lacking for lower valence vanadium compounds. Future studies will focus on developing repeated-exposure inhalation data for lower-valence compounds (e.g. V2O3).

Selenium (Se) and Tellurium (Te) are being used in increasing amounts in industry and agriculture. While Se is an essential trace element for selenium dependent enzymes, Te has not been found to have any physiologic role in human beings. Past research has reported that an excess of selenium can be neurotoxic. Previous work in our laboratory has demonstrated that Te compounds, diphenyl diselenide (DPDS) and tellurium tetrachloride (TeCl4) cause cytotoxicity in rat hippocampal astrocytes. The purpose of the present study was to evaluate the toxic effects of diphenyl diselenide (DPDS) and selenium tetrachloride (SeCl4), in astrocytes and to assess the status of mitochondrial respiration in cells treated with Se and Te compounds. Astrocytes were treated with DPDS at concentrations ranging from 0.2 to 125 μM and with SeCl4 at concentrations ranging from 0.2 μM to 250 μM. Significant decreases in cell viability were observed in concentrations of 0.9 to 125 μM of DPDS and in concentrations ranging from 1.9 to 250 μM with SeCl4. The LC50s of both compounds was found to be 7.8 μM. The LC50s of DPDT and TeCl4 have previously been documented to be 62.5 μM. Caspase activities were assessed upon exposure to Se compounds. Significant increases in caspase 8 activity were observed at the 7.8 μM and 15.625 μM concentrations with both Se compounds. Significant increases in caspase 9 activity were observed at the 62.5 and 125 μM SeCl4 concentrations and significant decreases in caspase 9 activity were observed in cells treated with the same concentrations of DPDT. The activities of complexes I and II of the mitochondrial respiratory chain were assessed with an ELISA assay. Significant decreases in activities of complex I (NADH Dehydrogenase) and complex II (succinate dehydrogenase) were observed at concentrations of 7.8 μM and 15.625 μM for Se compounds and 62.5 and 125 μM for Te compounds. These results suggest that Se and Te compounds cause apoptosis in astrocytes and inhibition of mitochondrial complex I and II.

**2476 SELENIUM AND TELLURIUM COMPOUNDS CAUSE INHIBITION OF MITOCHONDRIAL COMPLEX I AND II IN RAT HIPPOCAMPAL ASTROCYTES.**

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Selenium (Se) and Tellurium (Te) are being used in increasing amounts in industry and agriculture. While Se is an essential trace element for selenium dependent enzymes, Te has not been found to have any physiologic role in human beings. Past research has reported that an excess of selenium can be neurotoxic. Previous work in our laboratory has demonstrated that Te compounds, diphenyl diselenide (DPDT) and tellurium tetrachloride (TeCl4) cause cytotoxicity in rat hippocampal astrocytes. The purpose of the present study was to evaluate the toxic effects of diphenyl diselenide (DPDS) and selenium tetrachloride (SeCl4), in astrocytes and to assess the status of mitochondrial respiration in cells treated with Se and Te compounds. Astrocytes were treated with DPDS at concentrations ranging from 0.2 to 125 μM and with SeCl4 at concentrations ranging from 0.2 μM to 250 μM. Significant decreases in cell viability were observed in concentrations of 0.9 to 125 μM of DPDS and in concentrations ranging from 1.9 to 250 μM with SeCl4. The LC50s of both compounds was found to be 7.8 μM. The LC50s of DPDT and TeCl4 have previously been documented to be 62.5 μM. Caspase activities were assessed upon exposure to Se compounds. Significant increases in caspase 8 activity were observed at the 7.8 μM and 15.625 μM concentrations with both Se compounds. Significant increases in caspase 9 activity were observed at the 62.5 and 125 μM TeCl4 concentrations and significant decreases in caspase 9 activity were observed in cells treated with the same concentrations of DPDT. The activities of complexes I and II of the mitochondrial respiratory chain were assessed with an ELISA assay. Significant decreases in activities of complex I (NADH Dehydrogenase) and complex II (succinate dehydrogenase) were observed at concentrations of 7.8 μM and 15.625 μM for Se compounds and 62.5 and 125 μM for Te compounds. These results suggest that Se and Te compounds cause apoptosis in astrocytes and inhibition of mitochondrial complex I and II.
of Te compounds on gastrointestinal cells. The purpose of this study was to evaluate the toxicity of tellurium tetrachloride (TeCl4) and diphenyl ditelluride (DPDT) in transformed and non-transformed human colon cells. Viability was assessed by MTT and bioluminescent-based assays. Phase contrast and scanning electron microscopy were performed to observe morphologic changes consistent with toxicity. In order to investigate the mechanism of cell death, fluorescent microscopy and caspase 3/7 and activity were assessed in the HT-29 and C29-18C0 cells using concentrations ranging from 125 μM to 1mM for each compound. Fluorescent microscopy was done using labeled Annexin V and Ethidium homodimer III, which confirmed apoptosis with all DPDT exposures and oncosis with all TeCl4 exposures in HT-29 cells. Significant increases in caspase 3/7 and 9 activity were observed in DPDT concentrations of 500 μM and 1mM in HT-29 cells and in the 1mM concentration in C29-18C0 cells, indicating cell death by the intrinsic apoptotic pathway. No significant increases in caspases were observed in either cell line treated with TeCl4 at all concentrations. GSH/GSSG assays were performed on both cell lines at concentrations ranging from 62.5 μM to 1mM for both compounds. Significant decreases in the GSH/GSSG ratio were observed in all concentrations with DPDT and TeCl4 treated HT-29 cells. Significant decreases in the GSH/GSSG ratio was observed in C29-18C0 cells with 1mM DPDT treatment and from 250 μM to 1mM cells with TeCl4 treatment when compared to the control group, suggesting the involvement of oxidative stress. It was concluded that DPDT treatment resulted in apoptosis and TeCl4 resulted in oncosis in both cell types. Reduction in GSH/GSSG ratios suggests that oxidative mechanisms are involved in Te toxicity.

PS 2478 POTENTIAL ROLE OF STEM CELLS IN CADMIUM-INDUCED ONCOCgenic TRANSFORMATION OF HUMAN LUNG CELLS.
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Cadmium (Cd) is a lung carcinogen in both humans and rodents. In this study, we attempt to develop an in vitro model of Cd-induced lung carcinogenesis in order to help further define molecular mechanisms of Cd carcinogenesis. Here we provide evidence that chronic Cd exposure induces an acquired cancer phenotype in human lung epithelial cells and in particular, in putative stem cells (SCs). The human peripheral lung cell line, HPL-1D, an immortalized, non-tumorigenic epithelial cell line was used. The total HPL-1D cell population was first exposed to a non-toxic level (5 μM) of Cd to see when signs of acquired malignant phenotype would develop. Matrix metalloproteinase-2 (MMP-2) activity, colony formation in soft agar, invasion and expression of lung cancer relevant genes were used to assess oncogenic phenotype. By 20 weeks of Cd exposure secreted MMP-2 increased to 358% of control in the total cell population, levels considered indicative of cancer phenotype. Moreover, after 20 weeks of Cd exposure, cell invasion increased by more than 3-fold in total cells compared to passage-matched controls while colony formation similarly increased with Cd exposure. After 20 weeks of Cd levels of the tumor suppressor gene p16 were reduced to about half of control at protein and the transcript levels. Loss of p16 expression is typical in human lung cancer. CD44 positive putative cancer SCs (CSCs) were enriched from the total population by the magnetic bead technique at a time point after these indications of a cancer phenotype had occurred. CD44 is a lung CSC surface marker associated with various lung tumor types. CD44 positive putative CSCs isolated after 20 weeks of Cd exposure showed highly elevated levels of MMP-2 transcript (348% of control), secreted MMP-2 activity (256%) and markedly increased expression of SC markers such as SOX2, K18, EGFR and NOTCH-1. In summary, it appears Cd has altered lung SCs toward an oncogenic phenotype during carcinogenic transformation of lung epithelial cells.

PS 2479 CADMIUM TRANSPORT IN A MODEL OF NEONATAL INTESTINAL CELLS IS CORRELATED WITH MRPI AND NOT WITH DMT1 AND FPN1.
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Newborns have a higher gastrointestinal uptake of cadmium than adults. In adults, the iron transporters DMT1 and FPN1 are involved in the intestinal absorption of cadmium. In neonates, the transporters responsible for cadmium absorption are not identified and the mechanistic understanding of the age-dependent differences in cadmium absorption is lacking. We have investigated possible cadmium transporters in the neonatal intestine by applying a model of immature human intestinal epithelial cells. To mimic the continuous cadmium exposure via diet in neonates Coca-2 cells were allowed to differentiate for 7 days in cell culture medium supplemented with 1 μM CdCl2. Cadmium pretreatment caused a drastic upregulation of the MT1 gene expression, indicating a high sensitivity of the immature intestine to cadmium. Cadmium pre-exposure did not affect the passive diffusion of mannitol or the transepithelial electrical resistance. The basolateral efflux of a tracer dose of 109Cd was increased by cadmium pretreatment. The augmented transport of cadmium was correlated to an upregulation of MRPI gene expression and increased activity of the efflux protein MRPI. No effects were observed on gene expression of the efflux proteins MRP2 and Pgp or the iron transporters DMT1 and FPN1. Neither were the cellular localizations of DMT1, DMT1-IRE and FPN1 affected by cadmium pretreatment, indicating that the increased cadmium transport is not mediated by these iron transporters. In conclusion, our data demonstrate that continuous cadmium exposure increases the absorption of the metal in immature intestinal cells and that MRPI conceivably is involved in the intestinal cadmium absorption in neonates.

PS 2480 CADMIUM APPEARS TO INITIALLY KILL THEN SELECT FOR STEM CELLS IN A HUMAN PANCREATIC EPITHELIAL CELL LINE.
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Cadmium (Cd) has been potentially linked to human pancreatic cancer. Emerging data indicates cancer stem cells (CSCs) may be critical to the carcinogenic process. Our prior work suggests Cd produces CSC-like cells in human pancreatic ductal epithelial (HPDE) cells during oncogenic transformation, and in a prostate cell line Cd exposure initially targets stem cells (SCs) early in exposure, causing SC-specific cytorelativity prior to recovery with transformation and SC production. Thus, we determined if acute Cd induced similar loss and rebound of SCs using isolated non-adherent pancreaspheres (NAPAS), which are enriched in SCs. NAPAS were produced from normal HPDE cells using a low adherence culture system and compared with adherent cells (ACs) containing the normal array of pancreatic epithelial cells. The initial lethal concentration of Cd in 50% of the cells (LC50) was 30.5 μM in ACs and 13.5 μM in NAPAS. After treatment with a non-cytotoxic level of Cd (5 μM) for one week, the acute Cd LC50 increased to 149.0 μM in NAPAS, while the LC50 for Cd in ACs increased only to 49.4 μM. Thus, the SCs in NAPAS, although initially more sensitive, rapidly gain resistance to Cd. In this first week of Cd exposure, NAPAS showed an initial marked decrease in expression of SC markers CXCR4, OCT4 and PSCA, which then rebounded above control, indicating SC differentiation then, likely, aberrant de-differentiation back to stem-like cells (possibly CSCs). Expression of S100P, a marker for pancreatic cancer aggressiveness, showed a similar trend. SC-enriched NAPAS also secreted more matrix metalloproteinase-9, an indicator of cancer phenotype, than ACs after this Cd treatment. Thus, pancreatic SCs are initially sensitive to acute Cd, then rapidly gain resistance while undergoing a differentiation and then de-differentiation that coincides with indications of acquired cancer phenotype. This initial toxic impact of Cd on pancreatic SCs may act as "bottleneck" that could hasten the formation of CSCs.

PS 2481 DIURNAL SUSCEPTIBILITY TO CADMIUM TOXICITY.
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Diurnal susceptibility in drug sensitivity have often been demonstrated and known as chronotherapy. However, such observations especially about heavy metals are still scarce. We report the diurnal susceptibility to cadmium toxicity in mice. Male C57BL/6J mice kept in cages on 8:00-20:00 L/D cycle were adapted for 14 days time (ZT); ZT2, ZT6, ZT10, ZT14, ZT18 and ZT22. In case of dark period (10:00, 14:00, 18:00, 22:00, 2:00 and 6:00 h), describing as zeitgeber clock time (10:00, 14:00, 18:00, 22:00, 2:00 and 6:00 h), describing as zeitgeber clock time; ZT2, ZT6, ZT10, ZT14, ZT18 and ZT22. In case of dark period (10:00, 14:00, 18:00, 22:00, 2:00 and 6:00 h), describing as zeitgeber clock time (10:00, 14:00, 18:00, 22:00, 2:00 and 6:00 h), describing as zeitgeber clock time; ZT2, ZT6, ZT10, ZT14, ZT18 and ZT22. In case of dark period (10:00, 14:00, 18:00, 22:00, 2:00 and 6:00 h), describing as zeitgeber clock time; ZT2, ZT6, ZT10, ZT14, ZT18 and ZT22. In case of dark period (10:00, 14:00, 18:00, 22:00, 2:00 and 6:00 h), describing as zeitgeber clock time; ZT2, ZT6, ZT10, ZT14, ZT18 and ZT22.

In summary, it appears Cd has altered lung SCs toward an oncogenic phenotype during carcinogenic transformation of lung epithelial cells.
2482 CADMIUM DISRUPTS THE EXTRACELLULAR MATRIX SECRETED BY HUMAN OSTEOSARCOMA SAOS-2 CELLS.

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Cadmium is a toxic metal that leaches into the environment, most notably through the improper disposal of electronics. Bone is a target site for cadmium toxicity and studies have linked low-level, chronic exposure to cadmium with the development of osteoporosis and other bone diseases. Our lab works to elucidate the direct effects of cadmium in bone-forming osteoblasts. Osteoblasts secrete a bone matrix primarily comprised of a calcium-phosphate crystalline and a collagenous protein component. We hypothesize that cadmium exposure alters the nature of the extracellular matrix (ECM) produced by osteoblast-like Saos-2 cells. We induced cells to mineralize using osteogenic media containing β-glycerophosphate and an ascorbic acid derivative, and then treated with 1-10 μM CdCl2 for 3-10 days. The effects of CdCl2 on calcium, phosphate, and collagen deposition, as well as collagen secretion in the ECM were assessed using Creolsophthalein Complexone Methodology, von Kossa staining, and Sircol Collagen assay, respectively. Phosphate deposition in the ECM decreased in response to CdCl2 exposure for 5 or 7 days. Most notably, exposure to 1 μM CdCl2 increased collagen(μg) deposited in the ECM, which corresponded to a decrease in the amount of collagen detected in the media. When expressed as a ratio of ECM to media collagen, we observed that CdCl2 increased the ratio by nearly two-fold (control=0.87 compared to CdCl2=1.42 at 5-day exposure). Using a cadmium-specific Leadmium Green AM Dye, we have shown cells uptake cadmium. Current studies are being conducted using the same dye to assess cadmium deposition in the ECM. In addition, we are investigating potential binding interactions between cadmium and collagen. Collectively, these data suggest that the uptake of cadmium disrupts the various components of the extracellular matrix secreted by osteoblasts. Research funded by NIH-INBRE P20RR016454 and NIH R15ES015866 grants.

2483 ROLES OF METALLOTHIONEIN AND GLUTATHIONE IN PRO-ANTIOXIDANT EFFECT OF ZINC IN CADMIUM-TREATED CULTURED CHORDOID PLEXUS.

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We have shown in primary cultures of rat choroid plexus that cadmium (Cd) induces oxidative stress and stimulates apical choline uptake. Zinc (Zn) supplementation abates oxidative stress and modulation of choline uptake elicited by Cd. Our objective is to elucidate how Zn attenuates oxidative stress and disrupts stress modulation of choline transport. Glutathione (GSH) supplementation also abates oxidative stress and modulation of choline uptake in Cd-treated choroid plexus cells. We hypothesize that Zn abates Cd-induced oxidative stress and stress-modulation of choline uptake via induction of MT-1. Thus, in choroid plexus primary cultures we tested effects of GSH depletion and silencing of MT-1 on the efficacy of Zn supplementation to disrupt Cd-induced modulation of apical choline uptake. We analyzed GSH and GSSG (GSH/GSSG-Glo Assay; Promega) in cells treated with Cd (250 μM CdCl2, 18 h) or Zn-supplemented (25 μM ZnCl2, 48 h) then treated with Cd plus Zn. Cd-treated cells GSH concentrations exceeded those in controls irrespective of Zn; in Cd-treated cells GSSG concentrations exceeded those in controls. However, in cells treated with Cd plus Zn, GSSG concentrations were similar to controls; Zn alone did not alter GSH or GSSG. Cells were supplemented with Zn α- buthionine sulfoximine (BSO, 100 μM) then treated with Cd (500 nM Cd, 12 h). Cd stimulated 10 μM H-choline uptake (30 min) by 78%; Zn attenuated stimulation irrespective of BSO. Cells then were transfected with MT-1 siRNA, reducing MT-1 mRNA by 75%. In untreated cells, Cd stimulated choline uptake by 60%, and Zn abated stimulation; negative siRNA transfection did not alter modulation of uptake by Cd or Zn. However, in cells transfected with MT-1 siRNA Cd stimulated choline uptake by 100%, but Zn did not abate stimulation. These preliminary data suggest Cd increases GSH and GSSG, but efficacy of Zn to disrupt stress-modulation of choline uptake is dependent on MT-1 induction. Supported by NSF-IOS# 1053654.

2484 CELLULAR ACCUMULATION AND BEHAVIOR OF SILVER AND INDUCTION OF METALLOTHIONEIN IN HUMAN BRONCHIAL EPITHELIAL CELL FOLLOWING EXPOSURE TO SILVER NITRATE.

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Silver (Ag) possessed well-known antibacterial effects and has been used for wound dressing and deodorant powders. However, metabolic behavior and biological roles of Ag have not been well characterized in mammals. In the present study we investigated uptake and intracellular distribution of Ag, induction of metallothionein (MT), and generation of ROS following exposure to AgNO3 in human bronchial epithelial cells (Beas-2B). The cells were exposed to 0, 0.05, 0.1, 0.5, 1.0, 5.0, 10, and 100 μM AgNO3 for 3, 6, 12 and 24 h and cytotoxicity was assayed with a modified MTT method. The cell viability was decreased by AgNO3 in a dose-dependent manner and IC50 value of AgNO3 was calculated to be 2.5 μM. Concentration of Ag in culture media decreased with time and reached a plateau at 12 h after Ag exposure. Concentration of Ag in the cytosolic fraction was increased up to 3 h and then decreased, indicating that cytosolic Ag relocated to the insoluble fraction of the cells. HPLC-ICP-MS was used to determine distribution of Ag in the soluble fraction. The amount of Ag bound to MT in the soluble fraction was sharply increased up to 3 h and the amount of Ag-MT was decreased thereafter. The mRNA levels of major human MT isoforms, MT-Ia and MT-IIa, parallel with the amount of Ag-MT. BES-H2O2 was used for ROS imaging in the cells. This probe enables visualization of intracellular H2O2 distribution in live cells. The fluorescence intensity derived from H2O2 seemed to be elevated only at 24 h. These results suggest that the newly synthesized MTs inhibited the generation of ROS by exposure to Ag and then ROS level was increased with degradation of Ag-MT and relocalization of Ag from soluble to insoluble fraction or intracellular organelles.

2485 MTF-1 AND CARM1 PARTICIPATE IN A COMPLEX TO REGULATE METALLOTHIONEIN EXPRESSION AND METAL HOMEOSTASIS.

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Metallothioneins (MTs) are small metal-binding proteins that play important roles in the detoxification of nonessential metals and metal homeostasis. MT expression is inducible after exposure to a variety of stresses and is largely regulated by the metal-responsive transcription factor 1 (MTF-1). Previous studies have shown that MTF-1 translocates to the nucleus and binds to metal responsive elements (MREs) in the promoters of metal-inducible genes. During the transcriptional activation of MTs, MTF-1 participates in a multi-protein regulatory complex. To better understand the components of this multi-protein complex, a yeast two-hybrid analysis was performed to identify additional MTF-1 interacting partners. Results revealed that coactivator-associated arginine methyltransferase (CARM1) interacts with MTF-1 through amino acids 405-625, which contain proline-rich and serine/threonine-rich regions of the MTF-1 protein. To verify this observation in a mammalian system, FLAG-tagged MTF-1 and CARM1 were co-immunoprecipitated from HEK293T cells and also co-localize when examined by immunofluorescence. To determine if CARM1 could protect cells from metal-induced toxicity, wild type and CARM1 -/- cells were exposed to increasing concentrations cadmium chloride. Cell survival was evaluated by a Neutral Red cytotoxicity assay and revealed that CARM1-/- cells were hypersensitive to cadmium treatment, compared to wild type cells. This increased sensitivity to cadmium exposure correlated with a defect in MT induction. Real time PCR analysis showed robust induction of MT-1 mRNA in wild type cells after exposure to cadmium chloride; however, MT induction was reduced ~4-fold in CARM1-/- cells, compared to wild-type cells. Taken together, these results suggest that CARM1 may act as a chromatin remodeling member of the MTF-1 multi-protein complex to regulate the expression of MT genes in response to cadmium exposure.

2486 INHIBITION OF ENDOGENOUS MTF-1 SIGNALING IN ZEBRAFISH EMBRYOS IDENTIFIES NOVEL ROLE FOR MTF-1 IN IRON AND HEME HOMEOSTASIS.

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The MTF-1 transcription factor is considered to be a master regulator of zinc homeostasis. We previously characterized a constitutively nuclear, dominant-negative MTF-1/eGFP fusion protein (dnMTF-1). In vitro transcribed dnMTF-1 mRNA
was microinjected into zebrafish embryos (2-cell stage) and transcriptomic profiling was performed using an Affymetrix 4 x 44K array. A total of 594 and 560 probes were identified as differentially expressed at 24 hpf and 36 hpf, respectively. There were several main categories of genes affected by the inhibition of MTF-1 signaling including novel observations in iron and heme homeostasis. Heparin, a peptide hormone that regulates iron homeostasis, was significantly downregulated 24 h after treatment. Interestingly, heparin was significantly downregulated by all Cd doses at both timepoints while hepcidin was significantly upregulated only after 24 hours of Cd exposure. 72 hpf zebrafish embryos were further exposed to hemin concentrations (50-150 μM) for 4 or 24 hours and expression was determined by real-time PCR. Both hepcidin and metallothionein were significantly upregulated after 4 hours of heme exposure at the highest concentration, while heparin expression remained constant. However, after 24 hours of heme exposure, heparin was significantly downregulated at the highest heme concentration while hepcidin and metallothionein appeared unaffected. A search of 3kb upstream of the transcription start site identified multiple MTF-1 binding sites within the proximal promoter of hepcidin.

R. Ikeuchi1, T. Kido1, C. Sugaya1, M. Tsunoda1,1, R. Lin1,3 and X. Ren5

Both hepcidin and metallothionein were significantly upregulated after 4 hours of heme exposure at the highest concentration, while heparin expression remained constant. However, after 24 hours of heme exposure, heparin was significantly downregulated at the highest heme concentration while hepcidin and metallothionein appeared unaffected. A search of 3kb upstream of the transcription start site identified multiple MTF-1 binding sites within the proximal promoter of hepcidin.

2487 TRIMETHYLTIN CHLORIDE: INVESTIGATIONS INTO ITS ACUTE AND CUMULATIVE TOXICITY AND TOXICOGENIC ASPECTS.

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Methylthiin stabilizers are increasingly used as heat and light stabilizers in the production of polyvinyl chloride (PVC). Dimethylthiin (DMT) is the principle material to synthesize stabilizer used in the production of PVC, however, trimethylthiin (TMT) as a byproduct of DMT manufacture is often viewed as the main contributor for the neuropathological changes observed in poisoning patients exposure to methylthiin. Most previous studies have focused on the acute effects of TMT exposure, and limited information is available about the TMT cumulative effects and toxicokinetic properties. In the current study, we aim to further investigate the acute and particularly cumulative toxicity and to understand the toxicokinetic aspects of TMT exposure. TMT was given to rats or mice via gavage or intraperitoneal injection. Our results showed that administration of TMT in rats caused a high acute and cumulative toxicity, and that hypokalemia and neuropathological changes were the major characteristics of TMT exposure. Acute TMT administration in mice also induced significant toxicity, but its cumulative toxicity was moderate, suggesting that species differences exist. Moreover, we showed that TMT was well absorbed from the gastrointestinal tract and quickly distributed to various tissues in rats, but the highest concentration of TMT was found in erythrocytes of blood. The half-life of TMT in the blood of rats was relatively long (15 days), and TMT was detected in blood at 90 days later after the TMT administration. Given the vast production and use of PVC products, our results suggest that TMT exposure can constitute a health concern and pose potential health risks to occupational workers and the general population.

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2488 THE IMMUNOTOXIC EFFECTS OF TRIBUTYLtin ON SPLENOcyTES IN FEMALE F1 RATS BY CONTINUOUS EXPOSURE FROM THE FETUS STAGE THROUGH THEIR DEVELOPING STAGES.

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Immuno-toxicity is one of the major toxic effects of tributyltin (TBT). The problem remained to determine the immunotoxicity of exposure to TBT on the developing stages of F1 rats. We evaluated the immunotoxicity of TBT by continuous exposure from fetuses to 9 weeks of age by comparing the results of rats exposed to TBT via the placenta and their dams’ milk and those of rats exposed via only their food. The rats were exposed to TBT in utero and via their dams’ milk by the dams’ feeding on chow containing TBT at 125 ppm. After weaning, they were either fed chow containing TBT at 0 or 125 ppm. The pups were divided into 4 groups: the control (control-control, CC); the group exposed via their food (control-TBT, CT); that exposed via the placenta and their dams’ milk (TBT-control, TC); and the continuous exposure group (TBT-TBT, TT). The spleens were removed at 9 weeks of age, and the number of cells was counted. The spleenocytes were incubated with the antibodies of FITC-conjugated anti-rat CD3, PE-Cy5-conjugated mouse anti-rat CD4, PE-conjugated mouse anti-rat CD8a, PE-Cy5-conjugated mouse anti-rat CD45RA, and PE-conjugated mouse anti-rat NKP-1A and analyzed using a flow cytometer. The populations of pan T (CD3+), pan B (CD45RA+), NK (NKR-P1A+), helper T (CD3+CD4+), and cytotoxic T (CD3+CD8+) cells were calculated. The number of spleen cells of the paternal line of pan T, NK, and cytotoxic T in the CT, TC, and TT groups were significantly lower compared with those in the CC group. Changes in subsets of lymphocytes were observed in the 3 groups exposed to TBT. Immune system alterations among rats exposed to TBT via the placenta and their dams’ milk could possibly continue even after discontinuing exposure to TBT.

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2489 INSOLUBLE Ni2+ COMPOUNDS SILENCE DRIP80 GENE, ALTERING CA2+ ION DISTRIBUTIONS IN Ni2+/MCA-TRANSFORMED 10T1/2 MOUSE EMBRYO CELLS.

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Ni-containing sulficic/toxicic ores cause nasul/lung cancers in Ni refinery workers. Ni3S2/green NiO induces respiratory cancer in rodents. Ni3S2 and green/black Ni-containing sulfidic/oxidic ores cause nasal/lung cancers in Ni refinery workers. People working in industrial and manufacturing settings have an increased risk of exposure to these metals. The cytotoxicity of nickel and cobalt have individually been demonstrated, however the underlying mechanisms of coexposure to these heavy metals have not been explored. In this study, we investigated the effect of exposure of H460 human lung epithelial cells to nickel and cobalt, alone and in combination, on cell survival, apoptotic mechanisms, and the generation of reactive oxygen species (ROS). For simultaneous exposure cells were exposed to a constant dose of 150 μM cobalt or nickel, which was found to be relatively nontoxic in single exposure experiments. We demonstrate that cells exposed simultaneously to cobalt and nickel exhibit a dose-dependent decrease in survival compared to the cells exposed to a single metal. The decrease in survival was the result of enhanced caspase 3 and 7 activation and cleavage of poly (ADP-ribose) polymerase. Coexposure increased the production of ROS and the formation of double strand breaks. Pretreatment with N-acetyl cysteine alleviated the toxic responses. Collectively, this study demonstrates that coexposure to cobalt and nickel is significantly more toxic than single exposure and that toxicity is related to formation of ROS. These findings indicate that additional studies are needed to characterize the mutagenic and genotoxic effects of coexposure to cobalt and nickel.

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High environmental tungsten levels have been associated with a cluster of childhood pre-B acute lymphoblastic leukemia, although the causality between tungsten and leukemia has not been established. Our in vitro data suggest that developing B lymphocytes are susceptible to tungsten-induced DNA damage and growth inhibition. We therefore hypothesized that tungsten could alter the development of B-cells leading to a pro-leukemogenic phenotype. To test this, we exposed C57BL/6j mice over 16 weeks to tungsten-containing tap water at concentrations of 15, 200, and 1000 mg/L, levels that are representative of the environmental concentrations found in the area of the leukemia cluster. Exposure to 1000 mg/L tungsten resulted in a decrease in overall body weight, although mice appeared otherwise healthy and continued to gain weight at a rate similar to other treatment groups. Tungsten exposure, at all tested concentrations, decreased the number of peripheral white blood cells, including monocytes, granulocytes, and lymphocytes. Furthermore, thia tungsten concentration, as measured by ICP-MS, reached a dose-dependent plateau within 4 weeks. We examined changes in B cell development using flow cytometry and correlated this with tungsten exposure levels. Exposure correlated with an increase in immature B-cells (IgM<sup>+</sup>, B220<sup>−</sup>CD45R<sup>−</sup>) within the bone marrow, although no changes in B220<sup>+</sup>CD45R<sup>+</sup> populations in the thymus or the spleen were observed. The activity of B cell progenitors was increased by 4 weeks following tungsten exposure, as measured by preB colony forming assays. Finally, DNA damage, as assessed by COMET assay, was increased in the non-adherent bone marrow populations of exposed animals, with the greatest increase at the lowest tested concentration. Together, these data suggest that tungsten exposure may alter development of B lymphocytes, possibly promoting a pro-leukemogenic phenotype.
inner-city soil Pb was 438 mg/kg or 3.7 times larger than the median soil Pb of 117 mg/kg in outlying areas (p-value <0.0001). The prevalence of children (DUT) blood Pb ≥ 10/μg/dL was 22.9% in the inner-city vs. 9.1% in outlying areas of New Orleans (p-value <0.0001). The quantities of legacy Pb dust were calculated for paint and gasoline in New Orleans: Assuming removal of all Pb-based exterior paint by power sanding, the maximum release was 1811 metric tons (MT) Pb dust; leaded fuel use from 1927—1994 accounts for 9100 MT Pb dust. Accumulated Pb dust from gasoline additives plausibly explains the differences in soil Pb and children’s blood Pb between the higher traffic congested inner-city compared and the lower traffic congested outer areas of the city. The 20th century use of lead in paint and gasoline was national, and a similar inner vs. outer city pattern of environmental Pb dust contamination and childhood Pb exposures are expected in all cities.

This study demonstrates that the current soil Pb guideline where children live and play of 400 mg/kg is too large. Soils are both a sink and a continuing exposure source of Pb dust, and children need low Pb soil to prevent the toxicity of Pb such as behavior and learning effects. All cities have a Pb-safe source of soil for intervention; the median US non-urban background soil Pb is 16.5 mg/kg (range 10.3 to 30.1 mg/kg), and at least an order of magnitude smaller than soil in most cities. These results underscore the need for clean soil to protect children from accumulated Pb dust in soil resulting from the legacy of Pb use during the 20th century.

Environmental trace metals such as copper (Cu) are known inhibitors of olfactory function in rodents. Although the function of olfactory receptor genes has been linked to impaired homing to natal streams and a compromised ability to detect predators and prey, the underlying molecular mechanisms of metal-induced olfactory injury have not been elucidated. In the current study, we analyzed components of olfactory signal transduction on short-term Cu exposures that are associated with a loss of olfaction in coho salmon. Quantitative PCR (qPCR) analysis of the peripheral olfactory system (i.e., olfactory rosettes) and also the olfactory bulb and telecephalon (OBT) of Cu-exposed coho revealed a dose- and tissue-dependent modulation of key genes involved in maintaining G-protein coupled olfactory receptor signaling, cAMP mediated signaling and calcium signaling. Histological sectioning of coho olfactory tissues revealed the disruption of olfactory receptor neuron patterning at the highest dose of copper (50 ppb), whereas TUNEL staining revealed an increased cell death in olfactory epithelium (OE) at 25 and 50 ppb Cu. Similarly, a loss of immunoreactive type 3 adenylate cyclase (ACIII) protein expression was observed in the olfactory epithelial cilia of coho exposed to high doses of Cu. Exposure to Cu decreased intracellular cAMP levels in the olfactory rosettes, while increasing OBT cAMP. Similar effects of Cu were observed for OBT cAMP levels. Collectively, the results of our study indicate distinct morphological and functional changes within the olfactory system of coho salmon in response to environmental copper concentrations that are associated with the disruption of key cellular components responsible for maintaining olfactory signal transduction. This project was supported by NIEHS Superfund program project P42-040696.

Hexavalent-chromium [Cr(VI)] is a well-documented human carcinogen via inhalation route, however, the mechanism of Cr(VI)-induced carcinogenesis is unknown. The spindle assembly checkpoint (SAC) is a critical regulator of the metaphase-to-anaphase transition and ensures genome stability by preventing chromosomal missegregation events. SAC bypass can lead to genomic instability, manifested as aneuploidy, which eventually leads to tumor formation and cancer. Recent studies in our laboratory demonstrated that chronic exposure to zinc chromate induces SAC in a concentration- and time-dependent manner in human lung fibroblasts. To further study these events, we focused on the cell division cycle 20 (Cdc20) protein. Cdc20 has not been studied after Cr(VI) exposure, but other studies showed that overexpression of Cdc20 protein leads to aneuploidy. We investigated Cdc20 protein expression and localization in human lung fibroblasts treated with zinc chromate by using molecular techniques such as immunofluorescence, western blot and co-immunoprecipitation (Co-IP) studies. We found a concentration-dependent decrease in Cdc20 protein expression levels in both mitotic and interphase cells at concentrations of 0.1-0.2 μg/cm2 zinc chromate. In addition, we observed a decrease in Cdc20-kinetochore localization after 96 and 120 hours exposure in prophase and pro-metaphase cells. We further examined changes to Cdc20 function using Co-IP studies and found a dose-dependent decrease in the interaction of Cdc20 and Mad2 (mitotic arrest deficient related 2) protein. Altogether, the data indicates that zinc chromate exposure alters the expression and localization of Cdc20, which may
2500 HEXAVALENT CHROMIUM INDUCES CHROMOSOME INSTABILITY RESULTING IN A DNA DOUBLE-STRAND BREAK REPAIR-DEFICIENT PHENOTYPE AND NEOPLASTIC TRANSFORMATION.

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Chromosome instability (CIN) is a hallmark of lung cancer with cells exhibiting both translocations and severe aneuploidy. Hexavalent chromium (Cr(VI)) is a well-known respiratory carcinogen with the particulate form being the most potent form. These particles impact at bifurcation sites of the lung and over time dissolve. However, the ability of Cr(VI) to induce chromosomal translocations is unknown. We exposed human lung cells to lead chromate for three sequential 24 h periods, each separated by about a month. After each treatment, cells were seeded at colony forming density, cloned, expanded and treated. Each generation of clones was tested for chromosome sensitivity, chromosome complement, DNA repair capacity and ability to grow in soft agar. We found that after the first treatment, lead chromate-treated cells exhibited a normal chromosome complement though a few clones showed an increase in relative survival. After the second exposure, more than half of the clones acquired an abnormal karyotype including numerical and structural alterations. The third treatment resulted in more abnormal clones as well as previously abnormal clones acquiring additional abnormalities. Clones were treated with soluble Cr(VI) for 24 h followed by a 24 h recovery period to measure DNA double-strand break (DSB) repair. Abnormal clones showed persistent H2A.X and with soluble Cr(VI) for 24 h followed by a 24 h recovery period to measure DNA double-strand break (DSB) repair. Abnormal clones showed persistent H2A.X and ability to grow in soft agar. We found that after the first treatment, lead chromate-treated cells exhibited a normal chromosome complement though a few clones showed an increase in relative survival. After the second exposure, more than half of the clones acquired an abnormal karyotype including numerical and structural alterations. The third treatment resulted in more abnormal clones as well as previously abnormal clones acquiring additional abnormalities. Clones were treated with soluble Cr(VI) for 24 h followed by a 24 h recovery period to measure DNA double-strand break (DSB) repair. Abnormal clones showed persistent H2A.X and with soluble Cr(VI) for 24 h followed by a 24 h recovery period to measure DNA double-strand break (DSB) repair. Abnormal clones showed persistent H2A.X and

2501 SIMULATED MICROGRAVITY DECREASES CHROMIUM (VI)-INDUCED GENOTOXICITY IN HUMAN SKIN FIBROBLAST CELLS.

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Data collected during NASA's Reduced Gravity Student Opportunities program on the "Weightless Wonder" showed that altered gravity (microgravity and hypergravity combined) increased chromium (VI)-induced genotoxicity in human fibroblast cells. Other work at our lab showed that heavy metal-containing space dusts are toxic to these cells in normal gravity, suggesting that NASA's goal of space exploration could be potentially harmful to astronaut health. We want to determine if microgravity potentiates toxicity because astronauts will be exposed to microgravity in space for long periods of time. It is difficult to model microgravity on earth. Most microgravity (MG) studies have been in animal models such as fruit flies that do not represent human systems. In this study, we simulated microgravity using a bioreactor, which confines gravity and theoretically simulates different g forces depending on rotational speed. The lower the RPM, the lower the g force. For example, 25 RPM equals 0.01553g while 8 RPM is 0.00201g. We used human skin fibroblast cells (hHERTERT) and compared the effects of normal gravity and simulated microgravity on hexavalent chromium (Cr(VI))-induced toxicity after a 24 hour exposure. We found less Cr(VI)-induced chromosome damage in microgravity using sodium chromate as a representative Cr(VI) compound. Specifically, 0.5, 1, and 2.5 μmol sodium chromate damaged chromosomes in 17, 21, and 40 percent of metaphases respectively under normal gravity conditions, while the same treatments only damaged chromosomes in 3, 8, and 22 percent of metaphases under simulated microgravity. Future work will assess whether differences in uptake of sodium chromate are causing the decrease in damage, as well as study the effects of lead chromate as a particulate form of Cr(VI). This work was supported by NASA grant EP-08-01 (JPW Sr), the Maine Space Grant Consortium (MB) and the Maine Center for Toxicology and Environmental Health.

2502 EFFECTS OF ALTERED GRAVITY ON CHROMIUM-INDUCED GENOTOXICITY IN HUMAN LUNG CELLS.

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Trips to outer space are becoming more commercialized, and are starting to attract "space tourists." Yet the physiological effects of microgravity on humans are not thoroughly studied. Our lab has participated in NASA's Reduced Gravity Student Flight Opportunities Program, which provided us with the unique opportunity to conduct experiments on NASA's Weightless Wonder. The Weightless Wonder is a plane that simulates environments of microgravity and hypergravity by flying in a series of parabolas. Data we collected from these experiments showed a 2-3 fold increase in chromosomal damage, but a 2-3 fold decrease in chromium uptake was observed in flight compared to ground experiments. These results are very provocative for how the force of gravity affects cellular metabolism of chemicals. The next step we chose to take is to determine what is causing this difference – hypergravity or microgravity. We are able to simulate hypergravity using a centrifuge, and microgravity using a bioreactor. Our data from the bioreactor indicates that there is a decrease in the amount of chromate-induced chromosomal damage as the force of gravity approaches 0g. These data suggest that altered gravity inhibits the ability of chromium to get into cells. We are also looking into the effects of hypergravity and microgravity on chromium induced DNA double strand breaks and cytotoxicity. This work was supported by NASA grant ACD FSB-2009 (JPW Sr), a fellowship from the Maine Space Grant Consortium (JPW Jr) and the Maine Center for Toxicology and Environmental Health.

2503 ASSOCIATION OF ORGANOCHLORINE COMPOUND BODY BURDEN AND ADIPOKINES WITH PREVALENCE OF TYPE 2 DIABETES MELLITUS.

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Most organochlorine (OC) insecticides have been banned for over 30 years in the United States but still bioaccumulate due to lipophilic and environmental persistence, resulting in quantifiable levels in the blood and adipose of human populations. Some bioaccumulative OC compounds, notably p,p'-DDE, trans-nonachlor, and oxychlordane, have been observed association with increased prevalence of Type 2 diabetes mellitus (T2DM). Gas chromatography/mass spectrometry (GC/MS) analysis was performed to measure concentrations of these analytes in plasma from 149 subjects having T2DM and 151 non-diabetic subjects. Adipokines including leptin and adiponectin were measured by ELISA for all subjects and a subset of non-diabetic subjects was analyzed for metabolic hormone and inflammatory factors involved in development of T2DM. Overall 81% of subjects had measurable concentrations of at least one of the studied OC compounds. DDE was significantly higher in T2DM subjects (319.0 ± 40.6 ng/g) than in non-diabetic subjects (139.0 ± 19.2 ng/g), p<0.01. DDE was also higher in African-American (AA) T2DM subjects (315.9 ± 113.9 ng/g) compared to AA non-diabetic subjects (223.4 ± 59.5 ng/g) as well as in Caucasian T2DM subjects (234.1 ± 28.0 ng/g) compared to Caucasian non-diabetes (124.6 ± 19.8 ng/g). In the non-diabetic subset, significantly higher concentrations of leptin, TNF-α and MCP-1 were associated with higher plasma DDE concentrations (p=0.001, 0.08, and 0.002, respectively). Leptin was significantly higher (p=0.02) and adiponectin was significantly lower (p=0.03) in the T2DM subjects than in the non-diabetic cohort. In conclusion, the T2DM subjects in this study were found to have an association between higher OC pesticide levels and T2DM and that the T2DM population demonstrated altered adipokine concentrations compared to the non-diabetic study subjects. While causation is not known, the higher levels of OC compounds may be a useful biomarker of T2DM risk.

2504 DETECTION OF PERFLUOROALKYL ACIDS IN RIVERS AND TAP WATER FROM ALL OVER JAPAN.

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Water is expected to be the major exposure route of Perfluoroalkyl acids (PFAA) to humans. We measured Perfluorocarboxylates (from C5 to C12) and perfluorosulfonates (C54, C66, C58 and CS10) in the river water (RW) from 98 locations sampled in 2010 and those in the tap water (TP) from 142 locations sampled in 2007.
from all over Japan using LCMS/MS. Raw and purified waters sampled at 22 water purification plants (WPP) in 2009 were also measured. The results were compared with the data of RW collected in 2003 from all over Japan. In the RW, all the examined PFAs were detected with C8, C9, C6 and C88 showing high national average concentrations of more than 1ng/L in this order, and they were highest in Kinki. The average concentration of C6 in Kinki was about fifteen times as high as the national average, and extremely high concentrations (46 and 24 μg/L) were detected in the lower reaches at the foot of a fluorochemical plant, where extremely high concentrations of C8 (67 and 24 μg/L) were detected in 2003. From 2003 to 2010, both the nationwide average C8 and C9 concentrations decreased to about half, while Kinki showed drastic reduction of C8 concentration to one tenth. In TP, all the examined PFAs were detected with the high concentrations for C8, C88 and C9. The national average concentrations of C8, C88 and C9 in TP were about 20% of the corresponding concentrations in the RW, while C6 showed as low as 3.4%. The purification efficiency of C6 at the WPP did not differ from the other PFAs (about 20%). The distribution heterogeneity for C6 concentration in RW with extremely high sampling sites and the fact that the RW concentration was not reflected in TP may explain that the release of C6 to the environment began recently from some source(s) in Kinki. Even if the C6 release is cut off immediately, the level in TP (and thus the human exposure) will increase to the extent to C8 and C9. If the C6 release is continued, its concentrations in both RW and TP will exceed the levels of C8 and C88.

**2050** INVESTIGATION OF OXIDATIVE STRESS AS A MECHANISM FOR PBDE DISRUPTION OF NEURODEVELOPMENT.

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There have been several proposed mechanisms through which polybrominated diphenyl ethers (PBDEs) disrupt neurodevelopment. A primary proposed mechanism of disrupted neurodevelopment can be attributed to the formation of reactive oxygen species which damage neurons. Embryonic zebrafish were exposed to four different PBDEs (BDE 28, BDE 47, BDE 99, and BDE 100) then evaluated for cell death, apoptosis, and induction of antioxidant responsive genes. In addition, embryos were co-exposed with the glutathione inhibitor, buthionine sulfoximine, (BSO) or N-acetylcysteine (NAC), the rate-limiting substrate in glutathione synthesis. Previously, PBDEs were found to alter spontaneous movement activity of zebrafish during development at 24 hpf, alter swimming behavior at 120 hpf, and induce body curvature and mortality. In the present study, immunohistochemistry for caspase-3 was used to identify cells undergoing apoptosis in situ; however, there was no evidence indicating that the PBDEs tested induced apoptosis. Furthermore, testing with acridine orange did not reveal induced cell death for any PBDEs at concentration up to a concentration of 10 ppm. Glutathione-S-transferase (GSTpi), glutamate cysteine ligase catalytic subunit (GCLc), and cyclooxygenase 6 (COX6) were assessed using RT-PCR. Only COX6 was upregulated for all congeners. Conversely, GSTpi was downregulated for all congeners tested at 10 ppm. The results do not support a strong oxidative stress response following exposure to PBDEs. To further verify, co-exposures to NAC and BSO were conducted. Neither co-exposure altered rates of spontaneous movement at 24 hpf compared to the 10 ppm PBDE alone at 10 ppm. At 20 ppm, NAC decreased rates of spontaneous movement for all congeners except BDE 100. BSO and NAC did not alter rates of malformations or mortality at any concentration. In this study, oxidative stress was not a primary mode of action for PBDEs in the disruption of neurodevelopment.

**2051** THE USE OF TOXIC EQUIVALENCY FACTORS (TEFs) FOR POLYBROMINATED DIBENZODIOXINS (PBDDs) AND DIBENZOFURANS (PBDFs) IN RISK ASSESSMENT.


The WHO has established TEF values for polychlorinated dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) with a dioxin-like mechanism. Many governmental agencies use these for human and environmental risk assess-

ment. Recently, the presence of PBDDs and PBDFs in biotic and abiotic environmental samples was shown. As a result, it is suggested that PBDDs and PBDFs should be included in the TEF concept. Recently, UNEP and WHO jointly organized an expert consultation to assess this possible inclusion and evaluate the dioxin-like properties of PBDDs and PBDFs. Among others, it was concluded that toxicokinetics of PBDDs and PBDFs are not significantly different from their chlorinated counterparts. There is also a similarity between both types of compounds in Ah-receptor binding and associated transcription processes. Although much more limited than for PCDDs and PCDFs, available data indicate that most responses of PBDDs and PBDFs fell within the range of uncertainty associated with TEFs of their chlorinated analogues. Therefore, it is suggested to use similar interim TEFs for brominated analogues as for the chlorinated until more congener specific in vivo information becomes available. Using these suggested interim TEFs for PBDDs and PBDFs, their contribution may be as high as 10% of total TEFs in biological matrices relevant to humans. Fish specific relative potencies of PBDDs and PBDFs were also compared with TEFs derived from mammalian systems. It was concluded that similar to PCDDs and PCDFs, separate TEFs for fish should be used to avoid overestimation of PBDD and PBDF toxicity in aquatic ecosystems. For terrestrial wildlife, e.g., birds, there is no information. Thus, it is recommended by UNEP and WHO that 2,3,7,8-PBDDs and PBDFs should be included in the TEF concept.

**2052** IMMUNOLOGICAL EVALUATION OF THE RELATIVE POTENCY OF A SINGLE ORAL ADMINISTRATION OF BROMINATED AND CHLORINATED DIOXINS AND DIBENZOFURANS IN FEMALE B6C3F1 MICE.

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Increased use of brominated flame-retardants and incineration of brominate-containing materials has lead to an increase in the presence of polybrominated dibenzo-p-dioxins and dibenzofurans (PBDD/Fs) in the environment. Measurable amounts of PBDD/Fs have been detected in sediment, seafood, and human serum. In vitro studies indicate that some PBDD/Fs may have relative potencies equal to polychlorinated dibenzo-p-dioxins and dibenzofurans. Inhibition of the IgM antibody forming cell (AFC) response to sheep red blood cells (SRBC) following an acute single exposure is a sensitive indicator of toxicity for polyaromatic hydrocarbons. The objective of this study was to test the hypothesis that the relative potencies of PBDD/Fs are equivalent to their chlorinated analogs by evaluating the ability of each compound to alter humoral immunity in B6C3F1 mice as measured by quantifying antigen-specific antibody responses. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), 2,3,7,8-tetrabromodibenzo-furan (TBBF), 2,3,7,8-tetrachlorodibenzo-furan (TCDF), 1,2,3,7,8-pentabromodibenzo-furan (1PBF), 1,2,3,7,8-pentachlorodibenzo-furan (1PCDF), 2,3,4,7,8-pentabromodibenzo-furan (2PBF), 2,3,4,7,8-pentachlorodibenzo-furan (2PCDF), 2,3-dibromo-7,8-dichlorodibenzo-p-dioxin (DCDD) suppressed the IgM AFC response to SRBC, while 2,3,7-trichlorodibenzo-p-dioxin (TriDD) did not affect the response. The specific activity (AFC/106 spleen cells) data were fit to a Hill model to estimate the ED50 for inhibition. The rank order of potency for these chemicals was TCDD>DCDD>DBDF>FPBF>2PCDF>1PCDF>TCDF. While TCDD was the most potent of the compounds tested, the brominated analogs were more potent than their chlorinated analogs by factors of 2-20 with TBDF having the largest difference in potency compared to their chlorinated analog. This abstract does not necessarily reflect the policies or views of NIH.

**2053** EGCG PROTECTS ENDOTHELIAL CELLS AGAINST PCB 126-INDUCED INFLAMMATION THROUGH INHIBITION OF AHR AND ACTIVATION OF NRF2.

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Tea flavonoids such as epigallocatechin gallate (EGCG) protect against vascular diseases such as atherosclerosis via their antioxidant and anti-inflammatory functions. Persistent and widespread environmental pollutants, including polychlorinated biphenyls (PCB), can induce oxidative stress and inflammation in vascular endothelial cells. Even though PCBs are no longer produced, they are still detected in human blood and tissues and thus considered a risk for vascular dysfunction. We
These data suggest that EGCG provides anti-inflammatory properties via inhibition of NF-E2-related factor 2 (Nrf2)-controlled antioxidant genes, including glutathione S transferase (GST) and NAD(P)H:quinone oxidoreductase 1 (NQO1), in a dose-dependent manner. Pretreatment with all-trans retinoic acid, an inhibitor for Nrf2-associated signaling, resulted in an increase of Cyp1A1, MCP-1 and VCAM-1 expression, suggesting Nrf2-regulated antioxidant enzymes play a protective role against PCB-induced inflammatory responses in endothelial cells. These data suggest that EGCG provides anti-inflammatory properties via inhibition of AhR and elevated expression of Nr2f regulatory genes.

Polychlorinated biphenyls (PCBs) are persistent organic pollutants. The dioxin-like coplanar PCBs, such as PCB 77 and PCB 126, interact with the aryl hydrocarbon receptor (AhR), and previous work has demonstrated that coplanar PCBs initiate endothelial dysfunction which leads to atherosclerosis. Many of these animal studies utilized PCB 77; however, use of PCB 126, a coplanar PCB with a higher affinity for the AhR, has not been as well characterized. In order to determine the dose response relationship of PCB 126 toxicity in mice, a concentration range was selected including 0.0, 0.5, 5.0, 50.0, and 150 μmol/kg, also expressed as 0.0, 0.16, 1.63, 16.3, and 49.0 mg/kg (ppm). Each group of C57BL/6 mice was gavaged twice with the same concentration of PCB126 within a seven day period. After this acute exposure, the mice were sacrificed and assessed for toxicological and inflammatory endpoints of PCB 126 exposure. The goal of this study was to identify PCB concentrations which do not lead to wasting syndrome. Mice receiving the highest doses (50 and 150 μmol/kg) of PCB 126 exhibited a significant decrease in body weight, indicative of broad toxicity. The lowest concentrations of 0.0 and 0.5 μmol/kg did not contribute to weight loss or changes in inflammatory markers. All mice which received PCB 126 had significant increases in cytochrome P4501A1 (CYP1A1) mRNA expression, while those mice which received 5.0 μmol/kg of PCB 126 had significant increases in liver to body weight ratio and the inflammatory marker monocyte chemoattractant protein-1 (MCP-1). Exposure to 5.0 μmol/kg PCB 126 did not contribute to weight loss. These data indicate that a concentration of 5.0 μmol/kg PCB 126 may be used to study inflammation in C57BL/6 mice without causing overt toxicity.

Polychlorinated biphenyls (PCBs) were widely used as industrial chemicals and are ubiquitous human and environmental contaminants. They are carcinogens in rodents and one congener, PCB-95, was shown to induce mutations in vivo. PCBs can be metabolized to dihydroxy- and further to quinoid metabolites both in vivo and in vitro. Quinoid metabolites may form adducts at nucleophilic sites in important cellular proteins, like cytochrome c, which may be involved in the toxic and carcinogenic effects of PCBs. (Supported by NIEHS Superfund Program P42 ES013661.)

Firemaster 550, a mixture of four flame retardants that are either known to be toxic or lack adequate information, continues to be used as a replacement for polybrominated diphenyl ether (PBDE) flame retardants. Two of the four ingredients: 2,3,4,5-tetabromo-ethylhexylbenzoate (TBB) and 2,3,4,5-tetabromo-bis(2-ethylhexyl) phthalate (TBPB) have been found in blubber of marine mammals as far as the North Pole, and are also detected in house dust and sewage sludge from wastewater treatment plants. Sharing similar properties with the PBDEs, these new brominated flame retardants (new BFRs) are likely to enter through the food chain and/or via inhalation/ingestion of dust dust and, therefore, may pose health risks. We have developed an analytical method that can detect TBB, TBPB, as well as other commonly used new BFRs (2,4,6-trimethylphenyl ethyl allyl (ATE), 1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane (p,p'-TBECH), 2-bromoallyl-2,4,6-tri-bromophenyl ether (BATE), Pentabromotoluene (PBT), Pentabromoethylbenzene (PBEB), 2,3-dibromopropyl-2,4,6-trimethylphenyl ether (DPTE), Hexabromobenzene (HBB)) simultaneously with PBDEs. TBPB is the most problematic due to its sensitivity to acid treatment (e.g., sulfuric acid silica gel). By using liquid-liquid manual extraction in combination with a two-step Solid Phase Extraction (SPE) acid free cleanup procedure, we are able to measure these new BFRs in spiked bovine serum by high resolution gas chromatography coupled with high resolution mass spectrometry in electron ionization using a DB-5ms (15 m × 0.25 mm × 0.1 μm) capillary column. The mean recoveries of surrogate standards were 80% for PBDEs (13C BDE-28—13C BDE-209), 96% for 13C HBB, and 117.6% for 13C BTBPE. Matrix spike recoveries from bovine serum for all measured BDE congeners and new BFRs ranged from 65% for BDE-209 to 90% for BDE-66, and from 73% for BDE to 130% for TBPB, respectively. This method can be used in biomonitoring studies to monitor the levels and trends of new BFRs and PBDEs in human serum.
cific communities were obtained during the period 2005 - 2011. 475 game and fish samples were collected (161 game birds; 290 fish; 24 large mammals). Results and Conclusions: Preliminary results indicate that toxic metals and organochlorine contamination is negligible in most wild game, but several fish species had elevated levels of mercury. Nevertheless, as the school children seldom reported consuming fish, concern is tempered. When one takes into account the socio-cultural benefits of the hunting activities and the nutritional benefits of a traditional diet (second component in the present study), one cannot deny the importance of the traditional diet to First Nations adolescents.

2513 A COMPARATIVE TOXICITY STUDY IN RATS AFTER IN UTERO AND LACTATIONAL EXPOSURE TO PCB180 OR TCDD.

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In this study, the effects of in utero and lactational exposure of rats to highly purified PCB180 (99.9%) were compared to effects caused by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Pregnant Sprague Dawley rats were given PCB180 (2,2',3,4,4',5,5'-heptachlorobiphenyl) at daily oral doses between gestational days (GD) 7 to 10; total doses were between 0 and 1000 mg/kg b.w.; or a single oral dose of TCDD from 0, to 1.0 μg/kg b.w. on GD 11. One pup/sex/litter were sacrificed at PND7, 35 or 84 for PCB180 and at PND7, 35, or 70 for TCDD. Dose-dependent decreases in body weight of offspring were only observed after μgle oral dose of TCDD from 0, to 1.0 g/kg b.w. on GD 11. One pup/sex/litter were not affected. The most sensitive effects of this study were decreased hepatic atRA, codhRA, REOH and REPA along with accumulations of a retinyl ester was observed for TCDD. The EROD activity of the PCB180 was increased 2 – 10 fold in a dose dependent manner, starting from the dose of 10 mg/kg b.w. For comparison, CYP1A1 mRNA expression data from TCDD treated rats showed an induction from 13 – 2000 fold compared to control group, starting at a dose level of 0.05 μg/kg b.w. In conclusion, the response to PCB 180 differed remarkably as compared to the response to TCDD, the prototype DL-compound for most endpoints studied.

2514 RETINOID SYSTEM MODULATIONS INDUCED BY A PURIFIED DE-71 MIXTURE IN AN OECD TG407 RAT STUDY EVALUATED IN RELATION TO HUMAN EXPOSURE LEVELS.

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Pentabromodiphenylethers (pentaBDEs), constituents of technical DE-71, interfere with the endocrine system. Purified DE-71 was given by gavage to 5 male or female Wistar rats per dose-group for 28 days (OECD 407) and retinoids were determined by HPLC. Benchmark doses (BMD) were established for all trans-retinoic acid (atRA), 9-cis-4-oxo-13,14-dihydroretinoic acid (codhRA), retinol (REOH) and retinyl palmitate (REPA) concentrations. A multivariant partial least-squares regression between hepatic retinoid concentrations and toxicological observations was performed. Margins of exposure (MOEs) were computed based on animal tissue retinoid alterations by DE-71 and human BDE-exposure levels. Dose-dependent decreases of hepatic atRA, codhRA, REOH and REPA and decrease in serum REOH was observed in male rats, as in female rats, apart from renal REPA and serum REOH, which were not affected. The most sensitive effects of this study were decreased hepatic atRA and codhRA concentrations. Decreased hepatic retinoid concentrations were associated with decreased body weight reductions, liver weight increases, induced hepatic CYP1A, CYP2B and CYP3A, as well as reductions in serum total thyroxine. MOEs in the range of 0.03–2000 for general population, fish-consumers from a contaminated lake, and electronics dismantling workers were calculated (total assessment factor=25). The retinoid system modulations in regulatory toxicology studies occur at dose-levels that are of relevance to human exposure situations.

2515 SHAM SURGERY RECAPITULATES THE EFFECTS OF GONADECTOMY FOLLOWING PCB EXPOSURE ON STRIATAL Dopamine IN MALE AND FEMALE MICE.

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Polychlorinated biphenyls (PCBs) are widespread environmental contaminants that reduce dopamine (DA) concentrations in cells in culture, in laboratory rodents and in non-human primates. However, studies have neither examined the neurochemical effects of PCB exposure in both male and female animals nor the consequences of gonadectomy (GDX). We therefore determined the effects of long term exposure to PCBs in aducated rat chow (500 mg/kg) for 70 days in intact (non-surgically treated) and in GDX male and female B6 mice. Intact males, in a manner similar to that seen following exposure to MTPt or 6-OHDA, showed significant loss of striatal DA while intact females were not affected. In order to begin to determine the role of gonadal hormones in these sexually dimorphic responses, we again exposed GDX male and female mice to PCBs using the regimen described above. However, unlike other studies, we compared the effects of GDX using sham surgically-treated mice, rather than intact controls. Effects on striatal DA were again sexually dimorphic. Both GDX and sham-GDX female mice showed significant reductions in striatal DA compared to the lack of effect seen in intact females. In contrast, both GDX and sham-GDX male mice were protected against the loss of striatal DA compared to reductions seen in intact male. We suggest that these sexually dimorphic effects of surgery following exposure to PCBs may be due to interactions between PCBs and surgical stressors, both of which have been shown to reduce pituitary hormone levels that influence circulating gonadal hormones. These results underscore the importance of using appropriate controls when comparing the effects of DA neurotoxics following GDX as well as raising potential concerns related to the role of physiological and severe psychological stressors as potential risk factors in the etiology of Parkinson's disease. Supported in part by NIH grant R01 ES014675 to RFS.

2516 TELOMERASE REACTIVATION WITH INCREASED CMYC, HTER AND HTR GENE EXPRESSION REVERSES TELOMERE SHORTENING IN HUMAN SKIN KERATINOCYTES: A POTENTIAL MECHANISM OF PCB CARCINOGENESIS.

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Polychlorinated Biphenyls (PCBs), an environmental pollutant, are probable human carcinogens. Activation of telomerase activity and lengthening of telomeres are key steps in cancer initiation and progression. To explore if PCBs affect telomerase and telomeres, immortal human skin keratinocytes were exposed to PCB congeners 28, 126 and 153 at 5μM concentrations for 48 days. Cells were re-seeded every 6th day with fresh medium plus compound and telomerase activity, telomere length (pPCR), CMYC, HTER, hTR, TF1, TRF2, CYP1A1 mRNA (RT-PCR), CYP1A1 activity (EROD production), cell cycle distribution (flow cytometry), and superoxide (DHE oxidation), hydroperoxide (DFCH oxidation) level were determined. All PCB congeners reduced telomerase activity and telomere length, PCB126 caused the most prominent reduction of telomerase activity (50%), hTR and hTERT mRNA (10%), telomere length (40%) and cell growth, along with an increase in CYP1A1 mRNA (and activity), telomere protein TRF1 and TRF2 mRNA, and superoxide and hydroperoxide levels from day 6 to 48. Continuous treatment with PCB126 until day 90 resulted in an increase in cell growth, cMYC, hTERT, and hTR mRNA level (to 130%) along with re-activation of telomerase activity (to 100%) and re-elongation of telomere length (to 90%) from day 54 on. From day 66, a decrease in TRF1 and TRF2 mRNA levels was observed; Trf proteins restrict access of the telomerase enzyme to telomeres. The increase in cMYC, HTER, and HTR along with decrease in TRF1 and TRF2 transcripts after critical telomere shortening may be an indication of genomic instability. This study shows for the first time that PCBs initially reduce telomerase activity, telomere length, and
cell growth, with possible mechanistic connections to increased oxidative stress, but prolonged exposure lead to telomerase re-activation, telomere lengthening and increased cell growth, a hallmark in carcinogenesis. [Supported by NIEHS P42ES013661].

2519 ELIMINATION OF PCB11 AND THE FORMATION OF ITS HYDROXYLATED METABOLITE IN RATS AFTER ACUTE INHALATION EXPOSURE.

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The recent discovery of 3,3′-dichlorobiphenyl (PCB11) being released from paint and pigments stresses the urgency and importance to investigate the health risk associated with PCB11 exposure. The high level and ubiquity of PCB11 in Chicago air indicates that inhalation is very likely the major route of exposure. We performed an acute inhalation exposure study to PCB11 in rats to understand its biological fate. We generated vapor-phase PCB11 under carefully-controlled conditions into a moving airflow that was then diluted and supplied to a nose-only exposure chamber. Chamber outflow was sampled using XAD cartridges and characterized using GC-ECD. After 2 hr of exposure, the 3 groups of PCB-exposed Sprague-Dawley rats were serially euthanized at 0, 4 and 8 hr post exposure, while 2 groups of sham-exposed rats were euthanized at 0 and 4 hr post exposure. Lung, liver, blood, muscle, brain and adipose tissue were collected for PCB measurements. A pressurized liquid extraction and clean-up method was developed to reliably quantify PCB11 and hydroxylated (OH)-PCB11 from tissue samples using GC-ECD. This method allowed an efficient recovery of PCB11 (76 ± 8%) and its major metabolite 4-OH-PCB11 (58 ± 6%). In our low-level acute exposure (90 μg/m3), each rat received 2 μg PCB. A rapid decay of PCB11 was seen in lung (half-life = 2.6 hr), yet no 4-OH-PCB11 was found in lung tissue. In contrast, PCB11 was detectable in most other organs, the liver containing the most abundant PCB11 in lung. 4-OH-PCB11 was found in livers of most animals (40 mg/g tissue on average). The presence of the single metabolite was further confirmed by GC/MS. We conclude that inhaled PCB11 was rapidly eliminated in the lung and distributed to the liver as the primary site for metabolism. Further, we demonstrated that the lower-chlorinated PCBs can be metabolized to OH-PCB very quickly in vivo and therefore the metabolites are very likely the real driving force for toxicity of these compounds.

2520 SULFATED METABOLITES OF POLYCHLORINATED BIPHENYLs BIND WITH HIGH AFFINITY TO THE THYROID HORMONE TRANSPORTER TRANSTHYRETIN.

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Exposure to polychlorinated biphenyls (PCBs), a class of environmentally persistent and hazardous chemicals, is related to various adverse human health effects. Among the observed effects are pathological abnormalities in the thyroid and thyroid hormone levels. Certain hydroxylated metabolites of PCBs (OHPCBs) are known to be capable of displacing L-thyroxine from one of its transporter-proteins, transthyretin (TTR). In the human, TTR is assumed to be a mediator for the transport of thyroid hormones across the blood-brain barrier and the placenta. We have hypothesized that sulfated metabolites of OHPCBs also bind with high affinity to TTR. The sulfate esters derived from 2′-OH PCB 3, 3′-OH PCB 3, 4′-OH PCB 3, 4′-OH PCB 9, 4′-OH PCB 12 were examined for their ability to bind to human TTR. The observed Kd values for the high-affinity binding site in TTR ranged from 0.6 nM to 10 nM for these sulfated PCBs as compared to 0.9 nM for L-thyroxine. At the low affinity binding site in TTR, the sulfate esters of 4′-OH PCB 9 and 4′-OH PCB 12 displayed Kd values of 1.300 nM and 1.700 nM, respectively, while the comparable Kd value for L-thyroxine at this site was 900 nM. The corresponding OHPCBs were examined for their ability to serve as substrates for sulfation reactions catalyzed by rat and human forms of sulfotransferase 1A1, and all were substrates for both enzymes. Thus, our current results on the binding of PCB-sulfates to TTR suggest a potential relevance in PCB-mediated disruption of thyroid hormone homeostasis. [Supported by NIH P42 ES013661]
2522 USING YEAST FUNCTIONAL TOXICOGENOMICS TO DECIPHER THE TOXICITY OF ORGANOCHLORINATED PESTICIDES.

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Exposure to organochlorinated pesticides (OCPs) has been linked to neurotoxicity, endocrine disruption, and cancer, but the cellular mechanisms of toxicity behind these effects remain largely unknown. It was hypothesized that a chemical genomics approach using a Saccharomyces cerevisiae gene deletion library could help elucidate the cellular mechanisms by which various OCPs induce toxicity. Pools of deletion strains were exposed in triplicate for five and fifteen generations to the IC20, 50% IC20, and 25% IC20 OCP concentrations. The oligo sequences unique to each deletion strain were PCR-amplified and hybridized to TAG4 arrays to identify the knockout strains that showed the most growth. These results will refine the mechanism(s) in yeast and perhaps examine how the knockout or knockdown of orthologs in higher organisms, such as C. elegans or human cell lines, affects OCP toxicity.

2523 SHORT TERM PFOA ADMINISTRATION DOES NOT MARKEDLY AFFECT KEY LIPOGENIC AND ANTIOXIDANT GENE EXPRESSION IN ADIPOSE TISSUE.

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Perfluorooctanoic acid (PFOA) is a perfluorinated carboxylic acid commonly found in the environment. According to the EPA, low levels of PFOA are detectable in the blood of the general United States population. PFOA is a highly lipophilic molecule with a long half-life. PFOA is a known liver toxicant, development toxicant, and carcinogen found in serum and tissues of wildlife and humans worldwide. Studies suggest that PFOA increases body weight of mice even at low doses of 0.01mg/kg-0.30mg/kg. In another study, PFOA is considered an obesogen to mid-aged mice where insulin and leptin levels were altered at a very low concentration. PFOA is a potent activator of Ppar-alpha contributing to oxidative stress and fatty acid oxidation pathways in hepatocytes. Given its lipophilicity and persistence, the purpose of this study was to evaluate whether PFOA treatment affects fatty acid oxidation, lipid synthesis, and antioxidant response gene expression in adipose tissue. Adult male mice were treated with 1.0 or 3.0 mg PFOA/kg in corn oil for 7 days. Adipose tissue was collected and total RNA was isolated. Analysis of mRNA was completed by quantitative PCR. Results show findings of no significant difference between the control group and the treatment groups. Literature lacks data on PFOA in adipose tissue and in human health which continue to be discovered. Given its persistence, longer exposure periods and protein expression changes should be examined.

2524 PCBI26 INCREASES FAT MASS AND ATHEROSCLEROTIC PLAQUES IN APOE–/– MICE.

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Dioxin-like polychlorinated biphenyls (DL-PCBs) are ubiquitously found in the environment and in humans. Evidence in humans indicates that DL-PCBs may cause metabolic and cardiovascular toxicity, though the mechanisms through which this may occur are unclear. To study the possible toxicity of PCB on metabolic and cardiovascular functions, we orally exposed nine week old male atherosclerotic ApoE–/– mice to 50 ng 3,3′,4,4′,5-pentachlorobiphenyl (PCB126)/g body weight or olive oil control every week and a half for a total of seven doses. Mice were fed a diet high in fat (45% kcal) for the duration of the study to promote metabolic and cardiovascular stress. Although dietary intake was equivalent across treatment groups, mice exposed to PCB126 had an over 50% increase in perigonadal and inguinal fat mass (p < 0.05) presumably due to a reduction in energy expenditure. While fasting glucose levels trended to be higher in the PCB group, glucose tolerance was not altered. After the final dose, in vivo MRI imaging revealed that plaque area in the peritrochanteric area of mice exposed to PCB126 was more than doubled (p < 0.001). These preliminary findings are consistent with a role of DL-PCBs in increasing the risk of obesity and atherosclerosis in a mouse model of cardiovascular disease.

2525 IMIDACLOPRID PRODUCES MINIMAL CHANGES IN THE EEG OF LONG-EVANS RATS.


We have reported that the non-stimulus driven EEG is differentially altered by deltamethrin or permethrin (Lyke and Herr, Toxicologist, 114(S-1):265, 2010) as well as fipronil (Lyke and Herr, Toxicologist, 120(S-2):290, 2011). In the current study, we examined the ability to detect changes in EEG activity produced by imidacloprid, a neonicotinoid pesticide that binds to nicotinic acetylcholine receptors. Adult male Long-Evans rats were dosed with imidacloprid (po). A range-finding study (n=4/dosage: 50, 100, or 200 mg/kg in 0.5% methylcellulose / 0.4% Tween 80, v/v: 1 ml/kg) was conducted using observations of neurologal sign. The data suggested decreased open field rearing and mild to moderate tremors at 100 mg/kg or greater. Maximal effects were evident about 2 hours after dosing. Additional rats were implanted with epidural screw electrodes. After about 1 week recovery, non-restrained animals were gavaged with vehicle and tested for 2 days for acclimation. On day 3, the rats were dosed with vehicle, 50, or 100 mg/kg imidacloprid (n=14-15/dosage) and tested 2 hours later. EEG was recorded as 30 segments of 2 s durations, transformed using a FFT, and the spectra averaged. Treatment with either 50 or 100 mg/kg imidacloprid did not significantly alter the EEG spectra. A decrease in alpha amplitude and area (particularly between the visual cortex and frontal cortex; 35-19% decrease) and approximately 40% decrease was determined at the p<0.05 level. Approximately 23-57 animals/dosage would be required to detect these differences with 80% power. These results were different from the decreased gamma activity resulting from treatment with fipronil, increased gamma activity following treatment with permethrin, but similar to the lack of EEG changes after dosing with deltamethrin. The data show that imidacloprid produced minimal changes in CNS activity as measured by EEG, and the alterations may differ from some other pesticides. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

2526 CYTOTOXICITY INDUCED BY DELTAMETHRIN AND ITS METABOLITES IN SH-SY5Y CELLS.

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Deltamethrin, an α-cyano pyrethroid insecticide widely used throughout the world in agricultural applications, is a relatively potent neurotoxicant. Ester cleavage and oxidation, primary at the 4′ position are the two major means of deltamethrin metabolism. Esterases catalyse hydrolysis of the ester bond to form relatively non-toxic acid and alcohol moieties, whereas CYP450s catalyse aromatic hydroxylation of deltamethrin at various positions the 2′ position and notably the 4′ position, followed by glucuronidation. Although some aspects of the toxicity properties of parent compound deltamethrin have been reported in the scientific literature, limited information is available on the toxic actions of its metabolites. The aim of this study...
was to examine and characterize the neurotoxicity of the metabolites: 3-phenoy- 
benzoic acid (3-PBA), 2'-OH deltamethrin and 4'-OH-deltamethrin as the 
parent compound deltamethrin. Of the three metabolites tested in human neurob-
loma SH-SYSY cells, 3-PBA (0.01-1000 μM) did not show neurotoxicity. 
However, the metabolites 2'-OH-deltamethrin and 4'-OH-deltamethrin (10-1000 
μM) were more toxic than the parent compound deltamethrin (10-1000 μM). 
The levels of both nitric oxide (NO) and lipid peroxides measured as malondialdehyde 
(MDA) were found to be significantly increased in deltamethrin and 4'-OH-
deltamethrin-treated cells. These results suggest that oxidative stress observed in 
vitro is one of the major mechanisms of deltamethrin-induced neurotoxicity and it 
may be attributed in part to the disposition and metabolism of this chemical. This 
work was supported by projects Ref. BSCGHR58/08(UCM), Ref. No. 
S2009/AGR-1469(CAM) and Consolider-Ingenio 2010 No.CSD2007-
063(MEC), Spain.

2527 DIFFERENTIAL STATE-DEPENDENT MODIFICATION OF 
INACTIVATION-DEFICIENT RAT NAV1.6 SODIUM 
CHANNELS BY PYRETHROID INSECTICIDES. 


Pyrethroid insecticides disrupt nerve function by modifying the gating kinetics of 
transitions between the conducting and nonconducting states of voltage-gated 
sodium channels. Pyrethroids modify rat Nav1.6β1+β2 channels expressed in Xenopus oocytes in both the resting state and in one or more states that require 
channel activation by repeated depolarization. The state dependence of modifica-
tion depends on the pyrethroid examined and is consistent with the requirement of 
open state modification measured as the increase in the conductance of the pyrethroid-in-
duced sodium tail current. Resting state modification by tefluthrin was increased 
but prolonged depolarizations (up to 150 ms) caused a progressive increase in chan-
el activation, and S-bioallethrin modification is unaffected by repeated depo-
larization. Use-dependent modification by deltamethrin and tefluthrin implies that 
these compounds bind preferentially to open channels. We expressed Nav1.6Q3 
β1+β2 sodium channels in Xenopus oocytes to examine open channel modifica-
tion directly. The Nav1.6Q3 construct contains a mutation in the inactivation gate 
region that prevents fast inactivation and results in a persistently open channel. 
Deltamethrin did not detectably modify Nav1.6Q3 channels in the closed state, 
but prolonged depolarizations (up to 150 ms) caused a progressive increase in chan-
el modification measured as the increase in the conductance of the pyrethroid-in-
duced sodium tail current. Resting state modification by tefluthrin was increased 
during long depolarizations. By contrast resting modification by S-bioallethrin was not 
affected by prolonged depolarization. These studies provide direct evidence for the 
preferential binding of deltamethrin and tefluthrin (but not S-bioallethrin) to 
Nav1.6Q3 channels in the open state and imply that the pyrethroid receptor of 
resting and open channels occupies different conformations that exhibit distinct structure-activity relationships.

2528 A SINGLE DOSE OF SARIN AFTER A PROLONGED 
EXPOSURE TO STRESS AND LOW DOSES OF 
PYRIDOSTIGMINE BROMIDE (PB); DEET, AND 
PYRETHROID INSECTICIDES IN MALE RATS: 
NEURODEGENERATION IN THE CEREBRAL CORTEX 
AND HIPPOCAMPUS. 

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To understand the potential mechanisms of neuronal deficits in veterans exposed 
stress and chemicals during the Persian Gulf War, we investigated the effects of a 
single exposure of sarin (50 μg/kg/day, i.m.) on the adult male rat forebrain after a 
28-day exposure to stress and low doses of PB (0.13 mg/kg, oral), N,N-diethyl m-
toluamide (DEET, 40 mg/kg/day dermal), and permethrin (0.13 mg/kg/day, der-
mal). We quantified neuropathological alterations in four regions of the forebrain 
(cingulate, motor and somatosensory cortices, and hippocampus) in rats treated 
with: vehicle, restraint-stress, sarin, chemicals (PB + DEET + permethrin), stress + 
sarin, stress + chemicals, Stress + sarin + chemicals. Exposure to sarin, chemicals, stress + sarin and stress + chemicals caused a decreased forebrain-acetylcholinesterase 
(ACHE) and plasma butyrylcholinesterase (BCHE) activities; increased forebrain 
m2 muscarinic acetylcholine receptors; increased permeability of blood brain bar-
rier (BBB); and diffuse neuronal cell death with reduced MAP-2 immunoreactivity 
and increased glial fibrillary acidic protein (GFAP) immunoreactivity in the white 
matter. Interestingly, the above changes were not observed in animals treated with 
single treatments: chemicals, stress or sarin alone. Thus, a prolonged exposure to 
chemicals (PB, DEET and permethrin), alone or in combination with stress exacerb-
bates the vulnerability of adult rat forebrain regions responsible for motor, sensory, 
learning and memory functions to sarin induced neurodegeneration. (This study 
was supported, in part by the U.S. Army Medical Research and Materiel Command 
under contract project order DAMD 17-98-8027.)
duced into the soil and water of the surrounding environment and is highly toxic to aquatic organisms. Its toxicity to soil organisms is unknown. Neurotoxic effects of several organophosphates have been documented in the soil nematode, Caenorhabditis elegans. We hypothesized that there would be a dose- and time-dependent decrease in locomotive activity in C. elegans exposed to malathion. To test this hypothesis, we exposed C. elegans to K-medium with or without malathion (0.15 μM or 0.3 μM) for 0.5, 1.5, 6, and 24 hours. We found that the number of front body bends as a measure of the rate of locomotion. The data indicate that C. elegans exposed to 0.3 μM malathion exhibited significantly less locomotive activity within 1.5 hours of exposure. Nematodes exposed to 0.15 μM and 0.2 μM malathion demonstrated a gradual increase in locomotive activity, up to 4 and 1.5 hours respectively, which rapidly decreased thereafter. The results support our hypothesis and suggest that malathion is neurotoxic to C. elegans at concentrations lower than 0.15 μM.

**2533 MOLECULAR MECHANISM OF DICHLORVOS NEUROTOXICITY: OXIDATIVE STRESS-INDUCED CYT C RELEASE AND APOPTOSIS.**


Organophosphate (OP) compounds are potent neurotoxic chemicals that are widely used in medicine, industry and agriculture. Dichlorvos is an OP used throughout the world as an insecticide. Recent literature indicates that chronic exposure to OP compounds causes delayed neuronal cell death that involves free radical generation (ROS). These compounds also cause mitochondrial damage/dysfunction which results in generation of ROS, ultimately leading to oxidative stress. Brain is the most susceptible organ to oxidative damage because of high oxygen tension, low mitotic rate, high lipid content and low concentration of antioxidants. Considering the wide use of dichlorvos and susceptibility of brain to oxidative damage, in the present investigation we studied the effects of chronic dichlorvos exposure-induced mitochondrial oxidative stress and its role in inducing apoptotic neuronal cell death. Animals (male albino Wistar rat strain) were given 6mg/kg body weight of dichlorvos/day/sc for 12 weeks. We observed significant decrease in the activity of superoxide dismutase (SOD) accompanied by a decrease in the value of lipid peroxidation. Dichlorvos exposure also resulted in a significant decrease in glutathione peroxidase activity. The decreased level of both reduced and oxidized glutathione as observed in dichlorvos exposed animals is consistent with the GSH/GSSG ratio. All these events of oxidative damage caused damage of mtDNA, release of cytochrome c from mitochondria and activation of caspase 3. Thus, the present study suggests that chronic OP exposure may have the potential to disrupt cellular antioxidant defense system which may in turn trigger the release of cytochrome c and ultimately neuronal cell death by apoptosis that may contribute to its neurotoxic manifestations.

**2534 IMPROVING IN VITRO TO IN VIVO EXTRAPOLATION TOXICITY ASSESSMENT BY INCORPORATION OF TOXICOKinETIC MEASUREMENTS: A CASE STUDY WITH LINDANE INDUCED SEIZURES.**


In vitro toxicokinetic assessments are needed to maximize the capability of in vitro toxicity assays to predict in vivo outcomes. The purpose of this study was to determine the in vitro distribution of lindane, a non-competitive GABA receptor agonist, in rat primary neocortical neuron cultures and characterize its concentration-dependent effects on neural network activity as measured with multi-electrode array (MEA). These data were then compared to published blood and brain lindane concentrations associated with seizures. Using "faux MEAs" (identical culture dishes, but with no electrodes), the time (5-120 min) and concentration (0.1-250 μM) dependent accumulation of lindane into the neurons and the amount of lindane in the media and wells was determined by GC-μECD. Approximately 70% of the lindane was in the media while the amount in the cells never exceeded 5%. After the media and cells were removed, ~15% of the total applied dose was recovered from the wells when fresh media was added. Lindane accumulation in the cells was time and concentration dependent. Cell lindane levels were ~20-fold higher than the media concentration at the EC50, which was determined to be 1.9μM (0.58μg/ml) for an increased mean spike rate of spontaneous network electrical activity in rat primary neurons cultured on MEAs. The human (0.2-1.2 μg/ml) and rat (1.7-1.9 μg/ml) blood lindane levels associated with seizures were similar to the actual effective media levels determined in these experiments (0.4 μg/ml at the EC50). The published rat brain lindane levels associated with seizures were also ~20-fold (5-11 μg/ml) higher than the EC50 dose (11.1 μg/ml). These findings indicate that careful in vitro toxicokinetic measurements can greatly facilitate IVIVE and that in vitro MEA results are predictive of the in vivo dose-response neurotoxicity of lindane in rodents and humans. (This abstract does not reflect US EPA policy.)

**2553 THE ROLE OF MICROTUBULE ON DITHIOCARBAMATE CYTOTOXICITY.**


Environmental factors have been associated with the pathogenesis of neurodegeneration. Manganese ethylene-bis-dithiocarbamate compounds, Maneb (MB) and Mancozeb (MZ), are fungicides that have been widely used in United States. These...
Para-aminosalicylic acid, used as a potential manganese-chelating agent, had no significant effect on glutathione peroxidase (GPx) activity. Maneb-induced apoptosis was observed, including the level of glutathione thiol (GSH) and glutathione disulfide (GSSG), whereas the GSH/GSSG ratio was significantly decreased. Maneb had no toxic effect on the mitochondrial membrane potential. Fetal cells such as those treated with Maneb showed increased neurologic deficiencies. Animals were injected i.p. twice a week for 30 days, at 0, 15, 30, and 60mg of Maneb/kg body weight. Changes in body weight were found to be significantly decreased in the Nrf2 -/- mice at the 30mg dose and decreased in both the Nrf2 (+/-) and Nrf2 (+/+) groups at the 60mg dose. In addition, after 2 weeks treatment Nrf2 (+/-) mice exhibited hind limb paralysis at the 30 and 60 mg/kg doses of Maneb. Levels of total glutathione were significantly decreased in the Nrf2 (+/-) by the 15mg and 60mg doses of Maneb. Inhibition of SOD activity was significantly increased in Nrf2 (-/-) mice treated with 15 mg/kg. Manganese concentrations were significantly increased in Nrf2 (+/-) mice as compared to Nrf2 (-/-) mice at the 30 and 60 mg/kg doses of Maneb. These results indicate that Maneb may induce neurotoxicity by overwhelming oxidative defense mechanisms.

**2539 MANEB AND MANCOZEB ACTIVATE NF-KAPPA B SIGNALLING PATHWAY.**


Environmental factors have been associated with the pathogenesis of neurodegenerative conditions. Exposure to Maneb (MB), manganese ethylene-bis-dithiocarbamate, has been linked to the development of parkinsonian-like symptoms in agricultural workers. 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 and increasing inducible nitric oxide synthase (iNOS) expression represent a plausible mechanism for Mn-induced neurotoxicity. Since MB and mancozeb (MZ) both are Mn-containing compounds, it is interesting to know whether MB and MZ can activate NF-kappa B, thereby enhancing MPP+ toxicity. In this study, PC12 cells were treated with MB (20 uM) and MZ (20 uM) either alone or in the presence of SOD. The results showed that the nuclear translocation of NF-kappa B was induced after MB and MZ treatments. NF-kappa B-responsive luciferase Dual Glo reporter construct was used and confirmed the activation (about 20% increases) of NF-kappa B after MB and MZ treatments. Cells were also treated with NF-kappa B inhibitor SN-50 (25 ug/mL) for 1 hour prior MB and MZ treatments to block the activation of NF-kappa B. Cells pretreated with SN-50 showed significantly no cytotoxicity as compared to no SN-50 pretreated groups. The results demonstrated that MB and MZ activate NF-kappa B signaling pathway which is partially responsible for those pesticides induced synergistic effects on MPP+ cytotoxicity. The confirmation of MB-kappa B activation represents the first stage in elucidating a complete mechanism associated with neurotoxicity with potential application to the determination of treatment strategies of neurodegenerative diseases such as Parkinson's disease.

**2540 EXPOSURE OF RAT HIPPOCAMPAL ASTROCYTES TO ZIRAM INCREASES OXIDATIVE STRESS.**

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Pesticides have been shown in several studies to be the leading candidates of environmental toxins and may contribute to the pathogenesis of several neurodegenerative diseases. Ziram (zinc-bis(dimethylthiocarbamate)) is an agricultural dithiocarbamate fungicide that is used on a variety of plant diseases. It is also used as an accelerator in rubber manufacturing, packaging materials, adhesives and textiles. In spite of their generally acknowledged low toxicity, dithiocarbamates are known to cause a wide range of neurobehavioural effects as well as neuropathological changes in the brain. Dithiocarbamates are a family of highly reactive compounds due to their metal combining capacity and their ability to interact with sulphydryl-containing compounds. Astrocytes play a key role in normal brain physiology and in the pathologic 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. Astrocytes were exposed to 1uM of Ziram for one hour at 37 degrees C and then re-fed with complete media for 24 hours. Transmission electron microscopy showed the presence of numerous cytoplasmic lipid droplets and degenerating mitochondria which appeared swollen and contained heterogeneous content. The Ziram treated cultures were compared to controls. Numerous membrane bound inclusion bodies and vacuoles containing degraded structures were also seen in these cells. In addition, an increase of glu-
thionine peroxidase and a significant increase in oxidized glutathione were also observed in the Ziram treated cells. The ratio of oxidized to reduced glutathione calculated from the Ziram treated cells was also increased. As such, worms were first exposed to 2% glyphosate (as TD) for 30 min-utes (acute), washed, and further exposed acutely to either 10%, 12% or 20% Mn/Zn-EDTC (as TD) for the LC25, LC50 or LC75, respectively. Photomicrographs (n = 20 worms) were analyzed for fluorescence intensity, indicative of GST::GFP translation. One-way ANOVA demonstrated a statistically significant increase (**p < 0.001) in this intensity between all dual treatment groups relative to control. To determine whether up-regulation of GST::GFP was due to the dual exposure or TD alone, a second paradigm was tested in which worms were acutely exposed only to 2% glyphosate (as TD). One-way ANOVA demonstrated a statistically significant increase (**p < 0.001) in fluorescence intensity in the three dual treatment groups relative to 2% glyphosate (as TD) only, with TD similar to control. Taken together, these data suggest that dual exposure to TD followed by MZ increases oxidative stress in a non-dose-response manner in C. elegans, and the observed up-regulation of GST::GFP may be due predominantly to MZ exposure.

**2543 DOPAMINERGIC TOXICITY OF THE ATRAZINE DEGRADATION PRODUCT DINITRILITRIOAZINE AND THE THIOCARBAMATE PESTICIDES S-METHYL-N, N-DIETHYLTHIOCARBAMATE (DETC), MOLINATE, AND ETHYL N,N-DI-N-PROPYLTHIOCARBAMATE (EPCT) IN CAENORHABDITIS ELEGANS.**

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Parkinson's disease (PD) is the second-most common neurodegenerative disease in the United States, and is increasing in prevalence. PD is characterized by the loss of neurons in the nigrostriatal pathway and dyskinesia, as well as non-motor effects. Sporadic idiopathic PD is thought to develop through interactions between age, genetics, and environment, but exact mechanisms are unknown. Epidemiological studies have supported an association of environmental pesticides and heavy metals with an increased risk of PD. Atrazine and thiocarbamate herbicides are widely used throughout the United States and are known neurotoxicants, however their effects on dopaminergic neurodegeneration are not well characterized. Due to their genetic manipulability, a Caenorhabditis elegans model with GFP expressed under the control of the dopamine transporter dat-1 (BY200 strain, Pdat-1::GFP) was utilized, allowing for visualization of dopaminergic neurons after pesticide treatments in vivo. We hypothesized that treatment of C. elegans with either the environmentally persistent degradation product of atrazine, dinitrilitriazine (DNTA), or with the thiocarbamate pesticides, S-methyl-N,N-diethylthiocarbamate (DETC), molinate, or ethyl N,N-di-N-propylthiocarbamate (EPCT) would lead to dopaminergic neurodegeneration. DETC was found to be the most toxic to C. elegans (LD50 of 0.1147 mM), followed by EPTC (LD50 of 0.1450 mM), molinate (LD50 of 1.199 mM), and DACT (LD50 of 1.648 mM). Visualization of dopaminergic neurons using fluorescent microscopy revealed weak intensity of GFP fluorescence calculated from the Ziram teated cells was also increased. As such, worms were first exposed to 2% glyphosate (as TD) for 30 min-utes (acute), washed, and further exposed acutely to either 10%, 12% or 20% Mn/Zn-EDTC (as TD) for the LC25, LC50 or LC75, respectively. Photomicrographs (n = 20 worms) were analyzed for fluorescence intensity, indicative of GST::GFP translation. One-way ANOVA demonstrated a statistically significant increase (**p < 0.001) in this intensity between all dual treatment groups relative to control. To determine whether up-regulation of GST::GFP was due to the dual exposure or TD alone, a second paradigm was tested in which worms were acutely exposed only to 2% glyphosate (as TD). One-way ANOVA demonstrated a statistically significant increase (**p < 0.001) in fluorescence intensity in the three dual treatment groups relative to 2% glyphosate (as TD) only, with TD similar to control. Taken together, these data suggest that dual exposure to TD followed by MZ increases oxidative stress in a non-dose-response manner in C. elegans, and the observed up-regulation of GST::GFP may be due predominantly to MZ exposure.

**2544 BEHAVIORAL AND NEUROCHEMICAL ALTERATIONS CAUSED BY A SHORT-TERM EXPOSURE OF MALE C57BL/6 MICE TO THE HERBICIDE ATRAZINE.**

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Excessive exposure to the widely used herbicide atrazine (ATR) affects multiple organ systems, including the brain, dopamine (DA) circuitry in particular. Therefore, ATR could potentially be one of several pesticides linked to the etiology of neurodegenerative disorders affecting DA, like Parkinson’s disease. The present study aimed to investigate effects of short-term oral exposure to a dose-range (0, 5, 25, 125, or 250 mg/kg/day) of ATR on behavioral, neurochemical, and molecular indices of toxicity in adult male C57BL/6 mice. The experimental paradigm included open field, pole and grip tests (day 4), novel object recognition (NOR) and forced swim tests (day 9), followed by tissue collection 4 h post dosing on day 10. After 4 days of exposure, ATR decreased locomotor activity (125 or 250 mg/kg) and the average grip strength (250 mg/kg). On day 9, ATR-exposed mice exhibited dose-dependent decreased performance in the NOR test (25 or 250 mg/kg) and spent more time swimming during the forced swim test (125 or 250 mg/kg). Striatal DA homeostasis was the most sensitive neurochemical target to ATR, i.e., ATR increased striatal DA and DA turnover (its metabolite HVA and the HVA/DA ratio; 125 or 250 mg/kg). In the prefrontal cortex, DA was not affected by ATR; however, DA turnover and norepinephrine were increased (125 or 250 mg/kg). ATR also increased the serotonin metabolite 5-HIAA in both the striatum (125 or 250 mg/kg) and the prefrontal cortex (125 mg/kg). At the protein level, no change in the expression of key proteins associated with striatal DA homeostasis (TH, DAT, VMAT-2) by ATR was found; immunohistochemical (striatum and qPCR (substantia nigra) analyses are ongoing. At present, these results indicate that, in part resembling ATR’s effects in longer exposure paradigms, short-term ATR exposure targets monoamine pathways, specifically striatal DA, and alters both motor and cognitive behaviors, with performance in the NOR behavioral test being the most sensitive affected by ATR.
2546 GENE EXPRESSION ARRAY, HIGH-PERFORMANCE METABOLIC PROFILING AND THIOL ANTIOXIDANT OXIDATION CHARACTERIZE DIVERGENT MECHANISMS OF PARATHYRONE AND MANEB NEUROTOXICITY IN THE LUCOS COELEUS.

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Degeneration of noradrenergic neurons of the locus coeruleus (LC) can precede and surpass dopaminergic neuron degeneration in Parkinson’s disease, yet there is little understanding of the underlying mechanisms affecting the LC. This study investigates the toxic mechanisms of parathione (PQ) and maneb (MB) in a model of pesticide-mediated locus coeruleus degeneration. Investigation of third-order-antioxidant systems revealed that PQ caused oxidation of cellular glutathione, peroxiredoxin 1 and 3 and thioredoxin 2; however, maneb did not cause oxidation of these systems. Results from gene expression array analyses showed that MB had a greater effect on gene induction by inducing 10–15 times more genes compared to PQ. In addition to common stress-induced genes, like p21, reduced induction was observed for metallothionein, heme oxygenase 1, dna-inducible transcript 3 and tribbles homolog 3. Ingenuity Pathway Analyses showed specific networks of genes that are indicative of PQ or MB toxicity. Additionally, high-performance metabolic profiling revealed a greater number of metabolic features altered by MB compared to control and PQ. Taken together, these data provide mechanistic insights relating to PQ and MB neurotoxicity and demonstrate that these two toxic agents act through divergent mechanisms.

2547 PARATHYRONE AFFECTS THE HOMEOSTASIS OF DOPAMINERGIC SYSTEM IN PC12 CELLS.

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The broad application of parathion (PQ) has given rise to widespread public concern on its potential to damage the nigrostriatal dopaminergic system because its chemical structure closely resembles that of the well-known dopaminergic neurotoxicant MPP+. However, little is known about the relevance of dopamine homeostasis changes in response to PQ exposure as the underlying mechanism of the neurotoxicity. We used PC12 cell, a popular in vitro cell model system for characterizing dopaminergic neuron to examine the effects of PQ on the dopamine homeostasis. After 24 hours treatment with different concentrations of PQ (from 0 to 1000 μmol/l), MTT tests showed that cell viability decreased with the increase of PQ concentrations. Flow cytometry analysis also showed that PQ induced cell apoptosis in a dose-dependent manner. Enzyme-linked immunosorbent assay revealed that the intracellular dopamine content increased after PQ treatment. The mRNA expressions of genes associated with dopamine synthesis (TH), storage (VMAT2), transport (DAT and D2R) and degradation (MAO) were assessed using real-time PCR. Results showed that the expression of TH and COMT were unaffected while the expression of MAO was suppressed after PQ treatment. In addition, exposure to PQ reduced the expression of VMAT2 but up-regulated the expression of D2R. Moreover, the expression of DAT was up-regulated at low dose of PQ, and then decreased at higher dose. These alterations of gene expression of MAO, D2R and DAT are consistent with an increase of intracellular dopamine content. Together, these findings suggest that PQ exposure can influence the dopamine homeostasis, which may be partly related to the neurotoxicity of PQ. (Supported by NSFC81072324)

2548 PARAQUAT PRIMES MICROGLIAL ACTIVATION AND DOPAMINERGIC NEUROTOXICITY THROUGH NOX2 AND NF-κB P50.


Paraquat, an herbicide linked to PD, exerts neurotoxic effects via microglia-derived reactive oxygen species (ROS), and is reported to prime microglia to respond to additional neurological insult in a chronic, neurotoxic manner. However, very little is known regarding the intracellular pathways underlying paraquat-induced microglia priming. Here, we address how both NOX2 and NF-κB p50 are key to this process. Analysis with an immuno-spin trapping ELISA revealed that paraquat (0.5μM) elicited microglia in vitro. Primary rat microglia cultures pretreated with paraquat (0.5μM) for 30 minutes followed by LPS (2.5 ng/ml) showed synergistic TNFα production at NOX2+/− mouse mesencephalon neuron-glia cultures. However, TNFα priming was absent in the NOX2−/− mouse mesencephalon neuron-glia cultures. Mixed glia cultures pre-treated for 30 minutes with paraquat (0.5 μM) followed by LPS (2.5 ng/ml) showed synergistic TNFα production in NF-KB p50+/− cultures, but this priming was absent in the NF-KB p50−/− cultures. In vivo data demonstrate that a single paraquat injection (10mg/kg, i.p.) was sufficient to produce a deficit in motor performance on the accelerating rotarod in only the NF-KB p50−/− mice (7 days post-treatment) compared to saline controls, supporting that NF-KB p50−/− mice may already be primed for paraquat effects. Further, immunohistochemistry analysis using the IBA1 marker revealed that microglia in NF-KB p50−/− mice demonstrated a more activated morphology in response to paraquat. Together, these data support that paraquat priming is mediated by NOX2, ROS, and NF-KB p50.

2549 THE PARKINSON'S DISEASE-LINKED PESTICIDE, ZIRAM, CAUSES SYNAPTIC DYSFUNCTION IN A DROSOPHILA MODEL.

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Parkinson’s disease (PD) is a neurodegenerative disorder characterized by the loss of dopamine neurons. The majority of PD cases are sporadic and their etiology is poorly understood. Recent studies have linked an increased risk of contracting PD with lifetime exposure to environmental toxins such as pesticides. In particular, the pesticide ziram increases the likelihood of contracting PD up to three-fold. Previous studies have shown that ziram inhibits E1 ubiquitin ligase (E1), the ubiquitin-activating enzyme required for the initiation of both polyubiquitination, which tags proteins for degradation, and monoubiquitination, which modifies protein function. Thus, reducing E1 activity may have diverse effects and previous studies have shown that acute inhibition of E1 by ziram alters both spontaneous and evoked signaling at the synapse in primary neuronal cultures. However, the how the molecular mechanisms that link ziram exposure to an increased risk of PD in humans are not known. Our aim was to develop an in vitro model to examine the link between ziram and PD. We used pHluorin imaging and electrophysiological techniques at the Drosophila neuromuscular junction to examine the effects of E1 and proteasomal inhibition at the synapse. We show that treatment with ziram and the proteasome inhibitor lactacytin results in defects in endocytosis and exocytosis, functions essential for the maintenance of proper neuronal health and signaling. These findings suggest that ziram’s link to PD by characterizing the extent to which ziram induces synaptic dysfunction and identifying the molecular mechanisms involved.
The industrial chemical arsenite is candidate for inducing developmental neurotoxicity (DNT). In this study, we have examined the effects of sodium (meta) arsenite (AsNaO₂; AS) on the developing rat fetal brain. Pregnant rats were treated orally with AS via drinking water containing 50 mg/L, or via gavage with a dose of 20 mg/kg on gestation days (GD) 9 to 15, respectively. At GD16, the caesarian section was performed. We detected AS contents in the maternal liver (ave. 25 ppm), placenta (ave. 26 ppm), and fetuses (ave. 8 ppm) including fetal brain (ave. 5 ppm), indicating that AS is transferred to the fetal brain via the placenta. Further, the AS affected maternal body weight gain, including some biochemical landmards LDH, triglyceride, and ALP. However, the AS did not affect fetal body weight or fetal mortality. Morphologically, using the coronal serial sections of fetal brain AS was not found to induce excessive cell death. AS also did not affect the neural stem cell division or development of catecholamine neurons. Our results indicate that a high-dose of AS does not induce any remarkable morpho-histopathological changes in the fetal brain during early development. The results also suggest a possibility for existence of a critical period of AS-induced DNT at later developmental stages.

### 2551 BIPHASIC EFFECTS OF LEAD ON ANGIogenesis (AG): IMPLICATIONS FOR DEVELOPMENTAL NEUROTOXICITY.

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The elaboration of vasculature through AG is essential during neurogenesis. The effect of Pb on AG, as a contributing factor in developmental neurotoxicity was investigated using Pb, as acetate (12.5, 25, 50, 150, 200 and 500 μM), compared to vascular-endothelium growth factor (VEGF) or fibroblast growth factor (FGF) in the 10 day chick choiaoalantoic membrane (CAM). Test concentrations were placed on coverslips, added to the CAM and harvested after 3 days of incubation under optimal condition of O₂/CO₂ and humidity to quantify the perfusion area (A) and number of branch points (B). Significant dose-dependent increases in A and B at doses ≤ 100 μM, and declines at higher doses were observed. New vessels were thin and tortuous. They evidenced micro-hemorrhaging at high doses. The lack of patent vessel formation was not due to cytotoxicity as doses of Pb ranging from 0.1-1000 μM did not kill human endothelial cells. To confirm these findings, 100 μM of Pb was combined to matrigel with 10⁶ MCF7-DXR tumor cells, incubated with CAM and harvested after 7 days to determine tumor mass and hemoglobin (Hb) concentrations (mg/ml) as indices of AG. Pb treatment alone and in the presence of MCF7-DXR, significantly reduced Hb. The reduction of Hb was consistent with the α₂β₁-integrin receptor blocker XT-199 failed to reverse Pb effects, suggesting an intracellular mechanism. The mechanism of increased AG with Pb treatment, relative to VEGF and FGF, suggests a Pb-induced hypoxic response and the induction of additional trophic mechanisms. This is consistent with reports of Pb-induced release of VEGF and hypoxia-inducible factor. In addition, Pb-induced edema, contributing to encapheathy, may be mediated by VEGF and VEGF receptor phosphorylation. These results suggest that although Pb may induce AG at low doses, the potency of neovascularization is compromised, contributing to poor perfusion, increased permeability and producing ischemic conditions in the developing brain.

### 2552 CNS EPGENIC CHANGES FOLLOWING DEVELOPMENTAL LEAD (PB) EXPOSURE, PRENATAL STRESS (PS), AND COMBINED PB+PS.

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Epigeneic changes could contribute to the overlapping cognitive and mesocorticolimbic impairments associated with Pb, prenatal stress (PS) and combined Pb+PS and corresponding sex differences in these effects. This study explored hypoxic-cerebral glucocorticoid receptor epigenetic alterations in two rodent models: 1) mice given continuous Pb (0 or 100 ppm water from 2 mos before breeding; dam blood Pb 5-7 μg/dl), prenatal restraint stress (PS; 3x/day from estimated gestational days 11-19), or Pb+PS; 1, 2 rats given maternal Pb (0 or 50 ppm from 2 mos before breeding to weaning, dam blood Pb 7 μg/mL; pups 15 μg/dl at PND 5-6), PS (3x/day at gestational days 16-17), or Pb+PS. Hippocampus was collected at 2-3 mos of age. Hippocampal mRNA or cDNA was extracted (Qiagen Quacube) and glucocorticoid receptor gene Nr3c1 expression measured by RT-qPCR. Pyrosequencing was undertaken for methylation analysis of Nr3c1 promoter proximal to the NGFI-A domain (Qiagen PyroMark System). Hippocampal Nr3c1 mRNA expression levels were significantly increased by combined Pb+PS but not by Pb or PS alone in female mice, consistent with enhanced effects of Pb+PS in combination. Analysis of relative methylation levels on each Cpg dinucleotide across the sequenced region showed hypomethylation primarily in Pb and Pb+PS females, findings that may be consistent with the increased transcriptional activation of the hippocampal Nr3c1 gene. Hypomethylation in males was associated with PS and Pb+PS. In female rats, Nr3c1 mRNA expression levels were reduced by Pb, but not by PS, with the greatest reduction following Pb+PS, where expression levels were significantly lower (30%) than control, PS and marginally from PB groups. Male rat Nr3c1 expression was not affected by Pb, or Pb+PS. These findings suggest that differential epige-
netic alterations could contribute to sex differences in Pb+PS associated CNS ef-
fects. Grants RO1-ES015295 (JS) and ES05017 (DCS).

### 2553 DIFFERENTIAL INFLUENCE OF POSITIVE VS. NEGATIVE LIFETIME BEHAVIORAL EXPERIENCE ON THE CNS CONSEQUENCES OF CONTINUOUS LEAD (PB) EXPOSURE, PRENATAL STRESS (PS) AND THE COMBINATION.


Experimental models typically evaluate CNS effects of developmental insults in a static capacity. The human post-birth environment is highly dynamic, however, and all organisms undergo a variety of behavioral experiences that could either potentially enhance or ameliorate the consequences of early developmental insults. This study examined how the specific nature of behavioral experience influenced the CNS consequences of developmental exposure to prenatal restraint stress (PS), and Pb+PS. Mice exposed to 0 or 100 ppm Pb from 2 mos prior to dam breeding (blood Pb ~7 μg/dl), to prenatal immobilization restraint stress (30 min 3x day at 2 hr intervals to dams from approximate gestational days 11-19), or to Pb+PS, were subjected to a behavioral trajectory after birth of experience that was either negative (4 forced swim (FS) tests at 3 week intervals) or positive (food rewarded responding on a Fixed Interval (FI) schedule of reinforcement). Behavioral experience broadly differentiated catecholamine levels of control offspring in hippocampus, frontal cortex, striatum and midbrain, particularly in males. Further, Pb, PS and Pb+PS altered brain catecholamine levels differentially in relation to beha-
vorial experience. For example, norepinephrine was increased only by Pb+PS in frontal cortex, hippocampus, striatum and olfactory bulb in FI males, but by Pb and PS in FS males. Pb, PS and Pb+PS all increased striatal dopamine turnover in FS males, whereas no changes occurred in FI males. These findings demonstrate that the specific nature of lifetime behavioral experience can influence the ultimate neurochemical consequences of developmental Pb, PS and Pb+PS exposures and thereby also potentially modify the behavioral prognosis of early developmental insults. Elaborating the role of specific behavioral experiences in this capacity could facilitate understanding of mechanisms, and provide guidance on behavioral thera-
peutic strategies. RO1-ES05017
However, similar to ID, decreases in neuronal conduction velocity were noted with Pb at PND40 suggesting that ID and Pb in combination have the potential to act on neuronal conduction integrity in the developing CNS. Furthermore signaling dysfunctions have been reported in the hippocampus with both insults singularly that may correlate with learning and memory deficits observed in affected children. For this purpose, an in vitro hippocampal neuron culture system that allows combining ID and Pb exposure was utilized to study neurotrophic signaling as an underlying mechanism of hippocampal dysfunction. Decreases in neurotrophic protein levels of whole-cell neuronal lysates were observed with ID, an effect also reported with Pb indicating that disrupted neurotrophic signaling may play a role in hippocampal dysfunction. Our data suggests that Pb and ID combined have the potential to enhance toxicity to the developing CNS.

**2555 INFLUENCES OF THE MALE AND FEMALE HIPPOCAMPAL TRANSCRIPTOME: EFFECTS OF DIFFERENT LEVELS OF EXPOSURE DURING DIFFERENT DEVELOPMENTAL PERIODS.**

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Although the effects of lead (Pb) on the developing brain have been well studied, there are still gaps in our understanding of how Pb affects the brain on a molecular level to adversely influence brain development and function. The present study examined how variables of duration, timing and dose of Pb exposure might interact with sex to affect the rat hippocampal transcriptome. Long Evans dams were fed Pb-containing food (RMH 1000 with or without added Pb acetate: 0 ppm, 250 ppm, 750 ppm) prior to breeding and stayed on the same diet through weaning. Male and female pups were exposed to lead perinatally through postnatal day 25. Other animals were exposed to the same doses of Pb but exposure started on postnatal day 1 and continued through postnatal day 45. In all cases, at weaning rats were housed 4 to a standard cage and were otherwise handled similarly until they were euthanized for collection of brain samples. Hippocampi were rapidly removed, frozen on dry ice and stored frozen until processed. Approximately 5-15 µg of total RNA was extracted from each sample for subsequent microarray analysis (Affymetrix Rat Gene 1.0 ST Array). Gene expression data were analyzed using Partek Genomics Suite and GeneGo software. There were 978 transcripts (409 unique genes) differentially expressed regardless of sex, dose and type of exposure. A greater number of transcripts were affected by perinatal exposure (741) vs. postnatal exposure (546), regardless of sex. Often, the greatest effect was seen at the 250 ppm exposure level. A wide range of biological functions and pathways were affected, some common and some unique across the conditions. These results suggest a profound effect of developmental Pb exposure, particularly low level exposure, on the hippocampal transcriptome that is modified by sex and timing of exposure. Supported by NIH RO1-ES015295.

**2556 ROLE OF GENETIC VARIATION IN THE PATHOGENICITY OF LEAD EXPOSURE: DIFFERENT EFFECTS OF LEAD ON THE HIPPOCAMPAL TRANSCRIPTOME IN RATS WITH DIFFERENT GENETIC BACKGRONDS.**


Lead (Pb) is a potent developmental toxin and genetic factors may modify the outcome from Pb exposure. Some genes have been identified that may influence accumulation and toxicokinetics of Pb. However, there might also be as yet undefined genetic factors that may lead to different outcomes from Pb exposure. Such differences may in part underlie the lack of a “behavorial signature” for Pb toxicity. The present study examined how duration, timing and dose of Pb exposure may influence the hippocampal transcriptome in male or female rats of different strains (Long Evans (LE), Fisher 344 (F-344), Sprague Dawley (SD)). Dams were fed Pb-containing food (RMH 1000 with 0 ppm, 250 ppm, 750 ppm Pb acetate) prior to breeding and stayed on the same diet through weaning. Pups were exposed to lead perinatally through postnatal day 25. In other animals exposure started on postnatal day 1 and continued through day 45. At weaning, all rats were housed 4 to a standard cage and otherwise handled similarly until they were euthanized for collection of brain samples. Hippocampi were rapidly removed, frozen on dry ice and stored frozen until processed. Approximately 5-15 µg of total RNA was extracted from each sample for subsequent microarray analysis (Affymetrix Rat Gene 1.0 ST Array). Gene expression data were analyzed using Partek Genomics Suite and GeneGo software. There were 978 transcripts (409 unique genes) differentially expressed regardless of sex, dose and type of exposure in LE, 124 transcripts in F-344, and 79 transcripts in SD. The greatest effect in LE was seen with the 250 ppm perinatal exposure in both sexes. In comparison, the greatest effect in F-344 was seen in males with the 750 ppm postnatal exposure whereas in SD, the greatest effect was seen with 750 ppm postnatal exposure. Different pathways were affected, some common and some unique across the strains. These data show a significant role of genetic variation in the pathogenicity of Pb exposure. Supported by NIH RO1-ES015295.

**2557 EPIGENETIC MODIFICATIONS FROM DEVELOPMENTAL LEAD EXPOSURE: INFLUENCES OF SEX, EXPOSURE LEVEL, AND DEVELOPMENTAL TIMING OF EXPOSURE.**


Numerous prenatal and post-natal factors can produce epigenetic modifications (e., changes in DNA methylation) that influence functional development of the nervous system. Exposure to lead (Pb) may affect the epigenome at crucial stages of prenatal or postnatal development. The present study examined possible influences of sex, exposure level, and developmental timing of exposure of Pb in Long Evans rats on genome-wide DNA methylation profiles, using Rat NimbleGen 3x720K CpG Island Plus RefSeq Promoter Arrays. Dams were fed Pb-containing food (RMH 1000 w/0 ppm, 150ppm, 375ppm Pb acetate) prior to breeding and stayed on the same diet through weaning. Male and female pups were then exposed to lead postnatal day 25. Other animals had Pb exposure start on postnatal day 1 and continue until weaning. All dams had Pb exposure postnatal day 1 through day 45. At weaning, all rats were housed 4 to a standard cage and all were euthanized on day 45 when hippocampi were removed, frozen and stored until processed. Genomic DNA was extracted on a Qiagen Qicube prior to being fragmented and enriched by Methylated DNA Immunoprecipitation and whole genome amplification and hybridization to arrays. Preliminary analyses identified approx. 15000 unique peaks, indicating differentially methylated promoters, across all conditions compared to non-Pb exposed controls. All variables (i.e., sex, exposure level, developmental timing of exposure) significantly influenced gene promoter methylation profiles. Of particular interest, animals with the lowest Pb exposure level (150 ppm), showed significant changes in methylation of promoters downstream to transcription start sites for genes related to key biological functions such as estradiol metabolism, testosterone metabolism, retinoid metabolism, and signal transduction. These data suggest that developmental Pb exposure can modify the epigenome through DNA methylation in complex ways that depend on sex, level of exposure and developmental period of exposure. Supported by NIH RO1-ES015295.

**2558 SYNERGISTIC EFFECTS OF DEVELOPMENTAL LEAD (Pb2+) EXPOSURE AND MUTANT DISRUPTED-IN-SCHIZOPHRENIA 1 (DISC1) ON BEHAVIORAL ABNORMALITIES CONSISTENT WITH SCHIZOPHRENIA: A GENE-ENVIRONMENT INTERACTION STUDY.**


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Hypoactivity of glutamatergic NMDA receptors has been implicated in the pathophysiology of schizophrenia and related mental conditions. The developmental neurotoxicant, lead (Pb2+), is a potent and selective antagonist of the NMDA receptor. Recent human studies have suggested an association between prenatal Pb2+ exposure and the increased likelihood of schizophrenia later in life. It is widely recognized that schizophrenia results from interactions between genetic and environmental factors. This study examined the neurobehavioral consequences of developmental exposure to low levels of Pb2+ in mice with inducible expression of mutant DISC1 (mDISC1), a strong candidate gene disrupted by the chromosomal translocation and associated with schizophrenia and other major mental disorders in a Scottish family. We predicted that mDISC1 and developmental Pb2+ exposure would synergistically interact to produce schizophrenia-like neurobehavioral abnormalities. mDISC1 and control mice were raised and maintained on regular food or food containing low levels of Pb2+. We evaluated schizophrenia-related behavioral abnormalities in adult male and female mice. Although Pb2+ exposure produced increased anxiety in the elevated plus maze in control and mutant mice, significantly increased locomotor activity was found in mutant mice only, with stronger effects.
on female mutant mice. Both males and females mDISC1 mice exposed to Pb2+ exhibited impaired pre-pulse inhibition of the acoustic startle. No significant differences in the test for contextual or cue-dependent fear conditioning were found. Our data indicate that developmental Pb2+ exposure and mDISC1 interact synergistically to produce schizophrenia-like behavioral alterations in mice, possibly by affecting NMDAR neurotransmission. [Supported by ES066189 VICTER supplement to TRG]

2561 GENDER DIFFERENCES IN PARAOXONASE-2 EXPRESSION IN MOUSE PRIMARY STRIATAL NEURONS AND ASTROCYTES.

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The paraoxonase (PON) gene family consists of three members (PON1, PON2, and PON3). While PON1 and PON3 are mainly synthesized in the liver and are secreted in the plasma, PON2 is a ubiquitous intracellular enzyme, expressed in most tissues, but not present in plasma. In the mouse brain, the highest levels of PON2 are found in the nucleus accumbens, the substantia nigra and the striatum. PON2 levels were higher in astrocytes than in neurons in all brain areas. While all PONs play two roles, one in antioxidant defence and the other in neuroprotection, PON2 was shown to protect CNS cells from oxidative stress, as evidenced by a high susceptibility of cells from PON2 knockout mice. We also found that in all mouse tissues PON2 protein levels were higher in female animals. In primary striatal astrogocytes and neurons, PON2 protein levels in females were 4.8-fold and 2.5-fold higher, respectively. In accordance with the antioxidant role of PON2, the higher PON2 levels protected striatal neurons and astrocytes of female mice from the toxicity of the oxidants H2O2 and DMNQ. Brain cells from male mice were more susceptible to the toxicity of both compounds by 3.1 to 4.2-fold. We measured GSH levels in striatal neurons and astrocytes from female and male. GSH levels did not differ between cells isolated from the two genders, suggesting that the neuroprotective effect is due to the differential PON2 expression. In male mixed-sex striatal astrocytes, estradiol exposure induced a significant dose dependent increase in PON2 expression. In gender-specific cultures, after estradiol (100 nM) treatment, PON2 was significantly increased by 4.5-fold in male astrocytes and by 1.8-fold in female astrocytes. Our findings suggest a sex-dependent neuroprotective effect to oxidative stress-mediated neurotoxicity in the mouse CNS, due to a differential expression in PON2 (Supp. in part by ES04606).

2562 DIAZINON AND DIAZOXON IMPAIR ASTROCYTE ABILITY TO FOSTER NEURITE OUTGROWTH.

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Increasing evidence from animal and epidemiologic studies suggests that organophosphorus insecticides (OPs) are developmental neurotoxicants. The effects of the widely-used OP diazinon (DZ) and its active metabolite, diazoxon (DZO), on neuronal differentiation and function have not been fully explored and are the focus of this project. We have previously shown that astrocytes play a major role in fostering neurite outgrowth in neurons. We have also shown that oxidants impair the neurotogenic ability of astrocytes. The overarching hypothesis of this project is that by increasing oxidative stress in astrocytes, DZ and DZO may indirectly impair neuronal development by disrupting glial-neuronal interactions. Sub-cytotoxic concentrations of DZ or DZO (10 μM) caused a 2-fold increase in reactive oxygen species (ROS) in primary rat cortical astrocytes compared to untreated controls. Astrocytes previously exposed to DZ or DZO (0.1, 1, 10 μM) for 24 hr were incubated with primary rat hippocampal neurons for an additional 24 hr, and neurite outgrowth was measured morphologically. A 50% decrease in the length of the longest neurite was observed in neurons incubated with astrocytes previously exposed to 10 μM DZ or DZO, as compared to untreated controls. This effect was prevented when astrocytes were pre-treated with the antioxidants melatonin and N-t-butyl-alpha phenylisothiocyanate (PBN). Both DZ and DZO (10 μM) caused a 30% decrease in lysate levels of the neurotrophic protein fibronectin in astrocytes. Fibronectin is an extracellular matrix protein produced and secreted by astrocytes, and is recognized as playing a vital role in neurite outgrowth. Pre-treatment of astrocytes with melatonin and PBN also prevented this decrease in fibronectin. These results suggest that by increasing oxidative stress in astrocytes, DZ and DZO impair astrocytes’ ability to foster neuronal development, partly by decreasing levels of fibronectin produced by astrocytes. (Supported by ES07032 and P50ES09601)

2563 CHLORPYRIFOS DURING DEVELOPMENT REDUCES ANXIETY-RELATED BEHAVIOR IN ZEBRAFISH LARVAE.

H. Richendrfer and R. Creton. MCB, Brown University, Providence, RI, Sponsor: S. Pelkowski.

Neurobehavioral disorders such as anxiety, autism, and ADHD are becoming more prevalent even though the causes for these disorders are still widely unknown. Environmental toxins, such as organophosphate pesticides (OPs) are highly...
ubiquitous in the environment because of their efficacy in crop protection against destruction from insects. Ingestion of chlorpyrifos following the last dose and this level of inhibition persisted through 12hrs. By 24hrs, some recovery of activity was observed with the two higher dosages but not the lowest dosage. This persistent inhibition suggests that if daily exposure was occurring, the activities of these enzymes would not have the opportunity to return to control levels. Forebrain 2-AG and AEA levels were significantly elevated at these dose related manner and continued to increase to peak levels at 12hrs. Substantial recovery had occurred by 24hrs but levels remained significantly elevated above control levels. Thus, repeated exposure to CPS results in a persistent inhibition of the endocannabinoid metabolizing enzymes and a cyclic pattern of elevation of the endocannabinoids themselves. This alteration of endocannabinoid signaling during brain maturation could exert long term effects, leading to permanent alterations in neuronal brain circuits and behavioral responses.

2564 REGIONAL BRAIN DOSIMETRY FOR THE ORGANOPHOSPHORUS INSECTICIDE CHLORPYRIFOS IN THE PREWEANLING RAT.

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Infants and children may be sensitive to adverse effects of pesticide exposure associated with ingestion of residues on food. Organophosphorus (OP) insecticides, like chlorpyrifos (CPF), have been implicated as developmental neurotoxins; however, aspects of dose-response relationships are poorly understood particularly with regards to critical window(s) of vulnerability and target tissue (i.e. brain) dosimetry. Ongoing research is focused on evaluation of in vivo/in vitro brain chlorpyrifos dosimetry in the preweaning rat pup and for comparison in adult male rats. At post-natal day-10 (PND-10), both male and female pups were orally administered CPF at 1 or 5 mg/kg/day for 5 consecutive days and humanely sacrificed 4 hours after the last dose. Adult male rats were likewise orally dosed at 5 mg CPF/kg/day (5 doses). Whole brains and brain regions (forebrain, midbrain and cerebellum) were analyzed for CPF and its major metabolite trichloropyridinol (TCP), its major metabolite. In addition, brain region acetylcholinesterase (AChE) activity was determined in PND-15 pups. In vitro metabolism studies were conducted with hepatic and brain microsomes and whole brain homogenates prepared from naïve adult male rats. A comparison of whole brain dosimetry (5 mg CPF/kg/day) suggests that the concentration of CPF and TCP in the brain of preweaning rats is comparable to adults following oral exposure. In both male and female PND-15 pups, regional brain CPF concentration tended to be forebrain > midbrain > cerebellum; whereas, the concentration of TCP was fairly comparable across gender and brain regions. In vitro brain metabolism studies support both the bioactivation of CPF to the neurotoxic metabolite CPF-oxon and detoxification of CPF to TCP. The importance of localized brain metabolism is highly relevant for lipophilic pesticides that potentially sequester in the brain where localized brain disposition and metabolism may be critically important for understanding target tissue dosimetry. Supported by CDC/NIOSH grant R01 OH008173.

2565 PATTERN OF INHIBITION OF BRAIN ENDOCANNABINOID METABOLIZING ENZYMES FOLLOWING DEVELOPMENTAL CHLORPYRIFOS EXPOSURE.

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The endogenous cannabinoids 2-arachidonylglycerol (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. We have previously reported that developmental repeated exposure to low levels of chlorpyrifos (CPS) results in inhibition of these enzymes as well as accumulation of endocannabinoids in rat forebrain at 48hrs post exposure. However it is not clear if these effects are persistent or transient. To determine this, 10 day old rat pups were exposed daily for 7 days to either corn oil or increasing dosages of CPS (1, 2.5, or 5 mg/kg) by oral gavage. FAAH and MAGL activity was inhibited in a dose dependent manner to the last dose and this level of inhibition persisted through 12hrs. By 24hrs, some recovery of activity was observed with the two higher dosages but not the lowest dosage. This persistent inhibition suggests that if daily exposure was occurring, the activities of these enzymes would not have the opportunity to return to control levels. Forebrain 2-AG and AEA levels were significantly elevated at these dose related manner and continued to increase to peak levels at 12hrs. Substantial recovery had occurred by 24hrs but levels remained significantly elevated above control levels. Thus, repeated exposure to CPS results in a persistent inhibition of the endocannabinoid metabolizing enzymes and a cyclic pattern of elevation of the endocannabinoids themselves. This alteration of endocannabinoid signaling during brain maturation could exert long term effects, leading to permanent alterations in neuronal brain circuits and behavioral responses.

2566 EVALUATION OF DEVELOPMENTAL NEUROTOXICITY OF MANCOZEB IN RATS.

M. Aggarwal, S. Marty and R. Billington, Dow AgroSciences, Indianapolis, IN. Dow AgroSciences, Indianapolis, IN. The pyrethroid pesticides are a commonly used class of insecticides worldwide. Due to the unique susceptibility of developing animals to the toxicity of these compounds there is increasing concern over the potential developmental neurotoxicity of pyrethroid exposure. However, few data are available on molecular mechanism(s) of developmental pyrethroid neurotoxicity. Previous work from our laboratory has shown that in utero exposure to the type II pyrethroid deltamethrin can produce persistent downregulation of voltage-gated sodium channel (Na(v)) mRNA and acute exposure can transiently downregulate Na(v) protein in an age-dependent fashion. In this current work PND 1-10 rats were exposed to deltamethrin at 0 or 0.1 mg/kg in corn oil, by oral gavage daily. At PND 10 the animals receiving deltamethrin were divided: 1 group continued receiving 0.1 mg/kg daily, while the second group began receiving 0.5 mg/kg daily until weaning at PND 21. Animals were sacrificed at PND 30 and samples were probed for Na(v) protein levels via western blot. Animals showed approximately 30% reduction in total Na(v) protein at 0.1 mg/kg and approximately 40% reduction at 0.5 mg/kg in the striatum, Individual Na(v) isoforms were measured using isoform-specific antibodies. Na(v) 1.1 protein levels were reduced approximately 30% at 0.1 mg/kg and 45% at 0.5 mg/kg, while Na(v) 1.2 protein showed no such downregulation. These data show an isoform-specific response to developmental deltamethrin exposure that corre-
sponds to sensitivity, as Na(v) 1.2 has been shown to be the least sensitive to detyramethin in previous in vitro studies (Meacham et al, 2008). These data suggest that the sensitive period for altering Na(v) protein levels extends at least until weaning, which corresponds with our earlier acute studies. Unlike our acute studies, this study suggests that, at doses with no symptoms of pyrethroid toxicity, there are still alterations in Na(v) protein levels.

Permethrin and bioallethrin belong to the Type 1 class of pyrethroid pesticides. The primary mechanism of action is interference with nerve membrane sodium channels that results in increased neuronal activity. We have earlier reported on developmental neurotoxic effects after repeated, PND 10 to PND16, neonatal exposure to pyrethroids. The effects were manifested as altered spontaneous behavior, hyperactivity and reduced cognitive function and changes in cholinergic muscarinic/nico-
tinic receptors in the cerebral cortex of neonatal and adult mice. The present study was undertaken to compare repeated and single exposure to permethrin and bioal-
lethrin during the neonatal brain growth spurt (BGS) on adult spontaneous behav-
ior in a novel home environment. Neonatal NMRI male mice were given permeth-
rin, orally (0.55; 3.3; 6.6 mg/kg bw/day) on PND 10-14, or just a single oral dose of 6.6 mg/kg bw on PND 10. Bioallethrin was given as a single oral dose of 0.7 mg/kg bw on PND 10, and compared to earlier published data on repeated ex-
posure. Mice serving as controls received the 20 % fat emulsion vehicle.

Spontaneous behavior test (locomotion, rearing, total activity) in 2-month-old mice revealed a significant higher activity in mice exposed to repeated doses of 6.6 mg/kg bw/carbaryl on PND 10-14, or just a single oral dose of 6.6 mg/kg bw on PND 10. Bioallethrin was given as a single oral dose of 0.7 mg/kg bw on PND 10, and compared to earlier published data on repeated exposure. A single dose of 0.7 mg bioallethrin on PND 10 caused the same effects as a repeated dose of 0.7 mg between PND 10 to PND 16. This demonstrates that a single dose of these pyrethroids can cause the same developmental neurotoxic effects as that seen following repeated doses over one week during the neonatal BGS period in mouse. This research provides is consistent with previous findings that exposure during the BGS can result in persistent behavioral effects.

# 2568 COMPARISON OF SINGLE AND REPEATED EXPOSURE TO LOW DOSES OF PYRETHROIDS, PERMETHRIN AND BIOALLETHRIN, DURING NEONATAL BRAIN DEVELOPMENT ON ADULT SPONTANEOUS BEHAVIOR.

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Factors impacting sensitivity to chemicals across life stages include toxicokinetic and toxicodynamic changes. We systematically compared the dose-response (3, 7.5, 15, 22.5 mg/kg) and time-course (3 or 15 mg/kg at 30, 60, 120, 240 min) of acute effects of carbaryl (oral gavage) in preweaning (postnatal day, PND; 18) and adult male Brown Norway rats ranging from adolescence to senescence (1, 4, 12, 24 mo).

Motor activity (MA), brain and RBC cholinesterase (ChE) activity, and brain and plasma carbaryl concentrations were measured in the same animals. At the time of peak effect (40 min), PND18 rats were the most sensitive to the brain ChE-inhib-
hibiting effects of carbaryl, but 12- and 24-mo rats showed more MA depression even at similar levels of brain ChE inhibition. Time-course data showed that MA depression reached a maximum earlier, and recovered faster, in the 1- and 4-mo rats compared to the other ages; slowest recovery and maximal effects were seen in the 24-mo rats. The recovery of brain ChE inhibition varied with age only after 15 mg/kg, whereas recovery following 3 mg/kg as well as RBC ChE inhibition (both doses) has corresponded with sensitivity at our earlier acute studies. Unlike our acute studies, this study suggests that the sensitive period for altering Na(v) protein levels extends at least until weaning, which corresponds with our earlier acute studies. Unlike our acute studies, this study suggests that, at doses with no symptoms of pyrethroid toxicity, there are still alterations in Na(v) protein levels.

# 2569 CARBARYL EFFECTS ON OXIDATIVE STRESS IN BRAIN REGIONS OF ADOLESCENT AND SENESCENT BROWN NORWAY RATS.


Oxidative stress (OS) plays an important role in susceptibility and disease in old age. Understanding age-related susceptibility is crucial in assessing the human health risks of chemicals. Growing evidence implicates OS in carbamate toxicity in addition to cholinesterase-inhibiting effects. This study explored OS as a potential toxicity pathway for carbaryl exposure (a carbamate insecticide) and whether these effects were age-dependent. OS-related events included reactive oxygen species pro-
duction [NADPH Quinone oxidoreductase 1 (NQO1), NADH Ubiquinone re-
ductase (UBIQ)], antioxidant homeostasis [total antioxidant substances (TAS)], and oxidative damage (total aconitase).

Male Brown Norway rats (1 and 24 months) were dosed orally with carbaryl (0, 7.5 or 15 mg/kg) in corn oil. Frontal cortex (FC), cerebellum (CB), striatum (ST), and hippocampus (HP) were dis-
sected 2 hours after exposure, quick frozen, and stored at -80°C until analysis. Results indicate that substantial age-related increases in ROS production (-6x in UBIQ-RD and > 8x in NQO1), decreases in antioxidant homeostatic mechanisms (i.e. TAS ~2x), and decrease in aconitase activity (2-3 x) regardless of brain area. The effects of carbaryl treatment were age- and brain area-specific. In general, ef-
fecs on OS parameters were greater at 1 mo vs. 24 mo. Interestingly, measures of OS production were often in opposition to each other (e.g. UBIQ-RD decreased -26% in the striatum at 1 mo of age while NQO1 was increased -26%) possibly indicating activation of different compensatory pathways. Carbaryl generally de-
creased or had no effect on TAS. Also similar to the age effect, aconitase levels were decreased by carbaryl in some brain regions. These results indicate OS as a potential toxicity pathway, but the complex interaction between age and carbaryl exposure on OS parameters in different brain regions require further investigation. (This ab-
stract does not necessarily reflect USEPA policy).

# 2570 DOSE-RESPONSE AND TIME-COURSE OF MICROTOXICITY AND TISSUE CONCENTRATIONS OF CARBARYL IN BROWN NORWAY RATS FROM PREWEANING TO SENESCENCE.


Lysosomes, first discovered by Dr. Christian de Duve more than five decades ago, are membrane-enclosed compartments filled with acid hydrolytic enzymes that di-
gest macromolecules from the endocytic, autophagic, and phagocytic membrane-trafficking pathways. Lysosomes are central in ChE inhibition genetics and pharmacokinetics, including cholesterol homeostasis, plasma membrane repair, bone and tissue remodeling, and pathogen defense. Lysosomal malfunction as a consequence of gen-
etic deficiency of a lysosomal enzyme or membrane protein can trigger lysosomal storage diseases with various clinical abnormalities such as organomegaly and cen-
tral nervous system dysfunction. Reduced lysosomal storage mechanism has been shown to result in mitochondria-mediated apoptosis. Inducers of lysosomal mem-
brane permeabilization include, but are not limited to, oxidative stress, lipids, cas-
pases, microtubule toxins, and metals. A number of basic lipophilic compounds have been shown to accumulate into acidic organelles, including lysosomes, in a process known as lysosomal tropism. Lysosomotropic agents include structurally di-
verse chemicals that are used in clinical medicine such as chloroquine, amiodarone, imipramine, tamoxifen, and imatinib. In the past, phospholipidosis associated with drug lysosomal sequestration has been investigated extensively and prevailing the-
ory has been that the phospholipidosis is primarily an adaptive response rather than a toxic response. However, the relationship between physicochemical properties of compounds, lysosomal accumulation, cellular damage, impairment of membrane trafficking processes, including autophagy, and especially how this is associated with various toxicological manifestations has not been fully elucidated. The goal of this symposium is to highlight the potential role of the lysosome in drug-induced toxicity and recognize lysosome perturbation as a potential mechanism for organ toxicity.

# 2571 NEW VISIONS IN TOXICOLOGY: LYSOSOMES—ROLES IN DISEASE, TOXICITY, AND DRUG DEVELOPMENT.

S. Lu1 and J. M. Willard2. 1Pfizer Inc., San Diego, CA and 2US FDA, Silver Spring, MD.

The concept of the structure and function of the endosomal-lysosomal ap-
paratus has evolved from the pioneering work of de Duve and co-workers. The apar-
atus is responsible for the intracellular digestion of externally (endocytosis and phagocytosis) and internally (autophagy) generated macromolecules. Acidic lumen
of lysosome contain more than 50 known hydrolases, which sorted by the man
nose-6-phosphate receptor system. Understanding of lysosomal biology has
markedly increased during the characterization of lysosomal storage diseases
(LSDs). These are a group of more than 40 rare inherited metabolic disorders that
result from defects in lysosomal function. LSDs share a common pathogenesis: a
genetic defect in a specific lysosomal enzyme, receptor target, activator protein,
membrane protein, or transporter, leading to the accumulation of macromolecules
in cell lysosomes. This presentation will discuss different types of LSDs including
the mucopolysaccharidoses (e.g., Maroteaux-Lamy Disease, Hurler/Schie Disease)
and sphingolipidoses, (e.g., Gaucher's disease and Niemann-Pick disease). Their
critical clinical features and pathogenesis process will be introduced.

**2573 TRAIL-INDUCED LYSOSONAL PATHWAY OF APOPTOSIS IN HEPATOBIARY CELLS.**

Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN and
The Vollum Institute, Oregon Health & Science University, Portland, OR. S. Lu.

TRAIL, a death ligand, stimulates apoptosis in perturbed hepatocytes and mali
nant hepatocellular carcinoma and cholangiocarcinoma cells. Given the role of
TRAIL in the innate immune response and as a potential cancer therapeutic agent,
this pathway of lipoapoptosis is quite germane to hepatobiliary pathology and
cancer treatment. TRAIL stimulation of the lysosomal pathway is associated with
release of cathepsin B into the cytosol. Cytosolic cathepsin B then stimulates mito
chondrial dysfunction with subsequent caspase activation and cellular demise.
Recently we have explored the role of cellular inhibitor of apoptosis proteins (cIAP)
-1 and -2 in regulating TRAIL-mediated lysosomal dysfunction. Cellular elimina
tion of cIAP 1 and 2 by genetic or pharmacologic approaches, accelerate TRAIL
induced killing of hepatocytes. This cell killing is markedly attenuated in hepatocytes from
transgenic mice with catalytically deficient mice. Interestingly the cIAPs, which are E3 ligases,
appear to regulate the lysosomal pathway of cell death by ubiquitinating a trafficking
protein PACS-2. Ubiquitination of PACS-2 renders lysosomal trafficking in
competent, where as failure of PACS-2 ubiquitination promotes its trafficking to
lysosome. Upon TRAIL stimulation PACS-2 binds Bim which in turn activates
Bax promoting their association with lysosomes. Bax appears to oligomerize within
the lysosome membrane resulting in lysosomal release of cathepsin B into the cy
tosol triggering cell death. This information will help provide a frame work for the
development and novel therapeutic strategies effective for preventing TRAIL medi
ated human liver injury and promoting TRAIL employment as an anti-cancer agent.

**2577 OFF THE BEATEN PATH: PRECLINICAL APPROACHES TO SAFETY EVALUATION OF CELLS/GENE THERAPY, VACCINES, AND ADJUVANTS.**

J. E. Black, M. D. Green, T. MacLachlan, T. Chen and J. van

How a drug distributes within a cell is a therapeutically important consideration that can influence pharmacokinetic properties, potency and toxicity. Many
drugs contain weakly basic functional groups that can enable their extensive se
questration in highly acidic lysosomes through an ion trapping type mechanism. Some cationic amphiphilic drugs cause a lysosomal lipidosis whereas others cause
lysosomes to become swollen and vaculolated when presented to cells at relatively
high concentrations. During this presentation the mechanisms for amine accumu
lation in and egress from lysosomes will be discussed. Using quantitative assays we have established how drug-associated properties including pKa and permeability can predictably influence the degree of lysosomal uptake. In addition we have inv
vestigated the role of specific proteins and pathways in facilitating the vesicle-medi
ated trafficking of sequestered amines, and other cargo, out of lysosomes. The toxico
logical implications of this research will be highlighted.

**2578 VACCINES, ADJUVANTS, AND AUTOIMMUNITY.**

S. Gould. Toxolgy, Sanofi Pasteur, Marcy D'Estaile, France.

To predict the onset or potential development of autoimmune disease is a chal
lenge, not least because diseases are rare events, etiologies are unknown, causes are
multifactorial, and many factors (like genetics and hormones) can contribute to risk.
Even so, the concern of a risk of autoimmunity as a result of immunization is a
public and regulatory concern. What, from 'a toxicologist's perspective' can be done
to address this potential risk now or in the future? Today, there are no validated
models that accurately predict the potential risk; most current immunotoxicology
tools are directed at immunosuppression. This talk will explore several avenues of

**2576 PHOSPHOLIPIDOSIS: DRUG-INDUCED LYSONOMAL STORAGE DISORDER.**


Drug-induced phospholipidosis (PLD), characterized by intracellular accumulation of phospholipid-drug complexes, is believed to result from impaired phospholipid
metabolism following lysosomal accumulation of compounds. The development of
PLD is often proportional to the accumulation of the compounds in the cell or tis
sue. To identify the association of PLD with drug toxicity, a PLD working group at
the FDA has constructed a comprehensive database based on information from
IND and NDA submissions. Correlation of physicochemical property with PLD
will be demonstrated. Preliminary analysis utilizing the database will be presented
to illustrate the dose relationship between the appearance of phospholipidosis and
general toxicity signs in animal studies, and more specifically, the relationship of
phospholipidosis to QT prolongation, myopathy and hepatotoxicity. The use of
LAMP-2 biomarkers and BMP as a non-invasive biomarker of phospholipidosis
will be discussed in the context of differentiating PLD that results in toxicity from
those that are benign.
investment. One theoretical cause of autoimmunity is molecular mimicry, where a conformational similarity - between an infectious agent antigen and a self antigen - triggers an inappropriate immune response to self. This potential risk might be studied using in silico methods such as 3-dimensional molecular modeling and bioinformatics, and depending on the target, animal models of infection. Examples and a case study will be presented on how a risk from molecular mimicry was addressed for a prophylactic vaccine. Further, this presentation will touch on models used to assess safety of vaccine/adjuvants, models used to address autoimmunity, model reliability, assessment of the ever-evolving adjuvants, and what is being done in the scientific community to help address these issues (such as the HESI and IMI projects).

2579 ACUTE PHASE REACTANTS: TRANSLATIONAL BIOMARKERS FOR ADVERSE EFFECTS OF VACCINES.


Toxicity studies are conducted to support the preclinical safety assessment of clinical investigation for new vaccines which are intended to prevent various infectious diseases. A review and analysis of these studies was performed to develop new insights which might increase the potential utility of these studies. The submitted studies were used to investigate the relationships among various species between different adverse events such as pyrexia and weight loss to the presence of acute phase reactants in plasma following the injection of various candidate vaccines. Differences in the prominence and time course of changes in acute phase proteins were observed. These data suggest that C-reactive protein may be one of the more reliable biomarkers when using the rabbit as an animal model for toxicity testing. Nevertheless, the interpretation and applicability of these data are limited by the varying nature of the experimental designs, statistical approaches and means of analysis employed in the different toxicity studies. This talk presents an approach which aims at improving the risk assessment and extrapolation of nonclinical, toxicity data for various preventative vaccines.

2580 THE FLIP SIDE OF IMMUNITY TO VIRUSES—USE OF VIRAL VECTORS FOR GENE THERAPY.

T. MacLachlan. Toxicology, Novartis, Cambridge, MA.

The human immune system is exposed to and subsequently reacts to a variety of agents that can cause harm to the body. Among these are viruses that take advantage of cellular machinery to propagate and ultimately do damage, however a healthy immune system is effective in clearing these agents before they infiltrate or imparts a cascade of reactions that stops the spread of an active infection. Indeed, prophylactic and therapeutic vaccines are effective in triggering the immune system to protect the body from these foreign agents. However, the vectors by which these same viruses travel have proven to be very efficient means to deliver helpful therapies to cells by way of gene transfer—a means that could be very efficient and without adverse reactions if it were not for the immune system. Gene therapy viral vectors have given great promise to alternative therapy for human disease, but immune system reactions against these vectors have led to a host of consequences, ranging from lack of ability to infect cells to the observation of severe adverse events. Studies in animals have attempted to identify risk potential of either pre-existing immunity or post-dose immune reactions for certain viral vectors with mixed success—some work has suggested the possibility of severe systemic inflammatory events, but other data evaluating cellular immune responses suggests that humans and animals react in very different ways. This talk will review the current understanding in this area for adenoviruses, adeno-associated viruses and other vectors, and look to the future development of preclinical and clinical work at subverting these reactions.

2581 SAFETY ASSESSMENT OF THERAPEUTIC VACCINES/ADJUVANTS—REGULATORY CONSIDERATIONS.


Cancer and therapeutic vaccines for non-infectious diseases (hereafter referred to as ‘therapeutic vaccines’) are intended to treat a disease by inducing specific immune response directed against disease-related targets that are present in the patient’s tissues. The conduct of an investigative clinical trial is guided by the Code of Federal Regulations (CFR) Title 21, Part 312. According to 21 CFR 312.23 (a)(8), adequate information derived from pharmacology and toxicology studies is needed to support a trial that is reasonably safe and scientifically feasible. Therefore, the data from preclinical studies that guide the design of early phase clinical trials of therapeutic vaccine in humans should postulate: 1) a safe starting dose, a dose escalation scheme, and a dosing schedule; 2) a safe route of administration; 3) a safety and immune response profile following product administration; 4) subject eligibility criteria; and 5) an adequate clinical monitoring plan. Regulatory guidance for preclinical study design using therapeutic vaccines includes consideration of the characteristics of candidate product, the potentially induced immune response, and the proposed clinical trial design. The overall requirements for assessing the safety of traditional biologics or preventive vaccines may apply, modified approaches to understand the pharmacology and toxicology of therapeutic vaccines should be considered. The safety and/or immune response profile of the intended clinical product (vaccine and adjuvant), such as the target organs/tissues that express the target antigen, the humoral and/or cellular immune response, release of cytokines, and potential local and systemic risks, is important. This profile should be evaluated by in vitro and/or in vivo assessment prior to administration into the patient population. This presentation will discuss current CBER perspectives on preclinical evaluation of therapeutic vaccine/adjuvant combinations and discuss potential regulatory and scientific challenges.

2582 SIGNALS OF AUTOIMMUNITY—A REGULATORY PERSPECTIVE.


The 2009 pandemic resulted in rapid marketing authorization in Europe (and Europe) of several novel adjuvants (i.e. oil-in-water emulsions) – their use is suddenly more widespread. Furthermore, with HPV-vaccine and HB-vaccine, an adjuvant designated “MPL” (LPS-derived Lipid A domain) was introduced recently in the market. Some important adjuvants are characterized by their effects on the so-called “toll-like receptors”, pattern-recognition receptors found in a number of organs and cells. There is a general concern in the public that vaccines (especially combined with adjuvants) will give rise to autoimmunity based on various observations. Squalene has been used in animal models to induce autoimmune-related inflammatory signals. In the 1970s there was an association between a swine-flu vaccine and an increase in Guillain-Barré in the vaccinated population, but no adjuvant was included in the vaccine. In 2011, there were several epidemiologic signals that narcolepsy might have been increased by an H1N1 vaccine along with a specific adjuvant. Narcolepsy itself might be a type of autoimmune-related disease: it is associated with specific HLA-receptors, although the immune character of this relationship has never been proven. Is the antigen/adjuvant combination the most important association? Thus far, the human signals taken alone might be too weak to raise concern. However, better models are needed to evaluate if the risk is real, whether public concern is warranted, and if risks can be mitigated. What has been learned so far from the models we have, and how can they be improved? And from a regulatory perspective, when do signals have to be taken so seriously that regulatory measures against a product are needed?

2583 TRIVALENT ARSENIC METABOLITES AND ARSENIC TOXICITY.

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Inorganic arsenic (iAs) is a well-known environmental toxicant that could induce a variety of adverse health effects. iAs is enzymatically methylated in human tissues to mono- and dimethylated metabolites that contain trivalent arsenic (AsIII) or pentavalent arsenic (AsV). A recently identified arsenic (+3 oxidation state) methyltransferase (AS3MT) is the key enzyme in this pathway. Notably, trivalent methylated metabolites of iAs (MAIII and DMAAsIII) have been shown to play an important role in iAs-associated diseases such as cancer and diabetes. There is evidence that the AS3MT metabolic pathway, which was formerly considered a detoxification pathway, may significantly increase the toxicity of iAs. Ex vivo studies using tissue cultures have consistently shown that exposures to subtoxic concentrations of MAIII and DMAAsIII produce effects that mirror carcinogenic effects, and noncancerous conditions such as diabetes and cardiovascular diseases. However, the relevance of these findings for in vivo responses remains an area of ongoing investigation and debate. The complexity and lack of suitable methods has hindered the analysis of MAIII and DMAAsIII in biological samples. Lately, results of epidemiologic studies suggest that MAIII and DMAAsIII play a role in the onset of diabetes.
Critical levels of iAs exposure and other factors that are associated with an increased probability of these metabolites, and are linked/associated with increased prevalence of diabetes, have been characterized. The significance of these recent developments will be discussed in the context of physiologically based pharmacokinetic models, and the relationship between methylation and specific organ toxicity, as well as variations in methylation ability as a function of host factors.

2584 ROLE AND IMPORTANCE OF ARSENIC METHYLATION ASSOCIATED WITH TOXIC EFFECTS.
L. M. Del Razo, Toxicology, Cinvestav-IPN, Mexico, Distrito Federal, Mexico. Inorganic arsenic (iAs) is metabolized in humans to methylated arsenical species in a multistep process mainly mediated by AS3MT, which may catalyze both oxidative methylation of trivalent arsenic-containing substrates and reduction of pentavalent arsenic in methylated products. Hence, monomethylarsonous acid (MAsIII) and dimethylarsonous acid (DMAIII) conversion of iAs can significantly modify its toxic and cancer-promoting effects, primarily of skin, bladder and lung. Among these metabolites MAsIII and DMAIII are the most toxic arsenic species. This has to do with their reactivity due to the generation of reactive oxygen species oxidizing to sulfur containing compounds, like protein. Improved analyses have convincingly demonstrated the presence of such trivalent species in human urine where subjects who excrete higher proportions of MAs (particularly MAsIII) may have higher risks of iAs-associated skin and bladder cancer. However, results of recent studies indicate that the concentrations and proportion of iAs metabolites in urine do not necessarily reflect the concentrations and speciation of As in tissues targeted by iAs exposure. Current analytical technologies are sufficient to distinguish between DMAIII and DMAV in urine and biological tissues. Careful sample preservation and handling are required to ensure the stability of trivalent arsenical metabolites. Therefore, analysis of speciated As in target tissues could provide an effective tool for estimating internal exposure and for identification of individuals with an increased risk of developing specific diseases associated with chronic exposures to iAs. A better understanding of the role of phenotype, including trivalent methylated species, in the binding, disposition, and toxicity of iAs has become a critical issue for laboratory, epidemiological and risk assessment studies.

In the regulatory compliance, to address the impact of differential effects between trivalent and pentavalent arsenicals, we should be able to utilize more comprehensive quantitative biologically based models to better estimate the human risks from low level exposure to iAs.

2585 MODE OF ACTION OF METHYLATED TRIVALENT ARSENIC WITH A FOCUS ON THE POTENTIAL ACTION AS A CANCERGEN.
M. P. Walkees, C. Kojima, R. Ortizuela and E. J. Tokar, NTP, NIEHS, Research Triangle Park, NC. Developmental inorganic arsenic (iAs) exposure causes cancer in adulthood in humans and rodents. There is suspicion that biomethylation products of iAs, including MAsIII, are important carcinogenic species, but there has been little direct evidence. In recent work, pregnant mice were exposed to MAsIII at 0 (control), 12.5, and 25 ppm in the drinking water from gestation day 8 to 18, and tumors were assessed in the offspring. Treated female offspring developed ovarian and uterine tumors and adrenal adenoma. Treated male offspring developed hepatocellular carcinoma, adrenal adenoma and lung adenocarcinoma. Males showed unusual testicular lesions such as occasional retic testis carcinomas, adenomas, and hyperplasias, and interstitial cell tumors and hyperplasias. Thus, gestational maternal MAsIII exposure produced tumors consistent with similarly applied iAs, although the testicular lesions with MAsIII are distinct. Using target relevant cell lines in vitro (liver, bladder, skin, prostate), we find that cells that can methylate iAs, and thereby produce MAsIII, during chronic low level iAs exposure undergo malignant transformation at a rate half the time as those cells that do not methylate or poorly methylate the metalloid. Oxidative DNA damage (ODD) was measured using the immunoperoxidase trapping method. In cells that methylate iAs, ODD increased to the point of malignant transformation, but no ODD elevation occurred prior to transformation in cells that do not methylate iAs. If the various cells are exposed to MAsIII they all undergo transformation at a similar time point (~20 weeks) and show a similar level of increased ODD, regardless of ability to methylate. These data indicate iAs acts through multiple carcinogenic mechanisms, one of which involves ODD in cells that methylate the metalloid. MAsIII appears to be an active DNA oxidant species in the carcinogenic process in cells that methylate. Methylated iAs is not, however, obligate to acquire a malignant phenotype, but it may hasten the process. The ODD of biomolecules induced by MAsIII could have implications for diseases beyond cancer.

2586 ANALYSIS OF METHYLATED TRIVALENT ARSENICALS IN BIOLOGICAL SAMPLES.
M. Styblo1, J. Currier1, J. Saunders1, M. Svoboda2, J. Dedina3 and T. Matoušek3. 1Nutrition, University of North Carolina at Chapel Hill, Chapel Hill, NC, 2Charles University, Prague, Czech Republic and 3Institute of Analytical Chemistry of the ASCR, Brno, Czech Republic. Chronic exposure to inorganic arsenic (As) is associated with a variety of diseases. The methylated trivalent metabolites of iAs, methylarsonite (MAsIII) and dimethylarsonium (DMAIII), may play an important role in the etiology of these diseases. Thus, monitoring the production of these metabolites would provide critical information about the health risks associated with iAs exposure. MAsIII and DMAIII have been detected in fresh urines of subjects exposed to iAs. However, the quantitative analysis of these metabolites is complicated by a rapid oxidation that converts MAsIII to DMAV and DMAIII to DMAV. In addition, analytical methods requiring sample extraction or chemical modification may yield artifacts associated with the oxidation or losses of the methylated trivalent species. We have used hydride generation-cryotrapping-atomic absorption spectrometry (HG-CT-AAS) and HG-CT-inductively coupled plasma-mass spectrometry (ICP-MS) for the oxidation state specific speciation analysis of As in biological samples. Our results show that, in spite of their limited specificity, these methods represent efficient tools for the quantitative analysis of MAsIII and DMAIII in urine and in complex biological matrices, including cells and tissues. Here, hydrides from AsIII species are generated directly at pH 6; for analysis of AsIII-V species, the samples are pre-reduced with cysteine. Analysis of cell lysates or homogenates digested in phosphoric acid is used to control for As recovery. Using HG-CT-AAS or HG-CT-ICP-MS, both AsIII- and AsV- species can be quantitatively analyzed in cells and tissues with As recoveries of ~80-100% and comfortable detection limits. Moreover, both MAsIII and DMAIII are stable in cells and tissues for several weeks at -80°C, suggesting that the quantitative oxidation state specific analysis of iAs metabolites is possible even in frozen tissues and cells collected in laboratory or population-based studies.

2587 ROLE OF AS3MT POLYMORPHISM IN ARSENIC METABOLISM: TRIVALENT ARSENICALS.
Z. Drohna1, L. Del Razo2, G. Garcia-Vargas3, O. Valenzuela4, E. Hernandez Castellanos2, L. C. Sanchez-Pena2, D. Loomis1 and M. Styblo1. 1Nutrition, CBKU1, Charles University, Prague, Czech Republic and 2Toxicology, Cinvestav-IPN, Mexico City, Mexico, 3Juarez University, Durango, Mexico and 4Toxicology, University of Nebraska, Omaha, NE. Arsenic (+3 oxidation state) methyltransferase (AS3MT) is a key enzyme in inorganic arsenic (iAs) metabolism. In humans, sequential methylation of ingested iAs by AS3MT produces trivalent and pentavalent monomethylated (MAs) and dimethylated (DMA) arsenicals. Trivalent arsenicals are more toxic and reactive than their pentavalent counterparts. Urinary profiles of iAs and its metabolites are used to assess arsenic exposure and methylation capacity of individuals and to predict the risk of arsenic-related diseases. Inter-individual differences in iAs metabolism have been associated with AS3MT polymorphisms. Most of the studies associated AS3MT polymorphism with levels of total arsenic or pentavalent arsenicals in urine. In our studies AS3MT polymorphism is evaluated with respect to urinary profiles of trivalent methylated metabolites of iAs (MAsIII and DMAIII). A cross-sectional study was conducted in the Zimapán and Laguna regions in Mexico to examine the links between AS3MT polymorphism, urinary profiles of iAs metabolites, and premalignant skin lesions and diabetes. Among all tested genotypes T14458C (Met287Thr) individuals carrying the C (TC+CC) allele were at risk for premalignant skin lesions (OR=4.28; 95% CI 1.0-18.5). Carriers of C allele had significantly higher percentage of MAsIII and DMAIII in urine. When diabetes markers were analyzed as continuous variables, carriers of the TC-CC genotypes of Met287Thr had elevated levels of fasting blood glucose, 2-hour blood glucose level determined by oral glucose tolerance test, and glycated hemoglobin. Notably, the Met287Thr polymorphism is linked with another GC+CC polymorphism (G4965C) present in the promoter region of AS3MT. These findings suggest that Met287Thr increases the susceptibility to premalignant As skin lesions and diabetes, by increasing the production of methylated trivalent arsenicals in individuals exposed to environmental iAs.
Inorganic arsenic (iAs) is known to cause cancer and other chronic diseases and is suspected of causing diabetes mellitus. Our research examines associations of diabetes with exposure to iAs in drinking water and metabolites of iAs in urine in areas of Mexico where the concentration of iAs in drinking water has been historically high. We conducted a cross-sectional study among adults and children age ≥5 years living in Durango, Coahuila and Hidalgo states. Information on water supplies and residential and medical history was obtained by questionnaire and participants provided samples of drinking water and spot urine. Prevalent diabetes was classified by fasting blood glucose (FBG) (≥125 mg/dl), oral glucose tolerance test (OGTT) (≥200 mg/dl) and self-report of diabetes diagnosis or treatment. iAs and its metabolites in urine were analyzed by hydride generation-atomic absorption spectroscopy. Associations between diabetes and iAs and urinary metabolites of iAs were estimated by logistic regression with adjustment for age, sex, hypertension and obesity. Urinary creatinine was evaluated as a covariate in analyses of urinary metabolites. The odds ratio (OR) for diabetes increased about 1% per ng/ml of As in water (95% CI 1.01-1.02). Diabetes was associated with current iAs exposure, but not with cumulative arsenic exposure, or with total urinary arsenic concentration or total tri- or pentavalent iAs in urine. However, the odds of diabetes increased 7% per ng As/mI of urinary dimethylarsenite (DMAIII) (OR 1.07; 95% CI 1.01-1.13) after adjustment for creatinine and 5% per ng/ml without adjustment. Neither methylarsonate (MAIII) nor pentavalent methylated arsenicals in urine were associated with diabetes. This study links diabetes with exposure to iAs and is the first to suggest that the DMAIII may be a marker for the risk of developing diabetes.

Advancements in packaging technology, such as those that extend food shelf-life, agricultural products including pesticides and genetically modified crops, and a more integrated and global marketplace have led to increased food quantity and quality, but as a consequence have also led to concerns about food safety and potential risks to public health. These global food safety concerns range from incidental or deliberate food contamination from microorganisms or toxic substances, chemicals migrating into food from food containers, pesticide residues on food, and genetically modified foods. Our panel of experts will highlight the science-based approaches being used to regulate food safety in the food, chemical, and agricultural industries across the world. To underscore the important of these issues, we will identify opportunities for advancing technologies and science across many sectors including academia, industry, government, and public health organizations, to build confidence in the safety of our food to protect human health.

The challenges confronting food safety at an international level have increased dramatically over the last decade as the food supply has become more global. The potential hazards are varied and complex and encompass chemical, microbiological, and radiological threats. Chemical hazards include those that are naturally or are anthropogenic and are introduced into the food supply inadvertently or deliberately. Recent events like the melamine and analogues in pet food and infant formula and the tragic consequences of the Japanese earthquake and tsunami have highlighted the impact that these threats can have on an international level. In the past, these episodes have been usually confined to national boundaries and have tended to impact food safety on a country by country basis. With food now traveling great distances to new markets, the negative consequences have an impact around the world. The WHO is the sole international public health organization that can coordinate the appropriate public health response in dealing with these international food crises. Some of the issues that will be addressed and described will be the following: the integration and use of new science in the assessment of the hazards and risks of chemical threats, new approaches/methodologies to assess the safety/risk to the global food supply, and new technologies that are being brought to bear to facilitate the detection of chemically contaminated foods. The mechanisms and approaches that have been developed under the auspices of the WHO will be described and will include actual case monitoring strategies for proactively and retroactively detecting and responding to outbreaks of chemical food contamination.
tiered approach of targeted testing based on exposure estimates and structural con-
siderations allows for optimal utilization of resources to ensure human safety for
food contact materials.

2593 ADVANCES IN SAFETY/RISK ASSESSMENTS OF
PESTICIDE RESIDUES ON FOODS.
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Kingdom.

Data requirements for testing of pesticides are amongst the most rigorous in the
world. However, despite concern on the one hand that not all of the data generated
are necessary for the required risk assessments, on the other hand there is concern
that not all relevant effects are being assessed adequately, e.g. endocrine modula-
tion. There has been limited refinement of data requirements and approaches are
being developed for additional endpoints, but as yet there has been no concerted
overhaul. Whereas assessment of the active ingredient is now relatively thorough,
substantial questions remain on how to address cumulative exposures, with sugges-
tions that assessment groups should be much broader than heretofore. Similarly,
there is increasing concern regarding the appropriate toxicological residue defini-
tion, and how plant specific metabolites and environmental degradates should be
evaluated. It is not feasible to subject them all to full toxicity testing. Hence, screen-
ing strategies such as the threshold of toxicological concern are necessary, as is the
development and application of effective methods for chemical read-across.

2594 REGULATING THE SAFETY OF FOODS AND FEEDS
DERIVED FROM GENETICALLY MODIFIED CROPS.
B. Chassy. Food Science and Human Nutrition, University of Illinois at Urbana-
Champaign, Urbana, IL. Sponsor: N. Stapp.

Transgenic crops have been planted on more than one billion Ha by more than 15
million farmers in over 25 countries during the last 15 years. Farmers have found
that GM crops can increase yield and profitability while reducing pesticide use and
greenhouse gas emissions from agriculture. No credible report of adverse effects to
humans or animals has been documented to have occurred from the ingestion of
GM crops, although critics of GM crops have made numerous claims about the in-
cidence of adverse effects. Although the National Academy of Science has repeat-
estedly stressed that GM crops pose no new or different risks, GM crops are required
to pass regulatory review prior to their commercial release. The comprehensive
safety assessment that is applicable to new GM varieties includes a thorough molec-
ular characterization of the newly inserted DNA as well as any novel proteins and
metabolites. The products of inserted genes and their mechanism of action must be
established. Proteins and metabolites are subjected to toxicological evaluation, and
their potential allergenicity is assessed through bioinformatics analysis, evaluation
of their stability and digestibility, and if indicated through serum or other im-
munological tests. A comprehensive composition analysis is also performed. Whole
food feeding studies in laboratory and production animals may also be required in
some countries. With more than half of the world’s population now living in coun-
tries that have adopted GM crops, and with numerous other countries poised to
adopt them, it might be appropriate to reduce the regulatory scrutiny of GM crops
to a level that is commensurate with real risk.

2595 RISK ASSESSMENT AND MANAGEMENT OPTIONS
FOR CHEMICAL CONTAMINANTS IN A GLOBAL
FOOD SUPPLY.
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Park, MD.

The purpose of regulating chemical contaminants in food involves the promulga-
tion of risk management policies that will result in the elimination or reduction of
exposure to a chemical in food, whether anthropogenic or natural in origin. Two of
the most common policy formulations involve either setting a contaminant level that
is considered tolerable or proscribing particular food production practices. The
establishment of a contaminant level in food is generally determined on the basis of
a toxicological evaluation that identifies an exposure/dosage level deemed to be tol-
erable and an assessment of the potential dietary exposure to the chemical.
However, if the means of enforcement or compliance and costs are not considered,
the resulting policy of establishing a tolerable level of exposure may not be practica-
ble for several reasons. First, the required interventions (e.g., production practices)
to achieve a tolerable contamination level may be targeted by other intervention
approaches. Second, compliance practices are explicitly considered and their
impact assessed, regulatory policies that focus on particular production practices
may be easier to monitor than the enforcement of a concentration level. However,
the formulation and establishment of a regulatory policy that prescribes a produc-
tion practice is more difficult. In addition to a prerequisite hazard and risk charac-
terization of the relationship between exposure to a chemical and the attendant
health consequences, the establishment of a production practice that is intended to
reduce chemical risks must consider other factors such as consumer practices, mon-
itoring capabilities, and the effectiveness of specific interventions that may be taken
by the food industry.

2596 DISCOVERING NOVEL HYPOTHESES FOR
MECHANISMS OF TOXICITY FROM HIGH-CONTENT
DATA SETS.
N. Greene and A. Enayetallah. Pfizer Inc., Groton, CT.

For over a decade researchers have sought to apply technologies such as genomics,
proteomics, and metabolomics to either predict toxicity through the use of gene,
protein, or metabolite signatures or to further understand modes of action in toxic-
ity through chemical exposure. There have been some success stories in recent years
but largely these technologies have not lived up to the promises made when they
were first developed. The complex nature of biological systems and the multivariate
nature of a system’s response to a xenobiotic have made it difficult to pick apart
the true causes of the phenotypic changes that are observed. In addition, the explosion
of data available in the public domain has made it difficult for the human brain to
keep up with current knowledge and apply this effectively to a set of experimental
readouts to determine cause and effect relationships. Xenobiotics often induce their
biological effects via interactions with one or more biological targets, thus trigger-
ning whole cascades of events that culminate in adverse events in humans.
Understanding the nature of these events, coupled with consideration of their rele-
vance to the human mode of action and target context, will improve the scientific
basis and thus increase the accuracy of risk and safety assessments. In addition, bet-
ter understanding of toxicological modes of action will ultimately lead to the de-
velopment of more predictive models of in vivo biological responses. Where these take
the form of in silico toxicology prediction models and cell-based assays, it will increase
our understanding of the use of animals in laboratory experiments. This session will outline
some of the cutting-edge research and methodologies for distilling down the vast array of pub-
lic information into more manageable sets of knowledge and relationships. We will
discuss novel in silico approaches to mine these relationships and formulate hypo-
theses for modes of action of the compound or biological target under study and
present some applications of these methods in understanding toxicological mecha-
nisms.

2597 PREDICTING MECHANISMS OF CHEMICAL
TOXICITY USING THE COMPARATIVE
TOXICOGENOMICS DATABASE (CTD).
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The Comparative Toxicogenomics Database (CTD; http://ctd.mlib.org/) is a
freely available resource that provides curated and integrated data aimed at under-
standing the mechanisms of chemical-induced toxicity. Curated data consists of
chemical-gene/protein interactions as well as chemical-disease and gene-disease re-
relationships. These data are integrated with additional pathway and functional
information such that novel inferences can be made about the complex impact of
chemicals on both the etiology and treatment of human disease. Of particular rele-
ance for the prediction of toxicity are CTD’s ChemComps, inferred chemical-dis-
ease relationships, and enriched Gene Ontology (GO) and pathway annotations.
ChemComps uses a Jaccard index to identify similar chemicals based on common
gene interaction profiles. This measure allows users to a) identify chemicals with
potentially similar modes of action, despite differences in chemical structure and b)
gain additional insight about a chemical of interest by examining other chemicals
with similar modes of action. Inferred chemical-disease relationships are based on
common interacting gene sets. These gene sets are entirely unique to CTD and pro-
vide a mechanistic foundation for developing testable hypotheses about how a
chemical may contribute to the etiology or treatment of a disease. Enriched GO
and pathway annotations are calculated for chemicals based on common interact-
ging genes. These data provide insight into biological functions and canonical path-
ways that cannot be obtained by straightforward search. This presentation will provide a case study
that demonstrates how one might use CTD to investigate and develop predictive
models about mechanisms of chemical-induced toxicity.
Using computational methods to predict target-based toxicity early in drug development, before significant investment in drug discovery, can dramatically reduce cost, minimize candidate attrition, and improve the overall success rates of drug development. Perhaps more importantly, it has the potential to reduce patient exposure to harmful drug toxicities in early clinical development. To effectively assess target safety early in the process, it is important to understand the molecular mechanisms downstream of the target and the molecular mechanisms that underlie known drug toxicities. Using a database of prior knowledge as a substrate, we developed an application that uses novel path-search algorithms that comb through the knowledgebase to connect targets to pathologies. The application defines biologically plausible and relevant paths from target to pathology using a set of rules and parameters for path selection. It also includes metrics for path prioritization based on the types of connections between molecules within the path as well as on the path length. We will present examples of paths downstream of several targets that can be causally associated with drug-induced toxicities. The examples include proof-of-concept paths that recapitulate known networks, as well as novel biology through causal reasoning from a target to a toxicity. Since the identified paths are comprised of discrete molecular mechanisms downstream from a target that can lead to a pathology, they allow for the evaluation of target-induced toxicity with the ability to test and validate specific mechanisms in the laboratory.

In this study we used high-throughput quantitative proteomics involving stable isotope labeling with amino acids in cell culture (SILAC) leveraged by a recently developed systems biology approach (Causal Reasoning Engine, CRE) to investigate the effects of chemical compounds (thiazolidinediones) on the cellular proteome of HepG2 cells. SILAC is a metabolic labeling technique that involves growing two populations of cells, one in a medium that contains ‘light’ (normal) amino acids and the other in a medium that contains ‘heavy’ amino acids (containing 13C instead of 12C, 2H instead of H or 15N instead of 14N). This leads to a known mass shift that allows relative quantification by determining the ratios between heavy and light peptides identified by mass spectrometry. The quantitative proteomics analyses resulted in data that we then used to investigate cellular pathways affected by drug treatment using CRE. CRE is an algorithm that was originally designed to utilize microarray transcriptomic data. Here we adapted the algorithm to interrogate proteomics results in context of causal statements derived from the biomedical literature to infer upstream molecular events driving these protein changes. The inferred upstream events (also called hypotheses) are aggregated into biological models using a set of analytical tools that allow for evaluation and integration of the hypotheses in context of their supporting evidence. In comparison to more traditional proteomic analysis tools CRE results provided highly detailed molecular hypotheses that made up biological networks to explain the measured protein changes. These networks include oxidative stress, ER stress and protein folding abnormalities. Finally, the CRE hypotheses provide new insight into variation of potential toxic molecular mechanisms within the same compound class.

Compound-related injury to cardiac and skeletal muscle are common preclinical and clinical toxicities observed in drug development, and have resulted in the withdrawal of several pharmaceutical agents from the market. Current biomarkers for detecting muscle injuries can be both insensitive and nonspecific as well as poorly predictive. Improving the ability to detect drug-induced muscle injuries will facilitate preclinical and clinical drug development and help ensure patient safety. This session will focus on a consortium approach to qualify novel muscle biomarker candidates and seek regulatory endorsement for specific use claims, and will include presentations on (1) the biomarker qualification approach being used by the Critical Path Institute’s Predictive Safety Testing Consortium (PSTC) in partnership with FDA and EMA, (2) a pathological assessment of cardiac and skeletal muscle drug-induced toxicities, (3) a Biomarker Qualification Submission (BQS) for skeletal muscle biomarkers consisting of ~40 rat studies from consortium member companies, (4) a clinical qualification strategy for the proposed skeletal muscle biomarker candidates, (5) cardiac hypertrophy biomarker candidates based on clinical data and a reverse translational strategy for their qualification into preclinical use, and (6) FDA perspective on novel safety biomarker qualifications for preclinical and clinical applications. Data will be presented highlighting the qualification and implementation of these novel cardiac and skeletal muscle biomarkers in drug development, as well as discussion about the importance of agency endorsement to further promote translation to clinical applications and better enable drug development.
and patterns of toxic injury to cardiac and skeletal muscle will be discussed in order to provide a foundation of understanding for subsequent presentations. The nature and phase of the morphologic changes will be important determinants in the rational for considering particular molecules as candidate safety biomarkers. For example, it is reasonable to assume that sarcoplasmic proteins will likely require loss of myocyte viability or membrane integrity in order to appear in serum in concentrations high enough to provide a signal of myocyte injury. Therefore, for the assessment of these particular types of biomarkers, myocyte degeneration/necrosis will likely be a critical anchoring feature. When histopathologic changes are considered appropriate reference points for evaluation of biomarker performance, the lexicon for recording these changes should be adapted from that employed for general toxicity studies with recognition of the distinct goals of biomarker characterization efforts and designed to faithfully capture changes relevant to the likely capabilities of the various investigational biomarkers. While changes in caliber (hypertrophy and atrophy) are within the repertoire of both cardiac and skeletal myocytes, cardiac hypertrophy in response to drug-induced hemodynamic stress represents a particularly important biomarker need. Cardiac hypertrophy, however, is an example of a morphologic endpoint for which histopathology is not the most sensitive anchor; other assessments such as heart weight are more easily determined reference points for this change.

2604 QUALIFICATION OF NOVEL SKELETAL MUSCLE TOXICITY BIOMARKERS BY THE PSTC WORKING GROUP.

D. E. Watson, Investigative Toxicology, Eli Lilly and Company, Indianapolis, IN.

Skeletal muscle (SKM) toxicity is an undesirable side-effect of some valuable medications, including statins and fibrates. Existing biomarkers of SKM injury are helpful for the diagnosis of SKM injury, including serum creatine kinase (CK) and aspartate aminotransferase (AST) enzyme activities. However, CK and AST lack specificity for SKM, and can be non-responsive when SKM injury is detected by histopathology in toxicity studies. In recognition of these limitations the PSTC formed a Skeletal Muscle Working Group to begin qualifying novel SKM biomarkers for use in rat toxicity studies. The novel biomarkers include known sarcomeric and soluble proteins, including skeletal troponins T1 and 12, myosin light chain 3, fatty acid binding protein 3, parvalbumin, creatine kinase M, and myoglobin. Concentrations of these proteins were measured in serum and plasma (or urine in the case of myoglobin) from up to 40 rat toxicology studies that have used a large number of known and proprietary compounds. Sensitivity and specificity of AST, CK, and the novel biomarkers were evaluated relative to histopathology findings of degeneration and necrosis in a variety of SKM tissues, including soleus and quadriceps. The performance characteristics of the existing and novel biomarkers will be discussed, both individually and in combination with one another, as predictors of SKM injury. In addition, some biomarkers show preferential expression in Type 1 or Type 2 skeletal muscle fibers, as revealed by immunohistochemistry. The value of these biomarkers for discriminating between injury occurring in slow twitch (Type I) vs. fast twitch (Type II) skeletal muscle fibers will also be discussed.

2605 CLINICAL TRANSLATION OF SKELETAL MUSCLE BIOMARKERS.

W. Glaba, Merck Research Laboratories, West Point, PA.

Drug-induced skeletal muscle injury is a common preclinical and clinical toxicity observed in drug development, and has resulted in the withdrawal of several pharmaceutical agents from the market. Current biomarkers for detecting skeletal muscle injury are both insensitive and nonspecific, and improving the ability to detect drug-induced skeletal muscle injuries will help facilitate preclinical and clinical drug development and ensure patient safety. Qualification of novel skeletal muscle toxicity biomarkers is an on-going initiative using a consortium approach through the Critical Path Institute’s Predictive Safety Testing Consortium (PSTC). Preliminary preclinical data from the PSTC Skeletal Muscle Working Group support a Biomarker Qualification Submission (BQS) to seek regulatory endorsement from both the FDA and EMA, demonstrating the added value of these novel biomarkers in detecting drug-induced skeletal muscle injury. In order to translate these novel biomarkers from preclinical to clinical settings, the Skeletal Muscle Working Group has developed a clinical translation strategy. This presentation will outline the working group’s translational strategy, and review currently monitored clinical skeletal biomarker endpoints used routinely in the clinic. Identification of clinical assays for the novel biomarker candidates will be presented, as well as early validation work on these existing assays including available clinical baseline data. The presentation will conclude with identification of normal and/or disease populations needed to establish baseline measurements for the novel biomarkers, and the next steps needed to secure clinical samples from drug-induced skeletal muscle toxicities. Establishing these novel biomarkers in the clinic will be essential to fully leverage the preclinical qualification efforts and further enable clinical drug development.

2606 REVERSE TRANSLATING CLINICAL BIOMARKERS—A NONTRADITIONAL APPROACH TO QUALIFY NT-proANP.

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The current paradigm for the non-clinical development of molecules is lacking accessible biomarkers for identifying drug-induced hemodynamic perturbations. Many of these drug-induced cardiovascular changes can lead to cardiac hypertrophy, decreased left ventricular ejection fraction (LVEF) and ultimately heart failure. Histomorphology lacks the sensitivity to detect the early changes leading to pathologic hypertrophy. While functional analysis using echocardiography has the greatest ability to characterize hemodynamic changes, these imaging techniques are rarely utilized in routine and long-term toxicity studies. Natriuretic peptides (NPs) are hormones secreted by cardiomyocytes in response to stretch resulting from increased cardiac pressure or afterload. NP’s have been proven to be noninvasive clinical diagnostic and prognostic markers for the assessment of heart failure. Given their proven utility in humans, NP’s are candidates for circulating translational biomarkers that could be used during drug development in preclinical species. The Cardiac Hypertrophy Working Group (CHWG) of the Predictive Safety Testing Consortium (PSTC) initiated an effort to qualify N-terminal pro-natriuretic peptide (NT-proANP) for use as a non-clinical cardiovascular biomarker for compounds in development. The group has generated data on NT-proANP from rat toxicology studies, from which increased concentrations in the serum of rats were correlated with cardiac hypertrophy, defined by increased heart weight and/or left ventricular mass. The preclinical performance of NT-proANP will be investigated and compared in relation to the clinical performance of brain natriuretic peptide (BNP) for diagnosing cardiac hypertrophy and/or decreased LVEF. Given the current clinical use of BNP, this qualification will involve a unique reverse translational approach. The application of this approach and its potential to improve risk assessments for cardiovascular toxicity in patients will be discussed.

2607 A US FDA PERSPECTIVE ON SAFETY BIOMARKERS AND THE QUALIFICATION PROCESS.

M. Walton, Office of Translational Sciences, CDER, Food and Drug Administration, Silver Spring, MD. Sponsor: D. Robinson-Gravatt.

Drug related toxicities are important determinants of whether a drug candidate can be successfully developed for clinical use and how widespread the approved drug’s appropriate use will be. Cardiac and skeletal muscle injuries are among the types of toxicities that have been significant for drugs, and often are not fully appreciated until the post-marketing safety information is available. Improved techniques for detecting and evaluating muscle injury can aid drug development. This presentation will provide an overview of the Biomarker Qualification Process at the FDA and how this is intended to advance clinical drug development. Identifying a Context of Use for the biomarker is a critical step in the development of biomarkers as the data justifying acceptance of the biomarker must be relevant to the selected context of use. This will be discussed and how Context of Use relates to safety biomarkers. Novel clinical muscle injury biomarkers can have an important direct role in more efficiently developing therapies, but knowledge about the drug related injury performance of the biomarkers can also have an important role in the qualification of the biomarkers for clinical use. This presentation will also discuss the value of documenting and assessing the impact of the biomarkers that have been qualified and incorporated into drug candidate development.

2608 PROGENITOR AND STEM CELLS AS TARGETS OF ENVIRONMENTAL POLLUTANTS.

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Stem and progenitor cells, including hematopoietic stem cells (HSC), endothelial progenitor cells (EPC), and mesenchymal stem cells (MSC), are potential important targets of environmental pollutants due to their capacity to differentiate and divide. These progenitor/stem cells are mobilized from their niches (e.g., bone marrow and spleen) by a variety of stimuli to the circulation where they effectively regulate organ and tissue homeostasis and repair after injury. Epidemiological studies suggest these cells play an important role in the development of human disease and indicate environmental exposures affect both the number and function of circulating stem/progenitor cells. For example, exposures to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) are associated with an increased risk of specific hematological diseases, whereas inhalation of particulate and volatile air pollutants is associated with decreased and circulating endothelial progenitor cells, which is a risk factor of cardiovascular diseases. Thus, the level of circulating stem/progenitor cells could be a sensitive biomarker of pollutant exposure, as well as being a mechanism by which pol-
Acute and chronic exposure to elevated levels of fine particulate matter (PM_{<2.5}) is associated with a spectrum of cardiovascular disorders that likely arise from the development and maintenance of a dysfunctional endothelium. Damage to the endothelium is alleviated in part by a group of progenitor cells, the so-called endothelial progenitor cells (EPCs), which are mobilized from specific niches, home to sites of vascular damage and mediate repair through their terminal differentiation or paracrine effects. Indeed numerous studies have identified an inverse correlation between EPC number and CVD risk. To test the hypothesis that the cardioactive effects of PM_{<2.5} exposure results from defects in EPC-mediated repair, we initially quantified these defects in human EPCs. Interestingly, we observed that the highest levels of exposure were associated with a depletion of circulating EPCs. In parallel studies, mice exposed to concentrated ambient PM_{<2.5} (CAPs) also demonstrated reduced levels of circulating EPCs, but enhanced levels in the bone marrow. Treatment of these mice with VEGF/AMD3100 was not effective in reversing the PM-mediated effect on blood or bone marrow EPCs, suggesting that PM exposure contributes to a mobilization defect. Ongoing studies are focusing on the bone marrow progenitor cells that may ultimately lead to the development of various cardiovascular diseases.

Particulate air pollution, specifically nickel found on or in particulate matter, has been associated with an increased risk of mortality in human population studies and can increase vascular inflammation, generate reactive oxygen species, alter vaso-motor tone and potentiate atherosclerosis in murine exposures. With the discovery of endothelial progenitor cells (EPCs), a door has been opened which may elucidate these observed cardiovascular effects associated with inhaled air particles and nickel exposure. In order to further quantify the effects of inhaled nickel nanoparticles and attempt to elucidate how the observed findings from other studies may occur, whole body inhalation exposure experiments using nickel nanoparticles were performed. Exposure to inhaled nickel nanoparticles significantly reduced bone marrow EPCs while increasing their levels in circulation. Circulating endothelial cells were significantly upregulated suggesting that endothelial damage occurred due to the exposure. Tube formation and chemotaxis, but not proliferation, of bone marrow EPCs were significantly upregulated suggesting that endothelial damage occurred due to the exposure. Tube formation and chemotaxis, but not proliferation, of bone marrow EPCs was impaired in the nickel nanoparticle-exposed group. This decrease in EPC function coincided with downregulation of receptors for EPC mobilization and homing. Antioxidant plasma proteins were upregulated post-exposure and transferrin was downregulated as measured by proteomic analysis. In conclusion, these results indicate that inhalation exposure to Ni nanoparticles at below the current OSHA permissible exposure limit for Ni compounds can lead to alterations in bone marrow progenitor cells that may ultimately lead to the development of various cardiovascular diseases.

In contrast, a comparative study testing the effects of acrolein- or PM_{<2.5}-inhalation on the bone marrow showed that the decline in the blood was accompanied with an increase of Flk-1+*/Sca-1+ cells in the bone marrow. Culturing of bone marrow cells isolated from mice exposed to CAP or acrolein, likewise showed an increased outgrowth of colony forming units and Flk-1+*/Sca-1+ or acLDL+/UE-lectin+ bone marrow-derived cells. Combined VEGF/AMD3100 treatment to stimulate EPC mobilization from the bone marrow was inhibited by either CAP or acrolein inhalation. Investigations of upstream signal transduction events uncovered that both environmental pollutants inhibited endothelial VEGF-signaling. These findings indicate that particulate and volatile air pollutants similarly obstruct VEGF-mediated mobilization of EPCs from the bone marrow, which indicates a potential common mechanistic link between environmental exposures and CVD.

Recent data suggests an important function of the AhR in hematopoietic stem cell (HSC) biology. Epidemiological data has correlated accidental and occupational exposures to 2,3,7,8-tetrachlorodibenz-p-dioxin (TCDD) with increased risk for certain hematological diseases. There is also recent interest in the possible use of AhR antagonists for the expansion of HSCs to be used in bone marrow transplants. It is critical to further define mechanisms related to effects on hematopoietic stem/progenitor cell function and the signaling pathways that may mediate these alterations. In previous studies, we and others reported that TCDD treatment to mice compromised the competitive reconstitution of bone marrow into irradiated host animals. Further investigations revealed that although TCDD treatment alters numbers of phenotypically-defined stem and progenitor cells, there was no effect on the numbers of functional HSCs. However, TCDD treatment altered the trafficking of HSCs to bone marrow in vivo as well as to chemokine attractants ex vivo. Furthermore, TCDD treatment alters the expression of receptors involved in trafficking and genes involved in the regulation of this trafficking. Together, these data suggest a plausible and testable mechanism related to increased incidence of leukemia and lymphoma in humans exposed to TCDD. These results, along with other information in HSCs from AhR-null allele animals, also suggest a complex role of AhR signaling in HSC function. Further investigations will provide important information needed for our understanding of processes that regulate stem cells and their role in human disease.

Treatment with a single dose of dimethylbenzanthracene (DMBA) rapidly impairs bone marrow progenitor cells, as measured by colony forming assays, well before any decrease in bone marrow cellularity. The duration of impairment depends on the dose and route of administration, but extends beyond clearance of DMBA. In contrast, treatment with benzo[a]pyrene (BP) impairs bone marrow progenitor cell activity in vitro, but not bone marrow cellularity in vivo, because of an AhR-dependent restoration of progenitor activity. Microarray analyses of bone marrow cells revealed many more responses to BP than DMBA, including genes for oxidative signaling and AhR activation that may explain the AhR-dependent protection of hematopoietic progenitors. Bone marrow progenitor changes were reflected later in closely coordinated DMBA-induced changes (48-168h) in spleen and thymus weights, and total lymphocyte populations in those organs. Although bone marrow and spleen cellularity were not reduced by BP, total thymocyte numbers were equally suppressed by BP or DMBA, and suppression was independent of AhR. The proportions of T cell sub-populations in spleen and thymus were largely unaffected by a single DMBA or BP treatment. All of the above responses are dependent on CYP1b1 metabolism of PAHs. We infer that suppression and recovery of committed progenitor precursors (CLP) in bone marrow is sufficient to account for changes in total lymphocytes in BM, spleen and thymus by impairing their re-population by progenitor cells.
Increasingly, the T-cell dependent antibody response (TDAR) assay is used as a means to evaluate immunomodulation—immunopharmacology or immunotoxicology—in nonhuman primates (NHPs). This is primarily due to the plethora of immunomodulatory biopharmaceuticals in development. Our focus will be on several key topics relevant to the TDAR and other measures of immune responses to T-dependent (TD) antigens. Traditionally, the TDAR has been used as a means to evaluate immunosuppression. It is less clear whether it is possible or appropriate to use the TDAR to evaluate immunostimulation. In addition, although the read-out of the TDAR is by definition the generation of antigen-specific antibodies, T-helper cells, as well as antigen presenting cells, are also involved in the response, while poorly characterized in the context of this assay in NHPs. There is, for instance, little information on T-cell differentiation towards T-helper [Th]1 versus Th2 responses in this context. Cellular immune responses to TD antigens may also be evaluated using the delayed-type hypersensitivity (DTH) response; however, DTH is notoriously difficult to produce in NHPs and correlative data between the systemic and local responses to the TD antigen are lacking. Although immune responses to TD antigens are routinely evaluated nonclinically there is little understanding of translational data across species and to humans. Furthermore, the increased use of the TDAR in NHPs is associated with a disparity of protocols as well as methods for data interpretation; any discussions on standardization/best practices generally are fruitful and significant debate. The goal of this session is to share data and progress in our understanding of the measurable endpoints of the immune response to TD antigens and to provide a forum for discussion on the utility of these endpoints within drug development.

**Use of the T Cell-Dependent Antibody Response to Evaluate Immunostimulation.**

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Standard and specialized immunotoxicity tests have been developed and evaluated primarily to detect immunosuppression. The predictivity of specialized immunotoxicity endpoints, such as the T-cell dependent antibody response (TDAR) assay, in detecting immunostimulation remains to be determined. As with immunosuppression, drug-induced immunomodulation may be unintended toxicity or intended pharmacology. Unintended immunomodulation has been demonstrated using the TDAR assay in rodents treated with various pharmaceutical drugs or chemicals. With recent advances in immunomodulatory therapies, the utility of the TDAR assay as an experimental model to verify intended pharmacology also has been explored. These investigations may shed light on how alterations in animals translate to changes in humans. This presentation will focus on the recent data of the TDAR assay to detect drug-induced immunomodulation in rodents and nonhuman primates. The immunomodulatory potential of a monoclonal antibody to cytotoxic T lymphocyte antigen (CTLA4) on antibody responses to tetanus toxoid and keyhole limpet hemocyanin (KLH) in cynomolgus monkeys will be presented to highlight investigations in nonhuman primates. Discussion of immunomodulation of the TDAR in rodents will focus on what can be learned from rodent studies that can be applied to nonhuman primates and humans. Although additional research is needed, available data suggest a potential broad application of the TDAR assay to detect immunostimulation, as well as immunosuppression. However, the clinical relevance of unintended increases in antigen-specific antibody responses in toxicology studies remains to be determined.

**Beyond Antibodies: Characterization of the Cellular Immune Response to Keyhole Limpet Hemocyanin.**

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T-cell dependent antibody response (TDAR) assay is used as a functional assay to measure the effect of various compounds on the immune system. It is generally recommended as the first-line immune function assay in non-clinical evaluation. The humoral immune response to a foreign antigen is measured by evaluating the production of antibodies against this particular antigen. Although the production of antibodies is the principal readout in the TDAR, the generation of specific antibodies requires the participation of antigen-presenting cells, T lymphocytes and B lymphocytes. Therefore, it is assumed that alterations in the level of antibody produced to the specific antigen may reflect effects on any or all of the cell populations involved in the TDAR. To date, the cellular immune response in the TDAR has not been well characterized. Various laboratory are trying to understand the cellular response to well known antigens such as Keyhole limpet hemocyanin (KLH) and Tetanus Toxoid (TT) to better characterize the cell types affected when alterations in the TDAR are observed in non-clinical studies. Cell proliferation, cytokine production, delayed-type hypersensitivity responses are accompanied by immunohistochemical evaluation. T-cell and macrophage activation are all being investigated. The data from these experiments shows that T lymphocytes, macrophages and various cytokines are actively involved in the production of antibodies measured in the TDAR. This presentation will provide an overview of what is known about the cell mediated immune responses in the TDAR. Data gathered from various laboratories, including Lyon Poison Center, GSK, Pfizer, BMS, MedImmune and Charles River will be presented, where cell functions and responses were assessed in TDAR assays.

**Translation of the Immune Responses to T-Dependent Antigens Between Nonhuman Primates and Humans.**


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There are no identical evaluations of the immune responses to T-dependent antigens (TDAs) in humans and non-human primates (NHPs). To directly compare responses, a study was conducted in NHPs following a successful clinical protocol. The TDA keyhole limpet hemocyanin (KLH) was administrated subcutaneously on two occasions followed by an intradermal challenge to induce a DTH response. KLH-specific antibodies were measured in the peripheral blood and redness and induration were measured at the challenge site. Histopathology at the challenge site was assessed in NHPs only. Modulation of the DTH response by dosing with prednisolone was also evaluated. Finally, blood samples were collected to evaluate ex vivo T-cell responses to KLH re-stimulation. KLH immunization resulted in KLH-specific antibody responses and apparent DTH responses in both species; however, prednisolone treatment appeared more effective at inhibiting the DTH response in humans. The quality (immunoglobulin [Ig] isotypes) and quantity of the KLH-specific antibody response was difficult to directly compare due to the different assays used to detect the antibodies (human assay standards were not available/appropriate for use in NHPs), and lack of available reagents to detect NHP Ig isotypes. In NHPs, proliferative and cytokine responses were observed following ex vivo re-
stimulation of peripheral-blood mononuclear cells with KLH but no responses were elicited with human samples. In conclusion, KLH-specific antibodies and an apparent DTH response were induced in NHPs following a successful clinical protocol. The differential ability of prednisolone to modulate the DTH response in NHPs and humans implies that the responses may not have been mediated by the same cell types; however, this could not be further examined due to the lack of skin biopsy samples from the clinical study. Commercially available reagents to detect Ig isotypes in NHP are highly desirable to further our understanding of the TDAR response in NHPs.

2619 GOOD PRACTICES IN THE STUDY DESIGN, DATA ANALYSIS AND REPORTING OF T-DEPENDENT ANTIBODY RESPONSE STUDIES.

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The T-Cell Dependent Antibody Response (TDAR) assay is commonly used to evaluate effects of investigational small molecule drugs or biologicals on the immune system, including in nonhuman primates (NHPs). While this functional assay is recommended in the guidance document ICH S8 Immunotoxicity Studies for Human Pharmaceuticals, the assay is not standardized. An interlabatory retrospective analysis indicated that immunization of NHPs with either keyhole limpet hemocyanin (KLH), sheep red blood cells or tetanus toxoid lead to measurable primary and secondary antibody responses. Also, it was demonstrated that antibody responses can be obtained in response to immunization with bacteriophage. Interanimal variability in peak response values is significant with all antigens evaluated and studies need to be powered accordingly (8 animals per group [if all animals can be evaluated without interference from pre-existing cross-reactive antibodies] allows detection of 3-fold statistically significant differences between groups). Interanimal variability and peak antibody response values are similar in male and female NHPs and data from both genders may be combined for statistical analysis purposes (if appropriate based on pharmacokinetics and target biology) to increase the power to detect differences between treatment groups. In order to account for the kinetics of the antibody response, the area under the curve (AUC) defined by the power to detect differences between treatment groups. In order to account for the kinetics of the antibody response, the area under the curve (AUC) defined by the power to detect differences between treatment groups.

2620 KEY EVENTS OF THE INNATE IMMUNE RESPONSE AS TOOLS FOR IDENTIFICATION OF CHEMICAL SENSITIZERS IN VITRO: DO WE NEED MORE?

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Abstract: Sensitization is the toxicological endpoint associated with compounds having the intrinsic ability to adversely trigger the adaptive immune system. Martin et al. (2011) demonstrated that innate responses in keratinocytes (KC) and Langerhans cells (LC) are crucial. The presented study compared existing in vitro tests based upon innate key mechanisms for their capacity to properly label and classify chemicals. Methods: The performance of selected tests when exposed to well characterized sufficiently water-soluble skin sensitizers (N=35), respiratory sensitizers (N=9), and non-sensitizers (N=21) (including 5 irritants) (www.sens-it-iv.eu) was analyzed retrospectively. The tests included the IL-18 test (Corsini et al., 2009), the Keratinosens (Natsch et al., 2010), 2 reconstituted human epidermides (RHE) models (SkinEthic, CellSystems), the MUSST (Python et al., 2007), h-CLAT (Sakaguchi et al., 2009), VitoSens (Lambrecht et al., 2010), and GARD (Johansson et al., 2011). Results were expressed in terms of concordance (%) with the available in vivo data. Results: The Keratinosens and the IL-18 test identified the chemicals correctly as skin sensitizers in 87 and 95% of the cases. The MUSST and h-CLAT tests (overall: 78%) scored high with extreme and strong sensitizers (86-100%), but poorly with moderate and weak sensitizers (53-60%). Stronger irritants were found positive in these tests. The genomic marker profile of the VitoSens and GARD tests correctly labeled 89 and 98% of the chemicals. They performed equally well across the chemical potency classes and did not score the irritants on the list. The RHE models did not discriminate sensitizers from irritants, but irritative responses were found useful for classification of the chemicals (92%). Conclusion: Tests addressing multiple pathways (IL-18, VitoSens, GARD) performed best. The chemicals on the Sens-it-iv list with sufficient solubility in water were properly identified by one test or a combination of 2 tests (96-98%). In 92% of the cases a proper classification was obtained.

2621 GARD—A GENOMIC BIOMARKER ASSAY FOR PREDICTION OF SENSITIZERS USING A CELL-BASED IN VITRO ALTERNATIVE TO ANIMAL TESTS.

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Background: Allergic contact dermatitis and respiratory conditions such as occupational asthma are both caused by an adverse immune response towards chemical hapten. These diseases affect a significant proportion of the population, with increasing incidences, which leads to a substantial economic burden for society. Current tests for skin sensitizing chemicals rely on animal experimentation, whereas no such assays exist for respiratory sensitizers. New legislations on the registration and use of chemicals within chemical and cosmetic industries imply a need of alternative, human cell-based assays for the prediction of sensitization. The aim is to replace animal experiments with in vitro tests displaying a higher predictive power. Results: We have developed a novel cell-based assay for the prediction of sensitizing chemicals, called Genomic Allergen Rapid Detection, GARD. By analyzing the transriptome of the human cell line MUTZ-3 after 24 h stimulation, using well characterized skin sensitizing chemicals (N=20), respiratory sensitizing chemicals (N=9) and nonsensitizing chemicals (N=20), we have identified genomic biomarker signatures with potent discriminatory ability. Using a Support Vector Machine for cross-validation, the prediction accuracy of the assay is estimated to be 98%. The identified transcriptional biomarkers are involved in biological pathways with immunologically relevant functions, which can shed light on the process of human sensitization. Conclusions: Gene signatures have been identified and demonstrated to have the power to predict sensitization. This simple and robust assay has the potential to completely replace or drastically reduce the utilization of test systems based on experimental animals. Being based on human biology, the assay is proposed to be more accurate for predicting sensitization in humans, than the traditional animal-based tests.

2622 ESTABLISHMENT OF AN IN VITRO PHOTOCOASSAY TEST USING NCTC2544 CELLS AND IL-18 TO DISCRIMINATE PHOTOIRRITANTS FROM PHOTOALLERGENS.

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Purpose: Differentiation between photoallergic and phototoxic reactions induced by low molecular weight compounds represents a current problem. The use of keratinocytes as a potential tool for the detection of photoallergens as opposed to photoirritants is considered an interesting strategy for developing in vitro methods. We have previously shown that the human keratinocyte cell line NCTC2545 and the production of IL-18 is a good model for screening sensitizers. The main purpose of this work was to explore the NCTC2544 model as an in vitro model to identify photoallergens and discriminate from phototoxic chemicals. Methods: The effect of UVA radiation (3 J/cm2) over NCTC2544 cells irradiated and non treated, and treated with increasing concentrations of various compounds including negative compounds (irritants and allergens), ibuprofen and acridine (photoirritants); ketoprofen and chlorpromazine (photoirritant and photoallergen); benzophenone, 4-ter-butyI-4-methoxy-dibenzoylemethylene, 2-ethylhexyl-p-methoxyxcinnamate and 6-methylcumarin (photoallergens) was investigated. Twenty four hours after exposure, cytotoxicity was evaluated by the MTT assay, while the production of IL-18 was measured by a commercially available ELISA kit. Results: At the maximal concentration assayed with non cytotoxic effects, allergens and photoallergens induced and non irradiated, and treated with increasing concentrations of various compounds including negative compounds (irritants and allergens), ibuprofen and acridine (photoirritants); ketoprofen and chlorpromazine (photoirritant and photoallergen); benzophenone, 4-ter-butyI-4-methoxy-dibenzoylemethylene, 2-ethylhexyl-p-methoxyxcinnamate and 6-methylcumarin (photoallergens) was investigated.

2623 PYRIDOXYLAMINE REACTIVITY KINETICS AS AN AMINE-BASED PROBE FOR SCREENING ELECTROPHILIC CONTACT ALLEugiENS.

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Covalent protein binding is an initial step in dermal contact sensitization. We previously reported the utility of a nitrobenzenethiol (NBT) binding assay for screening thiol reactive haptenes (Chipinda et al Chem. Res. Tox. 2010). The present
The test results were assessed according to the UN-GHS, EU-CLP, and Brazilian substances have been tested including a wide range of different chemical classes. In vitro data is one type of test used for skin irritation testing by COLIPA (MacFarlane et al., 2009). We have used the KeratinoSens assay and the results indicate a good predictive value (~85%). In this study, we sought to further refine the applicability domain and predictivity of the assay by testing neat chemicals and mixtures of fragrances, preservatives, industrial solvents, and surfactants with a broad range of potencies for their ability to induce the luciferase gene. In parallel, cytotoxicity was assessed by both Neutral Red Uptake (NRU) and MTT assays. The results from the KeratinoSens assay and parallel cytoxicity assays were compared to the available correlation in vitro and human clinical data. The results indicate that the KeratinoSens assay may be used to evaluate a broad range of materials with reproducibility and a high predictive value.

Assessment of skin sensitization potential of chemicals is an important aspect of the safety evaluation process. Recent regulations, as well as responsible stewardship programs, have advocated for the development of non-animal approaches that can reliably predict skin sensitization potential for new chemicals. As the majority of chemical sensitizers are electrophiles that can react with nucleophilic sites on proteins, several assays have focused on characterizing this property in vitro for predictions of sensitization potential. Herein, we describe our evaluation of two such in vitro approaches, the KeratinoSens assay and the Direct Peptide Reactivity Assay (DPRA). The KeratinoSens assay uses a human keratinocyte cell line (HaCaT) in which activation of the Nrf2-ARE pathway is quantified via a luciferase reporter gene under the control of the antioxidant response element (ARE) derived from the human gene AKR1C2. This is a gene that is known to have an electrophile sensitive pathway. The DPRA exhibited 83% sensitivity, 100% specificity and 89% overall accuracy relative to available in vivo data. The DPRA Driven assay resulted in 99% sensitivity, 100% specificity, and 99% overall accuracy. However, integrating the results of the two assays improved the sensitivity, specificity and accuracy levels to 100% for the nine chemicals. The data sets also identified several characteristics for the conduct of these assays that need to be recognized to avoid false positives and negatives. Overall, the results provide support for the use of these in vitro assays to identify chemicals with skin sensitization potential and that integrating these results enhances the overall predictive capability.
result in serum ALT elevations much higher than define Hy’s Law. Moreover, several reports have indicated that necrosis is often preceded by a lack of hepatic necrosis in the first 12-24 h following the administration of hepatotoxic xenobiotics. Other studies in rodents treated with hepatotoxins have indicated that there is a correlation between a) liver glutathione and bilirubin levels and b) hepatic glutathione and ATP levels. These observations suggested the following hypothesis: bilirubin uptake and processing in the hepatocyte number effect is dependent upon both ATP availability and the number of viable hepatocytes. This hypothesis was tested using the DILys™ model, a mathematical representation of the physiological processes involved in DILI. The model has been validated with acetaminophen and methyfpirine data, and simulations reproduce DILI in mice, rats, and humans. Simulations including only the hepatocyte number effect did not produce increased bilirubin within 24 h after dosing of 960 mg/kg APAP in rats, whereas, simulations including the ATP effect were consistent with both the early and late elevation bilirubin data. The DILys™ model has helped provide substantiation for the hypothesis that hepatocyte ATP balance contributes to circulating bilirubin levels in the early period after dosing with hepatotoxic doses of xenobiotics. This mechanism may provide justification for the FDA’s definition of Hy’s Law.

2631 PREDICTING IN VIVO ENZYME INDUCTION USING PHYSIOLOGICALLY-BASED MODELING.

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Understanding the toxicokinetics and toxicodynamics of a xenobiotic is important in the pharmaceutical industry and for environmental chemicals. Cytochrome P450 (CYP) enzymes and transporter proteins play crucial roles in governing xenobiotic toxicity: metabolism can result in either toxification or detoxification, and transport can either remove xenobiotics from the body or cause increased tissue concentrations and hence induce toxic effects. Some xenobiotics affect their own toxicokinetics by altering the transcription of the genes coding for the metabolism and transporter proteins; this is mediated by the nuclear receptor (NR) superfamily. To investigate the effect of typical nuclear receptor activators on the induction of the enzymes that govern xenobiotic metabolism and disposition in human hepatocytes, a novel mathematical model for the in vitro and in vitro kinetics of chemicals has been developed. This model examines the induction of CYP enzymes mediated by the pregnant X receptor and constitutive androstane receptor, in response to phenobarbital and other CYP-inducing compounds. Unknown model parameters were estimated to a high level of confidence, and model simulations were compared to mRNA expression data of CYP and NR isoforms for primary human hepatocytes and found to give good qualitative agreement. The model has also been validated by comparing to mean fold induction data of the CYP3A4 isoform when induced by dexamethasone. To predict the effect of these NR activators in vivo, the hepatocyte model was linked to a whole body physiologically-based toxicokinetic (PBTK) model, which predicts exposure of hepatocytes to xenobiotics over various external exposure scenarios. This allows the in vivo effect of nuclear receptor activation to be quantitatively understood, and hence, 1) enables compounds that are expected to exhibit unacceptable NR activation in vivo to be screened out, 2) allows external exposure levels that can give rise to limiting adverse effects to be predicted.
Drug-induced liver injury (DILI) of an idiosyncratic nature is a significant concern in understanding drug safety. We hypothesized that sex and age-specific hepatic functional status would be determined by gene expression signatures at various ages and in both sexes. The aims of the study were to use hepatic transcriptional profiles from both sexes of F344 rats at ages of 2, 5, 6, 8, 15, 21, 52, 78, and 104 weeks to determine the functional status of the liver and to explore transcriptional mechanisms of status progression during the rat life cycle. Using pair-wise one-way ANOVA, the liver were divided into three classes-premature, mature and aged. Premature liver was observed in the two-week old female and male rats. Aged liver was identified in the 104-week old female and the 78 and the 104-week-old male rats. 243 differentially expressed genes (DEGs) were identified when comparing the premature to the mature liver. These DEGs are linked to developmentally related processes and significantly enriched for transcriptional factor binding sites (TFBSs), as determined using Genomatix software suite. The top 5 significantly enriched TFBSs included V$KLFS, V$SE22FF, V$MVB1, V$SPA6X, and V$XBBF which are potentially involved in regulation of hepatocytes, cell cycle progression, inhibition of apoptosis, and differentiation. Similar comparison of the gene expression profiles of the mature to the aged liver identified 388 DEGs which are involved in hepatic response to xenobiotics, cell death, and immune system processes. The top 5 significantly enriched TFBSs included V$NOLF, V$SNRF3, V$MIF1, V$SPA2F, and V$CTCF which potentially modulate transcriptional repression, adipogenesis and apoptosis. These findings suggest 1) a sexual dimorphism of hepatic aging progression during the rat life cycle and 2) the progression of hepatic status from premature to mature, and mature to aged, is likely regulated by distinct suites of transcription factors.
Components of combustion emissions are linked statistically with cardiovascular effects of exposure to air pollution, but exposures are seldom characterized in detail and it is not certain which pollutants actually cause the effects. To identify putative causal pollutants, apolipoprotein E knockout mice exposed to high-concentration diesel exhaust were exposed 6 hr/day, 7 days/wk for 7 wk to multiple dilutions of diesel or gasoline exhaust, wood smoke, simulated “downwind” coal combustion emissions, or to clean air. The highest concentrations were administered with and without filtration to remove particulate matter (PM). Exposures were analyzed in detail and components were grouped into 45 predictor variables. Aortas were assayed for transcriptional alterations of 8 markers of oxidative stress and vascular remodeling. Analyses of data from each study demonstrated differences in toxicity and little contribution from particles. The combined 4-study database was analyzed using the multiple additive regression tree (MART) method to identify the most important predictors of each response regardless of pollutant source. Sulfur dioxide (SO2), nitrogen oxides (NO, NO2), carbon monoxide (CO) and ammonia (NH3) were most consistently among the top 5 predictors for the responses, with various volatile organics less consistently among the top 5. MART analysis also yielded dose-response plots for the partial dependence on each predictor of the responses to the mixtures. Mice were then exposed to a mixture of the most dominant top 5 predictors as an initial confirmation that the responses to the mixtures could be largely or wholly reproduced by exposure to the top predictors. Based on responses to 1 wk exposures in previous studies, mice were exposed for 1 wk to a mixture of CO, NH3, NO, NO2, and SO2 at a single concentration each, representing the concentrations of ceium oxide particles in the air. The implications of the accumulating properties of ceium oxide particles for systemic toxicological effects after repeated chronic exposure via ambient air needs to be explored.

Carbon black is a spherical carbon amoneral where multiwall Carbon nanotubes (MWCNT) are cylindrical and graphene is a laminar allotrope of carbon. Processing and handling as well as abrasion processes can set free inhalableCNT particles. Results of rodent studies collectively show that regardless of the process by which CNTs are synthesized and the types and amounts of metals they contained, CNTs were capable of producing inflammation, epithelial granulomas, fibrosis, biochemical and/or toxicological changes in the lungs (Lam et al. 2004, Muller et al. 2005, Ma-Hock 2009, Pauluhn 2010). Graphene possess similar physical properties as CNT but may differ toxicological properties. We performed short-term inhalation studies in rats to compare the toxic potency of four different CNT, two graphenes and one carbon black. The materials are characterized thoroughly according to the OECD list. The four MWCNT caused morphological changes as described above. Several biochemical and cytological parameters in the bronchoalveolar lavage fluid were strongly increased consistent with the histological findings. Two MWCNT exhibited a higher toxicity than two other MWCNTs and findings caused by one Graphene typ were even less severe. The graphene with lower surface area as well as low surface area carbon black did not cause any adverse effects up to 10 mg/m3. The short-term inhalation studies were able to descriminate different toxic potencies of carbon-based nanomaterials and is hence used for the selection of less toxic materials for further product development as well as to fine and prioritize higher-tier toxicological testing of nanomaterials.

Identification of biomarkers assists in the disease diagnosis and environmental health risk assessment. Exposure to Libby amphibole (LA) has been associated with increased cardiovascular mortality. We hypothesized that rats exposed to LA would present a unique serum protein profile which could help elucidate biomarkers of LA-induced injury. In a series of experiments (various LA exposure scenarios and time points) healthy (Wistar Kyoto, WKY; and F344) and cardiovascular compromised rat models (spontaneously hypertensive, SH; and SH heart failure, SHHF) were intratracheally instilled with saline (control), or LA. Several markers of cancer, inflammation, metabolic syndrome (MetS) and acute phase response (APR) were analyzed in serum. All rat strains exhibited acute increases in t-2-macroglobulin and t-t-acid glycoprotein. Markers of inflammation, lipocalin-2 and osteopontin were increased in WKY rats after LA exposure while no LA effects were noted in the MetS markers adiponectin, insulin, leptin, or mesothelin. Quantitative Intact Proteomics profiling of WKY serum 1-day or 4-weeks after 4-week LA instillations indicated no oxytocin receptors modifications, however APR proteins were significantly increased. These included circulating serine protease inhibitor, apolipoprotein E, α-2-HS-glycoprotein, t-kininogen 1 and 2, ceruloplasmin, vitamin D binding protein, and serum amyloid P and decreased levels of serotransferrin, serum albumin, and fetuin 1-day after LA exposure. All changes were evident at 1-day and/or 4-week but were reversible thereafter during recovery. Thus, our comprehensive analysis indicated that pulmonary exposure to LA induces an APR and novel biomarkers of inflammation that could be useful in understanding systemic health effects of asbestos. (Does not represent US EPA policy).
A major question regarding the mechanisms underlying cardiovascular effects of inhaled pollutants is the route by which the toxicity is transferred systemically. We hypothesized that circulating factors, whether from the pollution or secondary biological intermediates, are a vital component of the findings of endothelial dysfunction and acute risk of myocardial events. To address this question in the most straightforward manner, we obtained plasma from healthy human subjects exposed to nitrogen dioxide (500 ppb) and filtered air on separate occasions (N=8) and also from volunteers exposed to diesel (100 µg/m3) or filtered air (N=8). Plasma samples were obtained prior to and after exposures, enabling pairwise, repeated measures analysis. The plasma was added to the media (1:3 or 3:1 ratios) of confluent human primary coronary artery endothelial cells and allowed to incubate for 24 hours at 37°C. After the incubation period, cells were washed and harvested for RNA isolation. ICAM, VCAM, and P-selectin mRNA were then quantified from the endothelial cells using qPCR. Plasma obtained following diesel emissions caused a 20% increase in VCAM expression compared with comparable plasma obtained before exposures and after filtered air exposures. Plasma obtained following nitrogen dioxide exposures caused a 40% increase in VCAM, a 30% increase in ICAM, and a 50% increase in p-selectin. These findings clearly show that, following exposure to diesel and nitrogen dioxide, circulating factors are present that can induce adhesion molecules crucial to vascular inflammation and injury.

**2645 THE ORIGIN, MAGNITUDE, AND IMPLICATIONS OF DIFFERENCES IN ASPERGILLOSIS CANCER RISK ESTIMATES DERIVED USING VARYING PROTOCOLS.**


The origin and magnitude of differences in aspergillus cancer risk estimates derived, respectively, using the "IRIS" protocol (currently used by regulators) and the newer Berman and Crump protocols were examined in a recent publication (Berman 2011). In that study, risks estimated by applying each protocol to real exposure data from both laboratory and field studies were compared to assess the relative health protective values of each protocol. The reliability of risks estimated using the protocols were also compared by evaluating the degree with which each potentially reproduces the known epidemiology study risks. Results from that study indicate that the IRIS and Berman and Crump protocols can be reconciled; while environment-specific, variation within fiber type is apparently due primarily to size effects (not addressed by IRIS) the 10 fold (average) difference between amphibole asbestos risks estimated using each protocol is attributable to an arbitrary selection of the lowest of available mesothelioma potency factors in the IRIS protocol. As a consequence, the IRIS protocol may substantially underestimate risk when exposure is primarily to amphibole asbestos. Moreover, while the Berman and Crump protocol is more reliable than the IRIS protocol overall (especially for predicting amphibole asbestos), evidence was presented suggesting a new fiber size related adjustment to the Berman and Crump protocol may ultimately succeed in reconciling the entire epidemiology database. However, additional data need to be developed before the performance of the adjusted protocol can be fully validated.

In this talk, the analysis and findings of Berman 2011 will be summarized along with the status of efforts underway to develop the data needed to finally and fully vet asbestos risk protocols. Implications for the ongoing assessments of asbestos-related cancer risks, particularly associated with natural occurrences of asbestos, will also be addressed.

**2646 EVALUATION OF DATA FOR A CAUSAL ASSOCIATION BETWEEN FORMALDEHYDE EXPOSURE AND MYELOID OR LYMPHOID MALIGNANCIES.**

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Recent risk assessments have been conducted relying upon the hypothesis that exposure to formaldehyde has the potential to result in myeloid or lymphoid malignancies. The biological plausibility of that conclusion must be reconciled with recent data that suggest evidence in contrast to this hypothesis. Formaldehyde is rapidly metabolized and highly reactive and, because it is an endogenous compound, detectable changes in the natural background levels are necessary to result in adverse effects. The development of sensitive assays of DNA adduct formation in several species has demonstrated that adducts derived from exogenous exposures to formaldehyde are confined only to the sites of contact. A recent study (Zhang et al. 2010) has provided results of aneuploidy in circulating stem cells that provide potential support for proposed mechanisms for formaldehyde to impact bone marrow. However, this demonstration of aneuploidy in human hematopoietic stem cells by the assay selected (CPU-GM) does not measure the proposed events in precursor cells for acute myeloid leukemia. In addition, evaluation of the supporting data indicate that the aneuploidy measured could not have arisen in vivo, but rather arose during in vitro culture, which together with numerical inaccuracies raise questions regarding the reported results. Statistical analyses of the supporting data suggest factors other than formaldehyde exposure may have contributed to the observed aneuploidy. These results, in combination with recent toxicological, pharmacokinetic, and mechanistic studies raise questions regarding the biological plausibility of a mechanism for a causal association between formaldehyde exposure and myeloid or lymphoid malignancies.
Human health risk assessment of environmental contaminants relies on evidence from animal toxicity testing and human epidemiological studies. High throughput methods are in demand for testing large number of chemicals known or suspected to pose risk to human health. Taking advantage of impedance based non-invasive high content screening system, and human cell lines with different organ origins, a cytotoxicity profiling project is under development to: (1) assess and validate the usefulness of this new in vitro screening system for identifying general toxicity of environmental hazards; (2) develop a biologically relevant human health risk assessment model of environmental contaminants; and (3) unveil possible mechanisms and toxicity pathways by which toxicants work. Cells were seeded in special E-Plate to record impedance signals, which were continuously monitored for ~100 hours to reflect cell growth and responses to testing chemicals. Each chemical was tested with 11 different doses in duplicate or quadruplicate, and repeated with at least one separate experiment. At selected time points, IC50 were calculated from each testing cell line. Preliminary testing of 14 chemicals spreading across 5 GHS categories revealed that the new assay has good window and reproducibility; prediction between in vitro parameters (IC50) and animal in vivo data (LD50) was reasonable. Preliminary testing of 30 chemicals fall into different cellular target groups, such as DNA damaging reagent, cytokine and cytotoxicity modifiers, revealed that this approach also help to discover mechanisms of actions from some testing chemicals. Further testing with more standard chemicals is planned. Taken together, this assay system has great potential to be a primary screening method in risk assessment, because of its high throughput, better extrapolation model, and better understanding of toxicity pathways.
bioassays, and the origin of the observed damage. The quality of the data and the nature of the genotoxic endpoint being examined and its sensitivity to toxicity and assay conditions were also important considerations. In each case, the organizations used a weight-of-evidence approach and, in most cases where an evaluation was done by more than one authoritative body, similar conclusions were reached. However, at times, significant differences were observed in how the evidence was evaluated and the conclusions reached. We conclude that a critical evaluation of the data as well as expert judgment is needed in reaching mechanism of action conclusions, and that these determinations should be made within the broader context of evaluating the chemical’s overall toxicology and carcinogenicity. Disclaimer: The views expressed are of those of the authors and do not necessarily reflect the views or policies of the US EPA.

**PL 2652**

**PBPK/PD MODELING OF KEY EVENTS IN A TOXICITY PATHWAY—IMPLICATIONS FOR DETERMINING POPULATION THRESHOLDS.**

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A source-to-outcome model was created for dietary exposures of chlorpyrifos by linking probabilistic dietary exposure models with a PBPK/PD model of an early key event, acetyl cholinesterase inhibition, in the toxicity pathway for the cholinergic effects of the compound. This modeling addresses several concerns raised in Chapter 5 of the NAS report “Science and Decisions”, as discussed at the Alliance for Risk Assessment workshop series. First, the modeling goes beyond MOE or HI approaches used in traditional non-cancer risk assessments and provides information on the fraction of the population affected by a given dose (i.e., exposure). Second, the model allows the quantitative investigation of inter-individual variations in both exposure and response (variation in sensitivity) for different age groups (adults, children, and infants). Third, the analysis provides an opportunity to investigate the impact of knowledge of mode of action (MoA) on two concepts that appear in the NAS report - the “marginal population” and the “background dose”. It is known that at some sufficiently high exposure, AChE inhibitors can cause a range of non-specific symptoms (e.g., cramps, nausea, and pupil dilation) and that there is background incidence for such symptoms in the general population. The modeling from this project demonstrates that current dietary exposures of chlorpyrifos are not expected to change the frequency of these symptoms as the exposure is insufficient to induce the key event (i.e., suppression of acetylcholinesterase). Thus even individuals who are predisposed to experience these symptoms are not expected to be affected by the current levels of dietary chlorpyrifos exposure. This suggests that a low-dose nonlinear individual and population-based dose-response model is most appropriate for this compound. This finding may be relevant for other compounds where MoA data indicate that current exposures do not exceed a threshold for a key event.

**PL 2653**

**DATABASE DEVELOPMENT AND ANALYSES OF EMERGENCY RESPONSE PLANNING GUIDELINES (ERPGs).**


Developed by the American Industrial Hygiene Association (AIHA), ERPGs are widely used as community exposure guidelines to make informed decisions about airborne chemical hazards. Currently, < 1% of commercially used chemicals have an ERPG assigned. It was proposed to estimate missing values from quantitative structure-activity relationship (QSAR) modeling. The goal of the present work was to develop a database of inhalation toxicity information for ERPGs that can be used for QSAR modeling, and to develop ERPGs to other common health guidance values: acute exposure guideline levels (AELGs) and concentrations causing 50% lethality (LC50s). A database for 140 ERPGs was developed from technical support documents (TSD). 270 AELGs were derived in-house, and 59 experimental rat acute inhalation LC50s were extracted from the TOPKAT software. ERPGs and AELGs were linearly correlated on the log scale with $R^2 = 0.69$, 0.93, and 0.93 at the -1, -2, and -3 severity threshold levels, respectively. For ERPG to rat LC50 analysis, the $r^2$ values were 0.52, 0.64, and 0.68, respectively. Deming regression log ERPGs and rat log LC50s suggested identity of the slopes (β) but not intercepts (α) at 99% confidence level (ERPG-1/LC50: β = 1.3 [99% CI: 0.84, 1.7], α = 4.1 [99% CI: -5.8, 2.4]; ERPG-2/LC50: 1.00 ± 0.13, -2.22 ± 1.12, and -2.22 ± 1.12, and ERPG-3/LC50: 0.98 [0.77, 1.2], -1.23 ± 1.5, -0.40, i.e. on average LC50s require scaling down by a factor of 10, 100, and 10 for deriving ERPG-1, -2, and -3, respectively. ERPG estimates derived using a preliminary ERPG QSAR model were 66%-75% and 74%--correlated with the actual data for ERPG-1, -2, and -3, respectively. When ERPG and AELG databases were combined, the predicted correlations were similar (73%-57% and 75% respectively), but the coverage of chemical space improved. These findings suggested that ERPGs are statistically identical to 1-hour AELGs and comparable to rat LC50s. These data can be used together to develop models for emergency assessment of airborne chemical concentrations.

**IS 2654**

**EVOLUTION AND IMPLEMENTATION OF COMBINED CHEMICAL EXPOSURES METHODS—INTERNATIONAL PERSPECTIVES.**

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The global risk assessment community, in response to recognition of the importance of the impact of combined exposure to multiple chemicals and other stressors, has been collaborating to coordinate efficient methods development. Important in these efforts is sharing of international expertise and experience gained in individual countries through global planning, strategic research, and coordinated assessment methodology. These efforts are being led by the World Health Organization/International Programme on Chemical Safety (IPCS), the Organization for Economic Cooperation and Development (OECD), and the International Life Sciences Institute (ILSI). The laws passed by United States and other governments have resulted in federal guidance for combined exposures assessment and joint toxicity assessment of multiple environmental contaminants/stressors. WHO and OECD have developed a framework for assessment of combined exposures with an emphasis on the critical content of problem formulation, the role of predictive tools in grouping of chemicals for consideration, and the importance of explicit delineation of uncertainty and sensitivity for tiered exposure assessment. The European Commission has supported the development of a state of art report for the predictive assessment of the toxicity of combined exposures in a regulatory context. ILSI’s Health Environmental Science Institute (HESI) has pursued efforts to study the likelihood of synergism at low dose levels and the application of the threshold of toxicological concern (TTC) in Tier-0 screening approaches. Our expert panel will discuss the evolution of methods, harmonization of efforts of the recent past, and the role of coordinated research in future developments.

**IS 2655**

**PROPOSITION 65: TWENTY-FIVE YEARS OF IMPLEMENTING CALIFORNIA’S UNIQUE AND FAR-REACHING LAW REGULATING ORGANIC AND METALLIC CARCINOGENS AND DEVELOPMENTAL/REPRODUCTIVE TOXINS.**

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Proposition 65 requires the Governor to publish, at least annually, a list of chemicals known to the state to cause cancer or reproductive toxicity. Proposition 65, the Safe Drinking Water and Toxic Enforcement Act of 1986, was enacted as a ballot initiative in November 1986. The Proposition was intended by its authors to protect California citizens and the State’s drinking water sources from chemicals known to cause cancer and birth defects or other reproductive harm, and to inform citizens about exposures to such chemicals. The first list of chemicals was developed in 1987. Since then over 530 chemicals have been listed for cancer, including Cr(VI), nickel, and arsenic compounds and many organic and metallic/metalloid carcinogens, and 300 chemicals have been listed for reproductive toxicity. The statute states that “no person in the course of doing business shall knowingly and intentionally expose any individual to a chemical known to the state to cause cancer or reproductive toxicity without first giving a clear and reasonable warning…” The Proposition has resulted in product warnings, reformulations, and the identification of toxic substances in products from candy to jewelry. The law has also resulted in extensive discussions of the nexus between science and the law. The session will discuss this nexus with regard to the law’s implementation and enforcement, its strengths and weaknesses, and its influence on science and toxicology. The session will include business and environmental perspectives on what works and what doesn’t.
2656 REFINING YOUR SCIENCE COMMUNICATION SKILLS.

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Attendee questions during a Science Policy Opportunities Education-Career Development Session at the 2010 SOT Annual Meeting highlighted the importance of effective communication as a key skill needed to succeed in the field of science policy. As scientists progress in their careers and/or transition into the science policy arena or into positions that require interactions with the public sector, effective written and oral communication becomes a vital skill. This session is designed to share information on key aspects and topic areas that are of critical importance when communicating science to fellow scientists and non-scientists alike. We will begin this session by covering the importance of effective communication skills and show students and postdoctoral fellows how to begin building a skill set, thus preparing themselves for careers in science, policy, or public health. From there, tips and advice will be provided on communicating science to the general public, as well as how to communicate science to other scientists—a skill that is often overlooked within academic environments. Rounding out this important information will be delivery of the art of the one-page memo, explaining how to summarize large scientific documents for the benefit of nonscientist decision makers. All the presentations will underscore the necessity of educating a target audience that needs to understand complex scientific concepts and critical issues regarding impacts on stakeholders—taxpayers, shareholders, etc.—in order to facilitate decision-making.

2657 ROLES OF RAT HEPATOCYTE MALIGNANT TRANSFORMING FACTOR (HMTF) IN RAT LATE-STAGE HEPATOCARCINOSIS.

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We have been trying to find genes involved in malignant transformation in rat hepatocarcinogenesis by DNA microarray. Recently we identified a novel gene which was overexpressed in hepatocellular carcinoma (HCC). Its function is unknown and we named it hepatocyte malignant transforming factor (HMTF). We found that HMTF gene was overexpressed in rat HCC cell lines, lymphocytes in the spleen, colon mucosal epithelia, spermatocytes, and granule cells of the hippocampus. Reduction of HMTF by RNA interference (RNAi) in N1 cells, an HCC cell line, caused suppression of cell proliferation, invasion and migration. Suppression of proliferation appeared to be due to cell cycle arrest without increased apoptosis. Decreased HMTF expression resulted in down-regulation of STAT3, PCNA and cyclin D1 and up-regulation of p27. These results suggest that HMTF is a new marker for rat HCC and is involved in HCC cell proliferation and may also be linked to cell proliferation in the spleen, colon, brain and testis.

2658 IMPACT OF INHALATION EXPOSURE REGIMEN ON THE POTENTIAL OF BENZENE TO INDUCE GENOTOXICITY IN MICE.

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Occupational exposure to benzene is associated with an increased incidence of aplastic anaemia, myelodysplasia, and acute myeloid leukemia after chronic exposure. Previous repeat dose studies in rodents have demonstrated that dose, route of administration, and exposure regimen (i.e. concurrent vs. intermittent) influence metabolite formation and genotoxic outcome following relatively high benzene exposures. The aim of this study was to evaluate the effect of exposure regimen for lower benzene exposures on metabolic formation, genotoxicity, and glutathione levels on mice exposed to the same total amount (ppm.hr) of benzene. To achieve the same total benzene exposure (60 ppm.hr), mice were exposed to varying concentrations either intermittently or continuously for 1 to 3 days as follows: 40 ppm benzene for 3 days (30 min once/day), 60 ppm benzene for 2 days (30 min once/day), 60 ppm benzene for 1 day (two 30 min exposures), 120 ppm benzene for 1 day (one 30 min exposure). Urine was collected from individual mice for 24 hours starting immediately after the last exposure. Blood and bone marrow were collected at necropsy 24 hours after the last exposure. No treatment-related changes were observed in blood glutathione levels (GSH or GSSG) or mucouicid (MN) induction in any of the benzene exposure groups. Mice exposed to 120 ppm for 30 min exhibited a statistically significant increase in urinary metabolite concentrations (hydroquinone, t-t-muconic acid, and s-phenylmercapturic acid) compared to all other treatment groups. Mice exposed to 40 ppm (3 days) or 60 ppm (for either 1 or 2 days) showed an increase in t-t-muconic acid concentration versus controls.

2659 ROLE OF MAMMARY EPITHELIAL AND STROMAL P450 ENZYMES IN THE CLEARANCE AND METABOLIC ACTIVATION OF 7, 12-DIMETHYLBENZ(A)ANTHRACENE.

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Exposure to environmental chemical carcinogens is a possible contributing factor in breast carcinogenesis. Microsomal P450 (P450) enzymes play a critical role in the metabolic activation of chemical carcinogens. P450 enzymes are expressed in both epithelial and stromal cells in the mammary gland. The specific roles of mammary epithelial and stromal P450s in carcinogenesis are poorly understood, although the importance of the interplay between the two cell populations in breast carcinogenesis has been increasingly recognized in recent years. The aim of this study was to test the hypothesis that mammary epithelial P450 enzymes are critical for the metabolic disposition of 7,12-dimethylbenz[a]anthracene (DMBA) in the target tissue, whereas both epithelial and stromal cells are capable of metabolic activation of this breast procarcinogen, leading to DNA adduct formation in the mammary gland. To test this hypothesis, we studied a new mouse model (named MEp- Cpr-null), in which P450 4A5 activities in the mammary epithelial cells are suppressed through tissue-specific deletion of the gene for P450 4A5 reductase (Cpr), an enzyme required for the activity of all microsomal P450 enzymes. The MEp-Cpr-null mouse was prepared by crossingbreeding the Cpr-lox mouse with the MMTV-Cre mouse. Comparisons between wild-type and MEp-Cpr-null mice showed that the suppression of P450 activities in the mammary epithelial cells resulted in a significant increase in the levels of DMBA and DMBA-DNA adduct (determined by [32]P-postlabeling) in the mammary glands, following a single i.p. dose of DMBA at 50 mg/kg. This finding not only demonstrated the critical role of mammary epithelial cells in the metabolic disposition of DMBA, but also revealed the capacity of mammary stromal cells in the metabolic activation of this breast procarcinogen in the target tissue. (Supported in part by NIH grant ES018884)

2660 CARCINOGENESIS STUDY OF TRIMETHYLOPROPYL TRIACRYLATE IN F344/N RATS AND B6C3F1 MICE.

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Trimethylolpropane triacrylate (TMPTA) is a multifunctional monomer with a wide range of industrial applications as an unsaturated reactive diluent and chemical intermediate. Repeated dermal exposure to humans or animals leads to contact dermatitis and irritation of the skin. Based on its high production volume and current use information, widespread human exposure in the workplace as well as to final product users is anticipated, mainly through the skin. To determine the carcinogenic potential of TMPTA, male and female F344/N rats and B6C3F1 mice were administered TMPTA (0, 0.3, 1.0, or 3.0 mg/kg) in acetone dermally for 2 weeks. There were no differences in the body weights and survival in the treated animals compared to controls. There were no clinical signs of toxicity related to
2661 DIFFERENTIAL EFFECTS OF CHRONIC INFLAMMATION ON DHPN LUNG CARCINOGENESIS IN F344, WISTAR-HANNOVER AND SD RATS.

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It is well known that chronic inflammation has the potential to promote carcinogenesis. In this study, effects of lung inflammation, caused by intratracheal instillation (i.t.) of quartz particles on N-bis (2-hydroxypropyl) nitrosamine (DHPN) lung carcinogenesis in F344, Wistar-Hanover and SD male rats were examined. In experiment 1, from week 0, lung tumorigenesis was initiated by DHPN in drinking water for 2 weeks and 2 mg quartz was administrated by i.t. at week 4. Rats were sacrificed after sampling their blood at week 24 for histopathological examination of lungs. In experiment 2, 2 mg quartz was administered by i.t. on day 0 and the rats were sacrificed and examined on day 1 and 28, thereafter. In experiment 1, carcinogenic potential was examined by comparing the numbers, areas and incidences of the lung tumors (adenomas, bronchial papillomas and adenocarcinomas). Lung inflammation was evaluated by scoring levels for the intensity of infiltration of lymphocytes, neutrophils and histiocytes, as well as pulmonary edema and fibrosis in experiments 1 and 2. Blood samples were examined hematologically and biochemically, parameters including interleukin-6 (IL-6). The numbers, areas and incidences of the tumors were increased with inflammation scores in F344 and SD rats, but in Wistar-Hanover rats decrease was noted. Quartz i.t. induced severe inflammatory changes in the lungs of F344 rats, whereas less influence was evident in Wistar-Hanover rats. No significant differences in IL-6 were apparent among the treatment groups. The present experiments indicated that rat strain differences are observed under lung inflammatory conditions caused by quartz instillation. Resultant chronic inflammation might have promoting effects on lung tumorigenesis in F344 and SD rats.

2662 POTENTIAL CARCINOGENIC TRANSFORMATION OF MYELOID PROGENITOR CELLS WITH FORMALDEHYDE.

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Formaldehyde (FA) is known to induce genotoxic and cytotoxic effects in cells or tissues directly exposed to the chemical and is associated with nasopharyngeal cancer in humans. Additionally, there are epidemiological data suggesting a positive correlation between occupational FA exposure and hematopoietic cancers in humans. Although the mechanism of FA-induced nasopharyngeal cancer has been extensively studied, it is poorly understood how inhaled FA exposure could lead to hematopoietic cancers. A proposed possibility is the transformation of local stem cells or circulating blood hematopoietic stem cells, which then undergo homing and lodgement similar to bone marrow transplantation. In this study, we use the normal murine myeloid progenitor cell line, 32Dcl3, to help define the carcinogenic potential of FA in this cell type. Secretion matrix metalloproteinase-2 (MMP-2), an enzyme that degrades the extracellular matrix and is associated with aggressiveness of leukemias, was used to initially assess cancer phenotype. HoxA9, a highly expressed Hox gene in hematopoietic stem cells that is often over-expressed in both experimental and human leukemias, was assessed as a molecular marker of transformation. 32Dcl3 cells in suspension culture were exposed to FA (10 μM) continuously with passage twice weekly. Although no change in MMP-2 secretion occurred at week 7 of FA exposure, by 10 weeks of exposure secreted activity increased by over 300%, indicative of an acquired cancer phenotype. In addition, in 32Dcl3 cells exposed to FA, a cell death phenotype of 3.2 fold was observed. These data provide initial evidence that FA may be able to induce an oncogenic phenotype in hematopoietic progenitor cells, although additional studies are required.
2666 EVALUATION OF THE UROTHELIAL CYTOXICITY OF PULEGONE.

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Essential oils, from mint plants including peppermint and pennyroyal oils, are used at low levels (<20 ppm) as flavoring agents in various foods and beverages. At high levels (>5 g) pennyroyal oil poisoning can cause adverse health effects including death. Pulegone, a monoterpeno ketone, is a major component of these oils. A major metabolite menthofuran, is implicated in its toxicity. In a 2-year bioassay, oral administration of pulegone slightly increased the urothelial tumor incidence in female rats. We hypothesized that pulegone causes urothelial cytotoxicity and increases urothelial cell proliferation, ultimately leading to tumors. We administered pulegone by oral gavage at 0, 75 or 150 mg/kg body weight to female rats for 4 weeks. Fresh void urine was analyzed for the presence of abnormal crystals. Urinary bladder tissues were evaluated by light and scanning electron microscopy (SEM), and the bromodeoxyuridine (BrdU) labeling index. In vitro, pulegone and its metabolites, menthofuran and menthone, were tested for cytotoxicity in MYP3 rat urothelial cells. Exposure to 1 mM 3-MA reduced soft agar colony formation of malignant UROtsa cells by 60.5%. Our data supports a potential role of altered autophagy in the malignant phenotype induced by chronic exposure to inorganic arsenic(III) in bladder epithelial cells.

2667 DISRUPTION OF AUTOPHAGY PRODUCES REVERSIBLE EVENTS IN THE MALIGNANT TRANSFORMED HUMAN UROTHELIAL-DERIVED CELL LINE UROTS.

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Chronic exposure to environmental arsenic has been shown to cause many different types of cancers, including bladder cancer. An immortalized human urothelial cell line, UROtsa has been widely used as a model of arsenic-induced bladder cancer. UROtsa cells have been malignantly transformed by chronic exposure to low concentrations of arsenic(III) to produce malignant UROtsa cell lines. Autophagy is a cellular degradation pathway that recycles damaged and superfluous proteins and organelles. Recent evidence suggests that autophagy may be a survival mechanism in malignant tumor cell growth. This led us to hypothesize that malignant UROtsa cells could have a higher autophagy rate than the parent UROtsa cells, and that inhibiting autophagy could reduce malignant potential in UROtsa cells. Using Western immunoblots, we found the malignant UROtsa cells have higher steady-state LC3II protein levels and correspondingly lower p62 protein levels compared to the parent UROtsa cells. When we blocked autophagic flux with 100mM of bafilomycin A1 (Baf-A1) in a 3-hour time course and measured LC3II protein levels we observed that malignant UROtsa cells have greater autophagic flux compared to parent UROtsa cells, suggesting a higher rate of autophagic turnover in the malignant UROtsa cells. Associated with this, the malignant UROtsa cells demonstrated a 50% increase in LysoTracker Red fluorescent levels measured by flow cytometry indicating more autophagic structures in the malignant UROtsa cells. Exposure of the malignant UROtsa cells to autophagy inhibitors (3-methyladenine (3-MA) or Baf-A1) resulted in significant inhibition of colony formation in soft agar, a key phenotype of arsenic-induced malignant transformation. Exposure to 1 mM 3-MA reduced soft agar colony formation of malignant UROtsa cells by 60.5%. Our data supports a potential role of altered autophagy in the malignant phenotype induced by chronic exposure to inorganic arsenic(III) in bladder epithelial cells.

2668 GAP JUNCTION ENHANCER INCREASES EFFICACY OF CISPLATIN TO ATTENUATE MAMMARY TUMOR GROWTH.

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Renal failure in cancer patients is a common problem. Cisplatin nephrotoxicity is clearly dose-related and used to be considered dose limiting. Cisplatin treatment has an overall 19% response rate in animal models with malignant tumors. A new class of substituted quinolines (PQs) possesses inhibitory activities against breast cancer cells through the enhancement of gap junctional intercellular communication. The objective of this study was to examine the effect of a combinational treatment of PQ and cisplatin in an animal model to show an increase in efficacy via the enhancement of gap junctions. Mice were implanted with estradiol-17beta (1.7 mg/pellet) before the injection of 1 x 107 T47D breast cancer cells subcutaneously into the inguinal region of mammary fat pad. Animals were treated intraperitoneally with DMSO (control), Cisplatin, PQ, or a combining treatment of PQ and cisplatin in an animal model to show an increase in efficacy via the enhancement of gap junctions. Mice showed that cell proliferation did occur. In summary, our results suggest that cytotoxicity followed by regenerative cell proliferation are the sequential key events that occur during exposure to diuron and may be induced by its urinary metabolites.

2669 MODE OF ACTION FOR THE SYNTHETIC PYRETHROID PERMETHRIN-INDUCED MOUSE LUNG TUMORS: EVIDENCE FOR CLARA CELL PROLIFERATION.

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It has been shown that diuron is carcinogenic to the rat urinary bladder at high dietary levels. The proposed mode of action for diuron is urothelial cytotoxicity and necrosis followed by regenerative urothelial hyperplasia. Cytotoxicity can be induced either by urinary solids or by chemical toxicity. However, diuron-induced urothelial cytotoxicity is not due to urinary solids, so it may be chemically induced. Diuron is extensively metabolized, and in rats, N-(3,4-dichlorophenyl) urea (DCPU) and 4,5-dichloro-2-hydroxyphenyl urea (2-OH-DCPU) were the predominant urinary metabolites; lesser metabolites included N-(3,4-dichlorophenyl)-3-methylurea (DCPMU) and trace levels of 3,4-dichloroaniline (DCA). In humans, DCPMU and DCPU have been found in the urine after cases of product abuse. The aims of this study were to investigate the cytotoxic effects of diuron metabolites toward a rat urothelial cell line (MYP3) and determine the time course of diuron-induced early cytotoxic and proliferative urothelial changes in male rats. In vitro, MYP3 cells were treated with different concentrations of DCA, DCPU and DCPMU for 72 hrs to determine the LC50 by the MTT assay. Results showed the following LC50s: DCPU-185uM; DCA-213uM and DCPMU-104 uM. In vivo, male Wistar rats were randomized into 2 treatment groups (40/group), control and 2500 ppm diuron. Rats were treated for 1, 3, 7, or 28 days. Scanning electron microscopy (SEM) showed cell swelling beginning at day 1. By day 28, SEM analysis detected extensive necrosis and exfoliation and piling up of cells suggestive of hyperplasia. Although there was no increase in the bromodeoxyuridine (BrdU) labeling index, the histopathological diagnosis of simple hyperplasia on day 28 showed that cell proliferation did occur. In summary, our results suggest that cytotoxicity followed by regenerative cell proliferation are the sequential key events that occur during exposure to diuron and may be induced by its urinary metabolites.
morphological alteration, focusing on time-course, dose-response, recovery, and sex, strain and species differences. Cell (7), i.e., as fed diet containing permethrin or ionized (as a reference mouse lung carcinogen) for 7 or 14 days.

Permethyl induced continuously increased Clara cell proliferation from an early stage of treatment (within one week) in female but not in male mice. There were strong parallels in the dose response for proliferation and the tumor appearance in female mice. Histopathological examination by light microscopy did not reveal any abnormalities but examination by electron microscopy revealed morphologic alterations of Clara cells in permethyl and ionized groups; dilation and/or proliferation of SER (smooth-surfaced Endoplasmic Reticulum), decreased secretory granules, increased incidence of Clara cells with blebs and Clara cells containing elongate-type mitochondria. All of these effects recovered after cessation of the treatment. The increased Clara cell proliferation was clearly observed in a susceptible mouse strain, but was not observed in rats. These data suggest that the MOA for permethyl-induced mouse lung tumors involves slight toxic effects on Clara cells resulting in stimulation of Clara cell proliferation in susceptible animals. Based on analysis using the ILSI/IPCS MOA and human relevance frameworks, including comparison with the results of ionized, acting by a similar MOA and being negative in epidemiological studies, it is reasonable to conclude that permethyl will not have lung carcinogenic activity in humans.

2670 FORMALDEHYDE INDUCES CENTROSOME AMPLIFICATION IN HUMAN LYMPHBLASTOID TKG CELLS.

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Formaldehyde, a primary industrial chemical and a ubiquitous environmental pollutant, has recently been classified as a human leukemogen by IARC and US National Toxicology Program (NTP). Though the mechanisms underlying its leukemogenesis remain elusive, DNA damage and chromosome alterations, including aneuploidy, frequently have been reported in formaldehyde treated animals and exposed humans. The centrosome is an organelle that serves as the main microtubule organizing center as well as a regulator of cell-cycle progression. During mitosis, two centrosomes form spindle poles and direct the formation of the bipolar spindles, which is essential for accurate chromosome segregation into daughter cells. The presence of more than two centrosomes (centrosome amplification) severely disturbs the mitotic process and results in chromosome segregation errors. Centrosome amplification is a common phenomenon in human cancers, including leukemia, and is thought to play an important role in carcinogenesis. To shed light on formaldehyde-induced leukemogenesis, we explored the induction of centrosome amplification by formaldehyde in human lymphoblastoid TK6 cells in vitro. TK6 cells were treated with 0-200 μM formaldehyde for 24 hours. After treatment, cells were washed and cultured in fresh medium for 24 hours and then harvested. Centrosome amplification was examined using immunocytochemistry. We also examined the effect of two established leukemogenic chemotherapy drugs, etoposide and melphalan, on centrosome amplification. The results showed that centrosome amplification rates in TK6 cells exposed to formaldehyde at 150, 175, and 200 μM were significantly increased compared to the control (p<0.05, p<0.001, and p<0.0001, respectively). Etoposide and melphalan also induced centrosome amplification in a dose-dependent manner. In conclusion, formaldehyde induces centrosome amplification in human cells and this may play a potential role in its leukemogenesis.

2671 RAPID INDUCTION OF COLONIC ADENOCARCINOMA IN MICE EXPOSED TO BENZO(A)PYRENE AND DEXTARN SULFATE SODIUM.

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Conceptually, cancer is well-known to be a genetic disease that is influenced by many epigenetic factors. However, mutagenic risk does not always correlate with that of carcinogenicity. The reasons for this discrepancy are poorly understood. Previously, we reported in a study using a transgenic mouse (MutaMouse), that the mutation frequency was markedly increased in the colon after the oral treatment of mice with a potent carcinogen, benzo(a)pyrene (BP); however this was not followed by tumor development. In the present study, dextran sulfate sodium (DSS)-induced colitis was induced in mice following BP exposure. Male C3D2F1 mice were given orally BP at 125 mg/kg/day for 5 days. Ten days after the last BP treatment, mice were exposed to 4% DSS in the drinking water for up to 2 weeks. Mice were examined weekly. Cell (11), i.e., as fed diet containing permethyl or ionized as a reference mouse lung carcinogen for 7 or 14 days. Permethyl induced continuously increased Clara cell proliferation from an early stage of treatment (within one week) in female but not in male mice. There were strong parallels in the dose response for proliferation and the tumor appearance in female mice. Histopathological examination by light microscopy did not reveal any abnormalities but examination by electron microscopy revealed morphologic alterations of Clara cells in permethyl and ionized groups; dilation and/or proliferation of SER (smooth-surfaced Endoplasmic Reticulum), decreased secretory granules, increased incidence of Clara cells with blebs and Clara cells containing elongate-type mitochondria. All of these effects recovered after cessation of the treatment. The increased Clara cell proliferation was clearly observed in a susceptible mouse strain, but was not observed in rats. These data suggest that the MOA for permethyl-induced mouse lung tumors involves slight toxic effects on Clara cells resulting in stimulation of Clara cell proliferation in susceptible animals. Based on analysis using the ILSI/IPCS MOA and human relevance frameworks, including comparison with the results of ionized, acting by a similar MOA and being negative in epidemiological studies, it is reasonable to conclude that permethyl will not have lung carcinogenic activity in humans.

2672 TEMPORAL CHANGES IN GENE EXPRESSION AND CELLULAR RESPONSES AFTER EXPOSURE TO MENADIONE, TERT-BUTYL HYDROPEROXIDE, AND HYDROGEN PEROXIDE IN HEPG2 CELLS.

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Oxidative stress plays an important role in hepatocarcinogenesis, however, our insight in the molecular responses to different oxygen radicals is fragmentary and incomplete. Since these cellular responses will differ in time, examining time-dependent changes in gene expression and correlation with phenotypic markers may provide new insights in responses to oxidants. Time and concentration-dependent cytotoxicity was investigated by the MTT assay as induced by three oxidants, menadione, tert-butyl hydroperoxide (TBH) and hydrogen peroxide (H2O2)/ferrous sulfate (FeSO4) in human hepatoma cells (HeP2). Cell division, apoptosis and cell cycle distribution were analyzed after exposure at 30', 1h, 2h, 4h, 6h, 8h, 24h. Cells exposed to menadione showed an increase in the level of apoptosis after 24 hours only, while no apoptosis was detected in cells exposed to TBH. However, exposure to TBH affected the cell cycle after 6, 8 and 24 hours by inducing 5-phase arrest. When cells were exposed to menadione or H2O2/FeSO4 no differences in cell cycle distribution were observed. In addition, gene expression profiling by micro-array showed similarities and differences in temporal gene expression changes, including related phenotypic changes. These results showed that it is of importance to study time-dependent changes in gene expression and phenotypic markers in order to understand sequential cellular responses towards different forms of oxidative stress.

2673 MODE OF ACTION (MOA) AND HUMAN RELEVANCE FRAMEWORK (HRF) ANALYSIS FOR FISCHER 344 RAT LEYDIG CELL TUMOURS.

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XI1422208 (CAS: 946578-00-3), a novel agrochemical, increased Leydig cell tumor (LCT) size and bilateral incidence (88%) relative to controls (6%) in a Fischer F344 DuCl rat carcinogenicity study. It was hypothesized that these effects represent LCT promotion via a hormone-based dopamine enhancement MoA with the following key events: 1) increased dopamine release via nicotinic acetylcholine receptor agonism at both dopaminergic neurons and dopamine receptors, 2) inhibition of prolactin (Prl) release by the anterior pituitary causing decreased Prl levels, 3) down-regulation of luteinizing hormone (LH) receptor gene expression in Leydig cells, 4) transient decreases in serum testosterone (T), 5) increased serum LH levels, leading to 6) promotion of Leydig cell tumorigenesis. These key events were experimentally examined to evaluate this MoA for XI1422208's promotion of F344 rat LCT. Direct data were generated for key events 1-6, which were used in combination with indirect data. A weight of evidence approach was used to evaluate these data, which included data for the other
8 known potential MoAs, based upon the Bradford-Hill criteria followed by subsequent application in an HRF. This hormonally-mediated, threshold based, non-linear MoA adequately explains the promotion of F344 LCT by X1142208. This MoA is considered not relevant to humans due to qualitative and quantitative species differences: 1) rat, but not human. Leydig cells express functional Prl receptors (PrlRs) on their surface, and 2) rat Leydig cells contain >10-fold more LH receptors compared to humans, which confers much greater sensitivity to changes in circulating LH levels. Stimulation of rat Leydig cells through PrlRs is a rat-specific mechanism by which LCT formation can occur. Therefore, this MoA is considered not relevant to humans and, in accordance with HRF analysis, should not be considered in the human health risk assessment.

2674 DIETARY ADMINISTRATION OF γ- AND δ-TOCOPHEROL INHIBITS MAMMARY CARCINOGENESIS.

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Dietary intake of vitamin E has been suggested to reduce cancer risk due to its antioxidant properties. Tocopherol, a member of the vitamin E family, consists of four forms designated as α, β, γ, and δ. Several large cancer prevention studies, which utilized α-tocopherol, have reported no beneficial results, but recent studies have suggested that γ- and δ-tocopherol may be more effective. Using a mammary carcinogenesis model in female Sprague Dawley rats induced with N-methyl-N-nitrosourea, the chemopreventive activities of individual tocopherols were assessed using diets containing 0.3% α-, γ-, or δ-tocopherol. At 11 weeks, the average tumor burden of the control group was 10.6 ± 0.8 g, whereas dietary administration of γ- and δ-tocopherol significantly decreased tumor burden to 7.1 ± 0.7 g (p<0.01) and 7.2 ± 0.8 g (p<0.01), respectively. Tumor multiplicity was also reduced in γ- and δ-tocopherol treatment groups by 32% (p<0.005) and 42% (p<0.0001), respectively. In contrast, α-tocopherol did not decrease tumor burden nor multiplicity. Tocopherol supplementation increased the levels of its corresponding tocopherol and short-chain metabolites in the serum, mammary gland, and tumor. In mammary tumors, the levels of Nrf2 were increased by tocopherol administration (δ > γ > α). Immunohistochemical analysis of mammary tumors showed a decrease in levels of nitrotyrosine and 8-OHdG when treated with tocopherol diet, with γ- and δ-tocopherol more effective than α-tocopherol. Both γ- and δ-tocopherol, but not α-tocopherol, appear to be promising agents for breast cancer prevention. (Supported by NIH R03 CA141756, ES005022, and the State Cancer Institute of New Jersey, New Brunswick, NJ.)

2675 AEROSOL-DELIVERED AKT1 SHRNA USING SPERMINE-BASED POLY(AMINO ESTER) SUPPRESSED LUNG TUMORIGENESIS.

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Polyethylenimine (PEI) has been widely used as a cationic polymeric gene carrier because of high transfection efficiency, however, the use of PEI has been hindered at the practical application because of its cytotoxicity. In this study, we study the development of poly(aminostar ester) (PAE) based on glycerol propoxylate triacrylate (GPT) and spermine (SPE) as an alternative gene carrier for lung cancer therapy. GPT-SPE copolymer was synthesized by Michael addition reaction between GPT and spermine. The molecular weight and composition were characterized using gel permeability chromatography (GPC) and 1H-nuclear magnetic resonance ((1H-NMR), respectively. The GPT-SPE could condense DNA with approximately 163nm size in yeast, and increased HQ genotoxicity in mouse bone marrow as measured by an in vitro micronucleus assay. Therefore, this MoA is considered not relevant to humans due to qualitative and quantitative species differences: 1) rat, but not human. Leydig cells express functional Prl receptors (PrlRs) on their surface, and 2) rat Leydig cells contain >10-fold more LH receptors compared to humans, which confers much greater sensitivity to changes in circulating LH levels. Stimulation of rat Leydig cells through PrlRs is a rat-specific mechanism by which LCT formation can occur. Therefore, this MoA is considered not relevant to humans and, in accordance with HRF analysis, should not be considered in the human health risk assessment.

2676 THE COMBINATION OF RALOXIFENE AND GEFITINIB IS SYNERGISTICALLY CYTOTOXIC TOWARD TRIPLE NEGATIVE BREAST CELL LINES.

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In recent years, numerous studies have shown that the selective estrogen receptor modulators (SERMs) tamoxifen and raloxifene reduce the risk of invasive breast carcinoma. However, while SERMs do prevent the development of many estrogen-receptor (ER)-positive breast cancers, few studies suggest a potential effect of these drugs in the prevention of the development of ER-negative breast cancers. Recently, studies perform in our laboratory demonstrated that raloxifene decreased the level of EGFR expression in triple negative xenograft tumors. Based on these results, we hypothesized that a combination of raloxifene and a tyrosine kinase inhibitor, gefitinib may improve cytotoxicity towards triple negative breast cancers. In the present study, we show that raloxifene (5 μM) in combination with gefitinib (6 μM) decreased MDA-MB-468 cell number by 90% and this was significantly greater than cell death elicited by either of the single treatments. This correlated with 25% of the cells undergoing apoptosis after 48 h, compared with 8 and 9% for raloxifene and gefitinib, respectively. Similar results were observed in MDA-MB-231 cells. Expression and phosphorylation pattern of proteins involved in cell proliferation such as NFκB, β-catenin, epidermal growth factor (EGFR) were also significantly decreased by the combination treatment, compared to single treatments. Furthermore, evidence of apoptosis activation was also observed by a 5-fold increase in the cleaved form of caspase-3, compared to single treatments. In vitro angiogenesis assays demonstrated that the combination also decreased cell migration, endothelial cell tube formation and cell invasion. In conclusion, these results suggest that there may be a role for the combination of gefitinib and raloxifene in the prevention of triple negative breast cancer. These results also provide us with the basis for future studies on the mechanism involved in raloxifene-gefitinib inhibition of triple negative tumor growth.

2677 FUNCTIONAL PROFILING REVEALS THAT MODULATION OF RAS SIGNALING ALTERS THE TOXICITY OF HYDROQUINONE.

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Hydroquinone (HQ) is an important benzene metabolite with ubiquitous environmental presence, leading to significant population exposure. We used a functional genomics approach to identify the genes that modulate the cellular toxicity of HQ in the model eukaryote Saccharomyces cerevisiae. This screen identified IRA2, a yeast ortholog of the human tumor suppressor gene NF1, as required for tolerance to treatment with HQ. Mutations in NF1 cause the disease neurofibromatosis type 1 (NF1) and pre-dispose individuals to cancers, including leukemia, due to increased Ras signaling. Activation of Ras family members causes increased cellular growth and differentiation. Benzene is an established human leukemogen, but the association between Ras status and HQ toxicity has not yet been defined. Here we show that the Ras status of cells modulates the toxicity of HQ, indicating potential synergy between Ras signaling and benzene toxicity. Specifically, enhanced Ras signaling causes increased HQ-related growth inhibition in yeast, and increased HQ genotoxicity in mouse bone marrow as measured by an in vitro erythroid micronucleus assay. Enhanced Ras signaling relative to WT also resulted in increased formation of mouse CLEL-GM bone marrow progenitor cells, the precursors to the malignant cells that cause benzene-associated leukemia, following treatment with HQ.
and Nutritional Sciences, University of Reading, Reading, United Kingdom, false negative compounds might become less significant considering their pharmacological negative tumor predictivity, and the toxicological significance of the tumors by compounds that meet the NEG CARC Rat criteria due to the sufficient accuracy of rat, were carcinoid by histamine H2 receptor antagonists and pheochromocytoma aminated modes of action of the true positive compounds, which did not meet the criteria.

The Japan Pharmaceutical Manufacturers Association (JPMA) carried out a carcinogenicity data survey using the same questionnaire as that of PhRMA, which involved pharmacological actions, drug-induced tumors in the rat and mouse, preneoplastic and proliferative/hyperplastic lesions in rat chronic studies, hormonal actions and genotoxicity of the marketed and nonmarketed drugs developed by JPMA companies during the past 25 years. The analysis of 64 compounds showed that 1) negative predictivity of the NEG CARC Rat criteria was 87.5%, whereas positive predictivity was 57.5%, 2) the estimated modes of action of the true positive compounds, which did not meet the criteria, and were carcinogenic in the rat, need to be related to enzyme induction, chronic inflammation, hormonal actions or secondary pharmacological actions, 3) tumors from three false negatives, which met the criteria but induced tumors in the rat, were carcinoid by histamine H2 receptor antagonists and pheochromocytoma in a vitamin D3 analogue. These results suggested little value for two-year rat carcinogenicity studies of the compounds that meet the NEG CARC Rat criteria due to the sufficient accuracy of its negative tumor predictivity, and the toxicological significance of the tumors by false negative compounds might become less significant considering their pharmacological actions.

Smokeless tobacco (ST) is growing in popularity due to: unsupported perception of safety, inducement of smoking bans, ability to conceal use, increased social acceptance, and reported ‘positive’ physiological effects, including relaxation, increased concentration, heightened alertness, and diminished hunger. Guthka, a type of ST, is particularly common amongst Southeast Asians, although the toxicological implications have not been delineated. This murine study examines the toxicological implications of Guthka use on generalized whole body effects, with a focus on enzymatic modifications in human colon tissue, both of which are linked to carcinogenicity. In a 2 year rat (Han Wistar) bioassay with Isopyrazam (IZM), a new pyrazole broad-spectrum fungicide (FRAC class 7) dosed at 100, 500 and 3000ppm, there was a higher incidence of hepatocellular adenomas in females only, at 3000ppm. This response was seen in the presence of substantial systemic toxicity (30% decreased body weight), considered in excess of an MTD. In sub-chronic studies, hepatocellular hypertrophy, hepatomegaly and induction of cytochrome (CYP) 2B activity were observed. In 2 year rat (Han Wistar) bioassay with Isopyrazam (IZM), a new pyrazole broad-spectrum fungicide (FRAC class 7) dosed at 100, 500 and 3000ppm, there was a higher incidence of hepatocellular adenomas in females only, at 3000ppm. This response was seen in the presence of substantial systemic toxicity (30% decreased body weight), considered in excess of an MTD. In sub-chronic studies, hepatocellular hypertrophy, hepatomegaly and induction of cytochrome (CYP) 2B activity were observed.
This study reports a comprehensive analysis of the subcellular proteome and lipidome of a hepatoma cell line (FaO) and its utility for monitoring sub-cellular changes associated with Non-Genotoxic Carcinogens (NGCs) exemplified by Mono(2-ethylhexyl) phthalate (MEHP).

This novel method determines the lipid distribution and protein localisation in organelles. The previously established Localisation of Organelle Proteins by Isotope Tagging (LOPIT) technique was expanded to accommodate a lipidomic analysis and the approach has been termed Lipid-LOPIT.

A self-generating density gradient was used to partially separate organelles into individual fractions. Fractions were selected based on their profile of proteins, measured by western blotting. Distribution was then determined by isobaric mass tagging, LC-MS and multivariate data analysis, based on proteins with known organelle locations. This analysis of enriched organelle fractions identified numerous lipid species, whose type and concentration varied between different organelles that could be used as subcellular markers. Following MEHP treatment, changes were seen in the observed distribution and concentration of the lipids including Phosphatidylethanolamine and Phosphatidylinositol throughout the gradient, supporting the hypothesis that the same of the changes in the lipid profiles (due to NGCs) are a result of induced organelle proliferation. We have also demonstrated that lipid homeostasis correlates with cellular proteins which suggest their role in regulating cell physiology.

The results represent the first use of Lipid-LOPIT in any cell line, the first application of LOPIT to a hepatoma cell line (FaO) and the first practical application of Lipid-LOPIT for determining early changes caused by a NGC.

This project is sponsored by MRC Integrative Toxicology Training Partnership in conjunction with Syngenta.
Historically, diagnoses of rat brain tumors have been based almost exclusively on histological features present in hepatocyte- and eosin-stained sections due to a lack of available ancillary techniques. Twenty-eight spontaneously-occurring glial tumors (astrocytomas, oligodendrogliomas, gliomas), nine brain tumors from a 2-year study of acrylonitrile and eleven treatment-related brain tumors from a 2-year chronic bioassay of a second chemical were evaluated using a panel of immunohistochemistry stains (RCA-1, Bba-1, OX-6/MHCII, Olig2, GFAP, neurofilament, PCNA). Based on staining characteristics, oligodendrogliomas were the most commonly diagnosed spontaneous tumor. Many of the spontaneous tumors previously diagnosed as astrocytomas were intensely RCA-1 and Bba-1-positive, and the diagnosis of microglioma is proposed for these neoplasms. In addition, three mixed tumors were identified with Olig2+ (oligodendrocytes) and Bba-1+ (macrophage/microglia) cell populations. In animals treated with acrylonitrile, all nine brain tumors were identified as microgliomas with staining properties similar to the spontaneous tumors. Gliomas from the second chemical study were most commonly identified as microgliomas (8/11). As compared to spontaneous neoplasms, microgliomas from the second chemical study exhibited less intense staining with RCA-1 and increased OX-6/MHCII positivity and contained larger numbers of stained Olig2+ and GFAP+ cells, which may indicate a difference in cellular activation or protein expression in these tumors. While samples were only available from two chemical studies, this may indicate that oligodendrogliomas are more common as spontaneous tumors, while chemically-induced neoplasms are more likely to be microgli/histiocytic in origin.

**2686 IMMUNOHISTOCHEMICAL STAINING OF SPONTANEOUS AND CHEMICALLY-INDUCED BRAIN TUMORS IN THE RAT.**

J. Hardisky and H. Kolenda-Roberts. EPL, Inc., Durham, NC.

**2687 ROLE OF INTESTINAL P450 ENZYMES IN THE METABOLIC ACTIVATION OF THE COLON CARCINOGEN AzoxyMethane in Mice.**


AzoxyMethane (AOM) is a potent colon-specific carcinogen in rodents. It undergoes sequential metabolic activation by P450 enzymes, forming the reactive methyldiazonium ion. The latter alkylates the DNA guanine, forming adducts, of which, O\textsuperscript{6}-methylguanine (O\textsuperscript{6}-MeG) is the most mutagenic and contributes to colon tumorigenesis. The mechanistic details of the in vivo metabolic activation of AOM remain unclear. The aim of this study was to determine whether P450 enzymes of the intestine, the target organ, contribute to AOM bioactivation in vivo. To accomplish this aim, we compared tissue levels of O\textsuperscript{6}-MeG adduct between A549 and HBECK-KT cells. In A549 cells the structures of B[a]P-7,8-dione DNA adducts were identified as hydrated N\textsuperscript{2}'-deoxyguanosine-B[a]P-7,8-dione and hydrated N\textsuperscript{2}'-deoxyguanosine-B[a]P-7,8-dione. In HBECK-KT cells the structures of B[a]P-7,8-dione DNA adducts were identified as hydrated 2'-deoxyadenosine-B[a]P-7,8-dione, hydrated N\textsuperscript{2}'-deoxyadenosine-B[a]P-7,8-dione and either unhydrated N\textsuperscript{2}'-2'deoxyadenosine-B[a]P-7,8-dione. In each case adduct structures were characterized by MS\textsuperscript{a} spectra. Adduct structures were also compared to those synthesized from reactions of B[a]P-7,8-dione with either deoxyribonucleosides or salmon testis DNA but were found to be different. (Supported by 1R01-CA39504 and P30-ES-013508 to TMF).

**2688 IDENTIFICATION OF COVALENT BENZ(A)PYRENE-7,8-DIOX-DNA ADDUCTS IN HUMAN LUNG CELLS.**

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Metabolic activation of the proximate carcinogen B[a]P-7,8-trans-dihydriodiol by aldo-keto reductases (AKRs) leads to benzo[a]pyrene-7,8-dione (B[a]P-7,8-dione) that is both electrophilic and redox-active. B[a]P-7,8-dione generates reactive oxygen species resulting in oxidative DNA damage in human lung cells. However, in formation on the covalent B[a]P-7,8-dione-DNA adducts is lacking. We studied covalent DNA adduct formation of B[a]P-7,8-dione in human lung adenocarcinoma A549 cells, human bronchoalveolar H358 cells, and immortalized human bronchial epithelial HBECK-KT cells. After treatment with 2 μM B[a]P-7,8-dione, the cellular DNA was extracted from the cell pellets subjected to enzyme hydrolysis and subsequent analysis by LC-MS/MS. Several covalent DNA adducts of B[a]P-7,8-dione were only detected in A549 and HBECK-KT cells the structures of B[a]P-7,8-dione DNA adducts were identified as hydrated N\textsuperscript{2}'-deoxyguanosine-B[a]P-7,8-dione and hydrated N\textsuperscript{2}'-deoxyguanosine-B[a]P-7,8-dione. In each case adduct structures were characterized by MS\textsuperscript{a} spectra. Adduct structures were also compared to those synthesized from reactions of B[a]P-7,8-dione with either deoxyribonucleosides or salmon testis DNA but were found to be different. (Supported by 1R01-CA39504 and P30-ES-013508 to TMF).

**2689 METHOD DEVELOPMENT FOR BUTADIENE TRIHYDOXYBUTYL=valine GLOBIN ADDUCTS (THB-“FIRE”) USING THE “FIRE” PROCEDURE\textsuperscript{TM}**

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Occupationally exposed butadiene workers have been biomonitored through the use of protein adducts as indicators of butadiene internal dose. Butadiene generates three major reactive epoxides: 1,2-epoxy-3-buten (EB), 1,2,3,4-diepoxysbutane (DEB), and 1,2-epoxy-3,4-butanediol (EBD) which all covalently bind to hemoglobin forming the N-terminal valine protein adducts pyr-Val, Hb-Val and THB-Val respectively. Immunoaffinity enrichment has been validated and used as the method of quantitation for pyr-Val and Hb-Val in rodents and humans exposed to 1,3-buta diene. Adducts from THB-Val have earlier been measured with a modified Edman degradation method using a Solid Phase Extraction GC-MS/MS method. The limits of detection and quantitation reported were 200 fmol of standard, 40 fmol on column, respectively, for EBD exposed rodents. A recently developed method known as the adduct “FIRE” procedure TM has been adopted to study the use of GC-MS/MS for LC-MS/MS. We propose to use the “FIRE” adduct procedure to accurately quantify both endogenous THB-Val adducts and butadiene specific THB-Val from 1,3-buta diene exposed rodents and human. THB-Val free- resin (thiohydrastin standards (THB-“FIRE”)) have been successfully synthesized and the initial LC-MS/MS data obtained has shown calibration curves with an R = 0.999 using pseudo SRM. The limit of quantitation is 3 fmol on column. Preliminary results on semipreparative LC purification with the corresponding fraction THB-“FIRE” LC-MS/MS method. The goals include quantitation of THB-Val adducts from both occupationally exposed human and rodents, to compare species and sex differences, to compare the results with earlier data and compare the three species following exposures similar to our occupationally exposed workers.

**2690 ARE CHEMICAL DISPERANTS USED IN THE GULF OF MEXICO CYTOXIC AND GENOTOXIC TO HUMAN LUNG AND SKIN FIBROBLASTS?**

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Chemical dispersants are chemicals compounds used to aid in the cleanup of crude oil spills. They became a significant public health concern in 2010 due to the BP Deepwater Horizon Oil Crisis when millions of gallons of chemical dispersants specifically Corexit\textsuperscript{®} 9527 and 9500 were used to break up the crude oil. They were dispersed via aerial spray and deepwater injection. The effects of these dispersants to humans is unknown. The primary route of exposure to these chemical dispersants is inhalation, direct dermal contact and ingestion; therefore, the objective of this study is to determine the cytotoxicity and genotoxicity of these two dispersants (Corexit\textsuperscript{®} 9500 and 9527) in human skin (B1H/TERT) and lung (W1TH6-B) fibroblasts. Cells were treated with and without 59 fractions with cofactors, because fibroblast cells do not readily express P450 enzymes necessary to metabolize the chemicals. Corexit\textsuperscript{®} 9500 was cytotoxic to skin cells. Specifically in skin, 50, 250, 350 and 500 ppm 9500 induced 95, 89, 52, and 3 percent relative survival, respect-
tively. S9-mediated metabolism increased toxicity inducing 78, 84, 39 and 2 percent relative survival, respectively. Corex® 9527 was cytotoxic to skin cells. Specifically in skin, 500, 650, 850 and 1000 ppm 9527 induced 89, 56, 73, and 24 percent relative survival, respectively. S9-mediated metabolism increased toxicity inducing 65, 60, 22 and 0 percent relative survival, respectively. Ongoing and future work will consider the genotoxic effects as well as the effects of dispersed oil.

2693 MODULATION OF PROSTATE CANCER CELL PROLIFERATION BY FATTY ACIDS AND EFFECTS OF ADIPOCYTE-CONDITIONED MEDIA.

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Obesity in men, particularly an abundance of abdominal fat deposits, has been correlated with increased incidence and poor prognosis of prostate cancer. Fat may also play a significant role in the therapeutic targeting of prostate cancer cells that metastasize to bone, as the bones of older individuals are composed predominantly of fatty tissue. Omega-3 polyunsaturated fatty acids (PUFA) are well characterized as effective agents in cancer prevention and therapy. Recent studies show an inhibitory effect of omega-3 PUFAs on PC3 prostate cancer cell proliferation. Additionally, we now demonstrate that other fatty acids, including certain omega-6 PUFAs, and both 10-trans,12-cis (10e12z) and 9-cis,11-trans (9z11e) isomers of conjugated linolenic acid (CLA), also inhibit proliferation of PC3 prostate cancer cells. To mimic fat cells in the body, and determine whether the presence of these fat cells would affect the inhibitory actions of fatty acids, we used a two-step model of media preconditioning and PC3 treatment. Culture media containing inhibitory concentrations of fatty acids was first conditioned on 3T3-L1 adipocytes, and then administered to PC3 cells. Here, we show that 3T3-L1 adipocytes modulate the effects of fatty acid treatment, generally rendering the fatty acids less effective in proliferative inhibition of PC3 prostate cancer cells. The lack PC3 inhibition by fatty acids following media preconditioning on fat cells may be significant to understanding the correlation between obesity and resistance to prostate cancer therapy.

2694 A RAT MODEL OF COLON CANCER REVEALS ZONES OF ENHANCEMENT OF TUMOR MULTIPURITY BY VITAMIN D.

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Colon cancer risk varies greatly around the world, indicating an environmental component to its pathogenesis. Epidemiological studies indicate that sunlight exposure and vitamin D are each associated with a reduced risk of colon cancer. However, studies in humans show conflicting efficacy of vitamin D supplementation, especially in individuals already sufficient for vitamin D. We have previously shown that supplementation with 25-hydroxyvitamin D3, [25(OH)D3], in two genetic models of familial colon cancer, the Apcmin mouse and the Apcmin rat, failed to reduce colon tumor multiplicity in either model (Irving et al, Arch Biochem & Biophys, in press). Notably, the daily dose of 25(OH)D3 tested in the Apcmin rat resulted in a statistically significant increase in the multiplicity of colon tumors. Because vitamin D supplementation has known beneficial effects, such as in prevention of osteoporosis, it is important to determine what range of doses does not sensitize for colon cancer. Apcmin rats were randomized to one of five doses of 25(OH)D3 ([range 0.66-4.5 mg/kg body weight]) or vehicle diet beginning at 33 days of age. Longitudinal endoscopic monitoring allows us to test 25(OH)D3 both for prevention of newly arising colon tumors and treatment of established tumors. Serum calcium and 25(OH)D measurements and intestinal tumor counts are obtained at study termination. Preliminary observations from endoscopy reveals two sensitization zones. Rats given a low or high dose of 25(OH)D3 have more colon tumors than rats given either vehicle alone or mid-range doses of 25(OH)D3. These data indicate that individuals sufficient for vitamin D may not gain added protection against colon cancer from supplementation, and may actually increase their risk with certain doses. Extension of these studies can be designed to predict safe ranges of vitamin D supplementation for health reasons other than the risk of colon cancer.

2695 DETERMINATION OF DOMINANTS OF LOW-DOSE FORMALDEHYDE TOXICITY BY FUNCTIONAL TOXICOGENOMIC SCREENING IN YEAST.

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Formaldehyde is a widely used, economically important chemical. There is extensive occupational and environmental exposure to formaldehyde all over the world, as it is released from household products, and is associated with nasopharyngeal, sinonasal and lymphohematopoietic cancer – specifically myeloid leukemias. At present the mechanistic basis of the carcinogenicity of formaldehyde is unclear. We
have used a functional toxicogenomics approach to identify the genes that modulate the cellular toxicity of formaldehyde in the model eukaryote Saccharomyces cerevisiae. S. cerevisiae has many tools available for genetic study, including a complete set of non-essential gene deletions, genetically tagged so that individual strains can be identified in competitive growth experiments. Using this set, parallel systematic analysis can be completed to identify mutant strains that are susceptible to toxicant treatment. We have used this technique to define the cellular processes targeted by formaldehyde. Data show that several proteins involved in mRNA processing, specifically the Ski complex and associated factors, are required for formaldehyde tolerance. Mutant strains deficient in the repair of DNA damage through multiple pathways were identified as having increased sensitivity, indicating that formaldehyde causes DNA damage at doses equivalent to endogenous levels of formaldehyde in human tissue. Several of the tolerance genes identified have human orthologs with conserved biological function, supporting the notion that the mechanisms of toxicity identified in yeast could be relevant to human diseases caused by formaldehyde. This study highlights S. cerevisiae as a simple but valuable model for mechanistic studies of toxicity, and for identifying biomarkers of genetic susceptibility to toxicant-related disease.

2697 COMPARATIVE EFFECT OF OLIVE AND PALM OILS ON BENZO(A)PYRENE-INDUCED COLON TUMOR DEVELOPMENT IN APCmin MICE.

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Colon cancer has been causing concern in terms of morbidity and mortality. In US alone, around 60,000 lives/year are lost to colon cancer. Diet and environment are implicated in the development of sporadic colon cancers. Therefore, understanding the role of environmental toxic contaminants of diet towards the development of colon cancers is important. One such prototypical chemical is benzo(a)pyrene (BaP) a polycyclic aromatic hydrocarbon (PAH) compound, which is formed in red meat cooked at high temperatures. Our studies thus far have shown the development of colon tumors in Apcmice orally exposed to BaP. In this study we investigated the effect of olive and palm oils (representatives of unsaturated and saturated fats, respectively) on BaP-induced colon carcinogenesis in Apcmice. All mice were allowed a 7-day acclimation period prior to being randomly assigned to a control (n = 7) or treatment group (n = 7). Treatment consisted of 50 and 100 μg BaP/kg bw dissolved in tricaproin (BaP-only group) or olive or palm oil administered daily to 7-week-old male Apcmice via oral gavage for sixty days. Post BaP exposure, mice were sacrificed; colons were retrieved and preserved in 10% formalin for observation under gross pathological changes. An increased prevalence of adenomas in colons of mice that ingested BaP through saturated dietary fat compared to unsaturated fat and controls (p<0.05) was recorded. Most of the polyps observed in the colon of BaP treatment groups were adenomas with low to high-grade dysplasia. Interestingly, we observed more adenomas with high-grade dysplasia in the BaP + saturated fat group, and these incidences were more frequent at the 100 μg/kg BaP dose. On the other hand, the BaP-alone and unsaturated-fat groups did not show significant differences either in the numbers of adenomas or invasive tumors in colon. In summary, our studies established that dietary fat, especially saturated fat, exacerbates the development of colon tumors caused by BaP in the Apcmice (funded by NIH grants 5R25GM059994-11 and 1R01CA142845-01A1).

2698 COMPARISON OF TAMOXIFEN (TAM)-DNA ADDUCT LEVELS IN UTERINE TISSUE FROM DIFFERENT SPECIES OF MONKEYS AND HUMANS EXPOSED TO TAM.

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Tamoxifen (TAM) is a selective estrogen receptor modulator used worldwide for adjuvant therapy and chemoprevention of breast cancer. Women receiving TAM have an increased risk of endometrial and myometrial cancer, which may be due to genotoxicity and/or receptor-related mechanisms. Controversy has surrounded the issue of whether or not TAM-DNA adducts form in humans, and we have examined this question in uterine tissues of aging Erythrocebus patas (patas) and Macaca fascicularis (macaque) monkeys given oral TAM dosing, as well as in endometrial and myometrial samples from women given TAM therapy. DNA adducts were determined by TAM-DNA chemiluminescence immunoassay (CIA) using an anti-lysine in vivo (both the free amino acid and when incorporated into proteins). Downstream reaction products of P450-catalyzed oxidation of furan are assumed to be formed non-enzymatically, therefore characterizing the in vitro kinetics of the reaction of BDA with known reaction partners is expected to provide insight into the mechanism of furan toxicity. An investigation of the kinetics of the reaction between BDA and GSH or N-acetyl-L-lysine (NAL) was performed to determine a) the half-life of BDA in the presence of reaction partners, b) the rates of competing reaction pathways, and c) the structural identification of intermediates. A time course of BDA reaction with either GSH or NAL was performed using a 700 MHz Bruker Avance II spectrometer equipped with a proton-enhanced cryoprobe. The peak areas of reagents, intermediates, and products were fit using kinetics software to determine the kinetic rate constants. We found that the half-life of BDA is ~3 minutes with either GSH or NAL. We also found that several intermediates were formed in the presence of GSH that persisted for >1 hour. We failed to see evidence for the formation of the pyrrolinone when GSH was present, indicating that GSH-BDA forms preferentially. These findings are consistent with the prolonged presence of reactive intermediates other than BDA, which may cause damage at sites distant from furan’s oxidation to BDA in the CYP2E1 active site.

2699 MEASUREMENT OF THE KINETICS OF REACTION OF cis-2-BUTENE-1,4-DIAL WITH GLUTATHIONE USING NMR.

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Furan has been identified as a possible human carcinogen. The first step in the biotransformation of furan is oxidation by CYP2E1 to give cis-2-buten-1,4-dial (BDA). BDA is known to cross-link glutathione (GSH) with cellular nucleophiles as a result of the formation of the pyrrolinone when GSH was present, indicating that GSH-BDA forms preferentially. These findings are consistent with the prolonged presence of reactive intermediates other than BDA, which may cause damage at sites distant from furan’s oxidation to BDA in the CYP2E1 active site.
ABERRANT METHYLATION AND GENETIC POLYMORPHISMS ARE ASSOCIATED WITH DECREASED EXPRESSIONS OF TUMOR SUPPRESSOR GENE P15 AND P16 IN BENZENE POISONING.

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Benzene is an important industrial chemical and a universal environmental pollutant that causes hematotoxicity and increased risk of acute myeloid leukemia (AML). The underlying mechanism of benzene poisoning (BP; i.e. hematotoxicity), however, is poorly understood. In AML, DNA hypermethylation has shown to be the primary mechanism that leads to the loss of expression of p15 and p16. Recently, single-nucleotide polymorphisms (SNPs) in gene promoter region have gained much importance because of their impact on gene expression. To explore genetic and epigenetic factors influencing the expression of p15 and p16, we carried out a case-control study in Chinese occupational benzene poisoning patients. 20 cases of BP, 17 healthy benzene-exposed workers and 19 matched unexposed controls were recruited. Six SNPs in CpG island of p15 and p16 promoter region were genotyped. Comparing to unexposed controls, both p15 and p16 mRNA expression levels were significantly down-regulated in BP (p<0.0001, p<0.0001, respectively) and also in benzene-exposed workers (p<0.01, p<0.01, respectively). Decreased expression of p15 is associated with homozygous genotype (GG) of rs3808845, as well as an increased level of methylation at the fifth CpG site (p<0.05). For p16, the methylation level at the second CpG site within the promoter region was significantly higher in BP compared with unexposed control (1.5% and 0.0%, p<0.05). Furthermore, increased methylation level at this CpG site is significantly associated with decreased expression of p16 mRNA (p<0.05). Interestingly, the second CpG site is located within the consensus binding sequence for the deformed epidermal ant regulator factor 1 (DEARF-1).

Therefore, hypermethylation and genetic polymorphisms are likely to contribute to the decreased expression of p15 and p16.

2702 ARSENIC IS CLASTOGENIC AND ANEUGENIC TO PRIMARY HUMAN UROTHELIAL CELLS.

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Arsenic is a known human carcinogen, inducing cancer in multiple organ systems. Exposure to arsenic is widespread with the general population being exposed to arsenic through both natural and anthropogenic sources. Arsenic contamination in drinking water is major concern in areas of the United States and around the world due to natural arsenic in the bedrock leaching into well water. Epidemiological data indicate that chronic exposure to arsenic drinking water increases the incidence of bladder cancer, however, the mechanism of arsenic-induced bladder cancer remains unknown. The purpose of this study is to determine the ability of arsenic to induce chromosome instability in primary urothelial cells. Arsenic induced a concentration-dependent increase in cytotoxicity in primary human urothelial cells with exposure to 2.5, 5 and 10 uM arsenic for 24 h inducing 99, 82 and 53 percent relative survival. Exposure to arsenic for 24 h also induced clastogenicity and aneuploidy. Arsenic induced damage in 22 percent of metaphases with a total of 28 damaged chromosomes and aneuploidy increased from 5 percent in the controls to 21 percent metaphases with aneuploidy in arsenic-treated cells. Future work is aimed at determining the effects of more chronic exposures to arsenic on chromosome stability in human urothelial cells. This work is supported by NIEHS grant ES016893 (J.P.W.).

2703 ADVANCES IN BRIDGING NONCLINICAL CARDIOVASCULAR DATA TO THE CLINIC. S. D. Pettit. HESI, Washington, DC.

Cardiovascular adverse events remain a significant cause of attrition during drug development as well as postmarket. The availability of translatable animal data that is more predictive and sensitive for clinical outcomes is essential to overcome this public health challenge. This symposium will feature four approaches to generating and assessing the concordance between nonclinical cardiovascular endpoints and clinical outcomes.

2704 THE ABPI-ANIMAL MODEL FRAMEWORK: CONCORDANCE OF DOG CARDIOVASCULAR TELEMETRY PARAMETERS TO SINGLE ASCENDING DOSE TRIALS IN MAN.

1AstraZeneca, Macclesfield, United Kingdom, 2Lilly, Indianapolis, IN, 3GSK, Hertfordshire, United Kingdom, 4Pfizer, Groton, CT, 5Amgen, Thousand Oaks, CA, 6Novartis, Basel, Switzerland and 7Aranex, Bever, Belgium.

Conscious dog cardiovascular telemetry is commonly used prior to first time in man administration of pharmaceuticals to fulfill ICH S7 guidelines. An understanding of the translation of this model to clinical outcome is therefore critical to ensuring safety of volunteers and patients. A framework approach (Valentin et al., 2009) has been developed by seven pharmaceutical companies to assess the degree of confidence in such preclinical animal models and in their translation to man. The companies have shared data from 114 proprietary small molecules tested in a conscious dog telemetry model and in a single ascending dose study in humans between the years 2001 and 2008. Five parameters (heart rate, blood pressure, PR and QRS intervals and QTc interval) were compared. Approximately 20% of compounds had at least one cardiovascular event in phase I. On the basis that the data consisted of true positive and true negative compounds, statistical measures of sensitivity, specificity and the model's predictive value (positive and negative) were calculated at a range of exposure multiples between animal and man. This presentation will showcase this novel data set and will discuss important findings and observations as well as discussing how this data will impact on decision making within the drug discovery and development process.

2705 THE ROLE OF PKPD MODELLING IN TRANSLATIONAL RESEARCH IN CARDIOVASCULAR SAFETY.

V. Dubois1, A. Chain1, P. van der Graaf2, D. Leishman3, D. Gallagher4, N. McMahan1, S. Visser4, M. Danhof5 and O. Della Paauw6,7.
1Pharmacology, LACDR/TI-Pharma, Leiden, Zuid-Holland, Netherlands, 2Pharmacometrics/Global Clinical Pharmacology, Pfizer Inc., Canterbury, United Kingdom, 3Global Safety Pharmacology, Eli Lilly and Company, Indianapolis, IN, 4Cardiovascular Safety, Johnson & Johnson, Bever, Belgium, 5Safety Assessment, GlaxoSmithKline, Ware, United Kingdom, 6Nonclinical Modelling & Simulation, AstraZeneca, Södertälje, Sweden and 7Clinical Pharmacology, Modelling & Simulation, GlaxoSmithKline, Stockley Park, United Kingdom. Sponsor: S. Pettit.

Background: Assessment of the propensity of non-antiarrhythmic drugs in prolonging QT/QTc interval is critical for the progression of compounds into clinical development. The objective of the current work is to demonstrate the advantages of...
Drug-induced QT interval prolongation and Torsades de Pointes remain serious public health issues in bringing safe new pharmaceuticals to the market place. Under the auspices of ILSI Health and Environmental Sciences Institute (HESI), a consortium involving representatives from pharmaceutical companies, regulatory agencies and opinion leaders from the scientific and medical research communities has been assembled. The objective is to assess the concordance between nonclinical repolarization assays and clinical measures of QT interval prolongation, i.e., establish a quantitative integrated risk assessment for each compound. To this end, the consortium is conducting a retrospective analysis of nonclinical and clinical data housed in the US Food and Drug Administration database. This presentation will provide analysis of the concordance of data extracted from thorough clinical QT studies and nonclinical studies submitted to support marketing approval of new pharmaceuticals. Nonclinical studies include an in vitro ion channel assay for effects on hERG current, action potential duration in isolated cardiac tissue, and an in vivo ECG studies. Concentration response data is compiled for each assay and compared across studies to examine concordance of nonclinical and clinical data. Data generated from several new pharmaceuticals will be shared along with estimates of concordance at various nonclinical to clinical concentration multiples.

**2708 EMERGING EVIDENCE FOR NOVEL NONCHOLINERGIC MECHANISMS OF ORGANOPHOSPHATE-INDUCED NEUROTOXICITY.**


Organophosphate (OP) compounds are used as pesticides and in more potent Chemical Defense, Aberdeen Proving Ground, MD. The highly toxic nerve agent VX undergoes an incomplete decontamination and can evaporate from surfaces long after the initial insult. As a consequence, low levels should be examined for their potential to induce functional impairments. We evaluated VX effects in rats exposed to 22.5, 13.5 and 2.25 μg/kg/day (0.5, 0.3, 0.05 LD50/day) for one month via implanted mini osmotic pumps. The rapidly attained continuous and marked whole-blood cholinesterase activity inhibition following 0.3 LD50/day (~85%), partially recovered 1 week post pump removal. Under these conditions, body weight, blood count and chemistry, water maze acquisition task, sensitivity to the muscarinic agonist oxotremorine, peripheral benzodiazepine receptors density and brain morphology as demonstrated by routine histopathology, remained unchanged. However, abnormal initial response in an Open Field test, and up regulation (~3 folds) in the expression of the exocytotic protein VAMP in hippocampal neurons was detected. Using MAP2 cytoskeletal protein immunolabeling demonstrated a decreased immunoreactivity in dentrites processes and an increased immunoreactivity in pyramidal cells soma in the CA2 sub region of the hippocampus, in the thalamus and in piriform cortex brain areas. GFAP labeling for astrocytes revealed an activated astrocytes in all brain regions. These changes could not be detected one month following termination of exposure. Our findings indicate that following chronic exposure to VX some important processes might be
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2711 SOMAN-INDUCED BRAIN DAMAGE AND NEUROLOGICAL DEFICITS: NEUROINFLAMMATORY DISORDERS AND CHANGES IN BRAIN METABOLISM: CONSEQUENCES FOR THERAPY.

P. Carpentier1, G. Testylier1, F. Dhoet1, K. Billon1, C. Piérard2, D. Béraudoché3, P. Filliat1, L. Barber1 and F. Dorandeu1.

Similar to other organophosphorus nerve agents, soman acts as an irreversible inhibitor of acetylcholinesterase (AChE) that results in a “hypercholinergic crisis” mostly explaining the observed broad spectrum of toxic signs. Depending on the dose, exposure can lead to hypersalivation, respiratory distress, fasciculations and muscle paralysis, cardiac-vascular dysfunction, convulsive epileptic seizures, coma and death. In experimental settings, surviving animals can present irreversible secondary brain damage (SBD) as well as long-term neurological deficits such as epileptogenesis and various cognitive impairments. The basic mechanisms that underlie these phenomena are not perfectly understood. We will present recent evidence that neuroinflammation and metabolic changes may play a role. We will also show results supporting that, at least partially, the glutamate receptor antagonist keta-mine associated with atropine could reverse or prevent these damage in a mouse model of soman-induced refractory status epilepticus.

S 2712 TEMPORAL, REGIONAL AND CELLULAR PROGRESSION OF NEUROINFLAMMATION FOLLOWING ORGANOPHOSPHATE NERVE AGENT-INDUCED STATUS EPILEPTICUS IN RATS.


Neuroinflammation occurs following nerve agent-induced status epilepticus (SE) seizures and may contribute to loss of neural tissue and impaired behavioral function. Limited transcriptional and translation al information on a small number of brain-expressed inflammatory mediators has been shown following nerve agent-induced SE. The purpose of this seminar is to present our data on the regional and temporal progression of the neuroinflammatory process following acute nerve agent-induced SE up to 72 hours following seizure onset. The protein levels of multiple cytokines and chemokines have been quantified using bead multiplex immunoonasays in damaged brain regions (i.e., piriform cortex, hippocampus and thalamus) and localized to resident brain cells using fluorescent immunohistochemistry. Significant concentration increases have been observed in all injured brain regions, with acute phase response (APR) cytokines and leukocyte chemokines playing prominent roles. The potential roles of these neuroinflammatory factors in neurodegenerative progression will be discussed along with potential interventional strategies. Increases in these potentially neurotoxic inflammatory factors likely play an active role in the progression of nerve agent-induced SE neuropathology though their exact role does require further study.

S 2713 NONCHOLINERGIC MECHANISMS IN THE TOXIC EFFECTS OF ORGANOPHOSPHATE POISONING—ATTEMPTS TO DESIGN EFFECTIVE ANTIDOTAL COUNTERMEASURES.


Potent cholinesterases (ChE) inhibitors such as soman and sarin induce an array of harmful effects including convulsions, cognitive dysfunctions and death. Due to the probability of various scenarios of military or terrorist attacks involving organophosphorus compounds, research has to be focused on finding the optimal countermeasures. A stepwise mechanism involving a cholinergic crisis and a late glutamatergic activation, yielded treatments consisting of an anticholinergic and an oxime. This approach provided increased survival rates but did not abolish convulsions or the ensuing brain damage and cognitive dysfunction. The specific antimuscarinic drug scopolamine, given together with pyridostigmine, prophylactically completely abolished soman-evoked convulsions. However, behavioral tests revealed significant memory impairments. Moreover, scopolamine did not prevent the decreases in glutamate receptors densities observed following exposure. Under the same paradigm, caramiphen, an antimuscarinic drug with distinct antiglutamatergic properties, prevented seizure activity, brain damage and behavioral deficits. Notably, studies with benactyzine and caramiphen highlighted their superior protective effects in prophylactic and post exposure therapies. These findings indicate a rapid activation of glutamatergic pathways accompanying the initial ChE blockade. The data also support the notion that since excitatory amino acids play a crucial role in neurodegeneration, partial antiglutamatergic characteristics of certain cholinolytic drugs appear to be the basis for their improved ability to prevent the toxic effects of organophosphates.

S 2714 NEUROTOXICOLOGICAL EFFECTS OF EXPOSURE TO ORGANOPHOSPHATE (OP) COMPOUNDS: THE SIMILARITIES AND DIFFERENCES BETWEEN LOW AND HIGH DOSES.


Organophosphate (OP) compounds are used as pesticides and in more potent forms are chemical warfare nerve agents that have also been used as terrorist weapons. Low dose, non-lethal exposures to these compounds is known to produce changes in a number of neurobehavioral functions, to include impaired cognitive abilities that are subtle and may be reversible. However, the relationships between the OP dose, magnitude and duration of effects and the impact of repeated exposures over long periods such as may occur in agricultural workers are still areas of active investigation. Also, the long-term effects of low level exposure to nerve agents such as occurred in the Tokyo subway sarin attacks show that these effects may persist for many years, long following the recovery of normal cholinergic functioning. Exposure to near lethal doses of nerve agent can cause a cascade of effects triggered by cholinergic overstimulation, but affecting other neurotransmitter systems to cause toxicities that are not cholinergically mediated. Some examples are the role of the glutamatergic system in mediating seizures and brain damage following nerve agent exposure and the role of catecholamines in producing cardiac abnormalities. In addition, the role of inflammatory processes in both the periphery and central nervous system to further exacerbate these toxicities, and as potential therapeutic targets, are just now being realized. This talk will provide an overview of current research in each of these areas to and identify areas for future work.

S 2715 EMERGING MECHANISTIC TARGETS IN LUNG INJURY INDUCED BY COMBUSTION-GENERATED PARTICLES.

M. W. Fitts1 and A. Ghio2. 1Adiria Client Services, Richmond, VA and 2US EPA, Chapel Hill, NC.

Environmental combustion-generated air pollutants are a global concern, and the adverse effects of such materials on human health, particularly respiratory and cardiovascular health, are firmly established. Despite strong epidemiological evidence linking ambient air pollutants to specific human diseases and general health decline, a significant gap remains in our understanding of precisely how such materials produce nonneoplastic respiratory diseases. This symposium will highlight recent advances in our understanding of the molecular and chemical mechanisms that govern lung and lung cell toxicity by common respiratory forms of complex ambient particulate materials originating from the combustion of fossil fuels, wood, or tobacco. Presentations will describe the current understanding of nonneoplastic respiratory diseases induced by combustion-derived particulate matter (PM); the participation of transient receptor potential (TRP) channels in detecting and initiating responses to unique forms of environmental combustion-derived PM to produce acute lung inflammation/injury/remodeling; the role of disrupted cell and mitochondrial iron homeostasis in wood combustion-derived PM toxicity, and the isolation and chemical-physical characterization of insoluble nanosized particles from cigarette smoke condensate including their cytotoxic potential. Attendees of this symposium session will gain up-to-date knowledge of novel cellular processes and PM components that appear to regulate the acute toxicological effects of particulate air pollutants. These effector molecules may represent important targets for future therapeutic strategies to mitigate the adverse impact of inhaled combustion-generated pollutants.
Incomplete combustion of fossil fuels and biomass burning are the largest sources of air pollution worldwide which poses serious risks to human health. The complex and dynamic aerosol mixtures from combustion are comprised of particles, semivolatile matter and gases whose makeup is governed by the source material, combustion conditions, control technologies and atmospheric aging and mixing. While exposure to high concentrations of combustion related gases such as NOx and CO can result in acute lung injury and poisoning, concomitant exposure to particles have less directly debilitating effects on the respiratory system, in part because of the lung’s effective clearance and detoxification processes. Depending on the dose and duration of exposure, however, particulates from combustion emissions do accumulate in the lung in association with pathological changes and may also impart effects on other organ systems to for example, worsen cardiovascular disease; alter the immune system to increase infectious and allergic diseases; and adversely affect in utero development. This presentation will provide an overview of the chemistry effects on other organ systems to for example, worsen cardiovascular disease; alter the immune system to increase infectious and allergic diseases; and adversely affect in utero development. This abstract does not reflect EPA policy.

The biological effect of particles can be associated with a disruption in cell iron homeostasis. We have demonstrated that an initiating event in oxidative stress and biological effect after mineral oxide particle (i.e. silica) exposure is a sequestration of mitochondrial iron. Organic compounds can similarly complex source of host cell iron to disrupt iron homeostasis. We tested the postulate that complexation of cell iron by woodsmoke particle (WSP) results in a biological effect. Exposures of respiratory epithelial (BEAS-2B) cells to 1) HBSS, 2) 100 μg/mL WSP, 3) 200 μM ferric ammonium citrate (FAC), and 4) both 100 μg/mL WSP and 200 μM FAC demonstrated significant elevations in cell iron concentration after co-exposures relative to incubations with FAC alone (0.71±0.06 and 0.38±0.03 ppm respectively). Co-incubation was associated with an increased cell import of metal. Complexation of mitochondrial iron was studied using 75 cm2 flasks of BEAS-2B cells pre-exposed to 1.0 μM 57Fe FAC and ICP mass spectrometry. Incubation with 100 μg/mL WSP resulted in diminished mitochondrial 57Fe concentrations in the particle-exposed BEAS-2B cells (0.19±0.03 and 0.29±0.04 ppm in WSP and HBSS exposed cells respectively) as the WSP complexed host metal from the organelle. Pre-incubation of respiratory epithelial cells with 200 μM FAC increased nuclear and mitochondrial metal concentrations and prevented significant iron loss from mitochondria exposed to WSP. Finally, exposure of BEAS-2B cells to 100 μg/mL WSP increased interleukin (IL)-8 release relative to incubations with HBSS (78±16 and 23±10 pg/mL) and this elevation was inhibited by 4 hr pre-treatment with 200 μM FAC (31±11 and 66±15 after pre-treatments with FAC and HBSS respectively). We conclude that biological response following exposure to WSP is associated with complexation of host mitochondrial iron; increasing available iron in the cell diminished the biological response to the particle. An initiating event in the cell response to a particle appears to be a complexation of requisite iron by the particle surface.

Human respiratory epithelia function such as mucociliary clearance of airways and formation of an epithelial barrier have recently been implicated in sensory functions in response to tants such as bitter. These epithelial cells are directly exposed to air pollution, amongst other pollutants, particulate matter. Of these, diesel exhaust particles are a representative and well-studied component of urban smog with world-wide relevance. This presentation will tender evidence that human respiratory epithelia also possess proteolytic signaling machinery, whereby proteinase-activated receptor-2 (PAR-2) activates Ca++-permeable TRPV4, which leads to activation of disease-enhancing Matrix-Metalloproteinase-1 (MMP-1), a signaling cascade initiated by DEP. We also demonstrate that it is indeed the organic fraction of DEP which exerts this effect.

The chronic obstructive pulmonary disease (COPD) is a multisystem disorder which results from the enhanced production of protein-protein complex formation between PAR-2, TRPV4 and phospholipase-C beta3 in human airway epithelia by confocal microscopy. These results are complemented by protein biochemistry which demonstrate an enhancement of protein-protein-complex formation between PAR-2, TRPV4 and PLC beta3 when cells are exposed to DEP. In sum, DEP evoke a uniquely protracted Ca++-influx via TRPV4, enhanced by the COPD-predisposing polymorphism, TRPV4P19S. This response reprograms mal-adaptive inflammatory and extracellular-matrix-remodeling processes in human airways. The novel concept of air pollution-responsive signal transduction from PAR-2 to TRPV4 will accelerate rationally-targeted therapies for this global health problem.

The role of the transient receptor potential (TRP) calcium channel family in acute inflammation is an active area of research. The sensory components of respiratory epithelia also possess proteolytic signaling machinery, whereby proteinase-activated receptor-2 (PAR-2) activates Ca++-permeable TRPV4, which leads to activation of disease-enhancing Matrix-Metalloproteinase-1 (MMP-1), a signaling cascade initiated by DEP. We also demonstrate that it is indeed the organic fraction of DEP which exerts this effect.

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ence cigarette smoke condensate. These particles have chemical properties similar to those reported for HULIS, possess stable carbon centered radicals and are cytotoxic in vitro. Additional studies are required to confirm the presence of these insoluble nano-sized particles in cigarette smoke as well as their potential role in respiratory diseases.

2721 REALIZING THE VISION OF 21ST CENTURY TOXICITY TESTING: GENETIC APPROACHES TO PATHWAY ANALYSIS.

C. Corton and L. Burgoon. US EPA, Research Triangle Park, NC.

The US National Academy of Science (NAS) report, “Toxicology Testing in the 21st Century,” outlined a vision that virtually all routine toxicity testing would be conducted in vitro using high-throughput analysis of toxicology endpoints. Realization of this vision would require a major increase in the flexibility of the screening platform, throughput and reduction of costs. We have developed a novel cell spot microarray (CSMA) method for production of high density siRNA reverse transfection arrays. The CSMA platform is distinguished from the other RNAi microarray techniques by its broad applicability and the high sample density that allows rapid query of gene functions with assays otherwise difficult or not applicable to high-throughput screening. This feature of the platform will be demonstrated with a comparative phenotypic analysis of DNA damage response activation and cell survival in human cells from six different tissues of origin following exposure to UV-irradiation. Knockdown impact of 690 genes directly associated with DNA repair mechanisms was compared to build a functional compendium of the early DNA damage response pathways.

2722 A REVERSE ENGINEERING APPROACH TO CONSTRUCTING NETWORKS OF CHEMICAL RESPONSE.


The molecular biology revolution led to an intense focus on the study of interactions between DNA, RNA and protein biosynthesis in order to develop a more comprehensive understanding of the components of the cell. One consequence of this focus was a reduced attention to whole-system physiology, making it difficult to link molecular biology to disease. Equipped with the tools emerging from the genomic revolution, we are now in a position to link molecular responses of toxicity pathways using high-throughput tests. Dose-response modeling of perturbations of pathway function would be organized around computational systems biology models of the circuitry underlying each toxicity pathway. Although there is a growing consensus that this vision will one day become reality, the difficult task of linking changes in the expression or modification of components in pathways to toxicity have yet to be fully realized. There is a clear need to better incorporate new and existing genetic tools that can be routinely used by toxicologists allowing relationships between chemical exposure, genetic networks, and phenotypic responses to be better understood. This symposium brings together experts to discuss genetic analysis of pathways that can be generally applied to toxicology and as such will help move us toward realizing the vision of the NAS report.

2723 EXPANDING THE SCOPE OF LOSS-OF-FUNCTION GENOMIC SCREENING WITH RNAI CELL MICROARRAYS.

J. Rantala. Oregon Health and Science University, Portland, OR. Sponsor: C. Corton.

High-throughput screening of cellular effects of RNA interference (RNAi) libraries is now being increasingly applied to explore the role of genes and signaling pathways in specific cell biological processes and disease states. However, the technology is still limited to specialty laboratories and assay types, due to restrictions of the traditional multwell-based screening platforms. In the future, alternative screening platforms will be required to expand functional analysis of toxicology pathways and states associated with disease. This talk will focus on reverse engineering approaches that incorporate information from genome-wide association studies as well as genomics and proteomics data to build realistic models in which constellations of genetic and environmental perturbations affect the molecular states of networks that in turn affect disease risk.

2724 USE OF PREDICTIVE MARKERS OF CANCER MODE OF ACTION THROUGH INTEGRATION OF GENETICS AND GENOMICS.

C. Corton. EPA, Research Triangle Park, NC.

Many drugs and environmentally-relevant chemicals activate xenobiotic-responsive transcription factors. Identification of target genes of these factors would be useful in predicting pathway activation in in vitro chemical screening and in assessment of their involvement in disease states. Starting with a large compendium of Affymetrix files (>2000 cell files and >530 comparisons), we developed a genetic and genomic strategy to identify sets of signature genes that are dependent on xenobiotic-responsive transcription factors (TF) including AhR, CAR, FXR, glucocorticoid receptor (GR), Nrf2, PPARalpha, and PXR. Target genes were identified by comparing the profiles after exposure to activators of a TF in wild-type and TF-null mice. We also used the mouse liver compendium to identify sets of genes associated with phenotypic readouts linked to liver toxicity/cancer including feminization/masculinization, hypoxia, inflammation and cytotoxicity. Validation of the identified genes was carried out by characterization of the expression of the signature genes under independent conditions of chemical exposure or perturbation known to alter the TF.

2725 RAPID IN VIVO ASSESSMENT OF CHEMICAL-GENE INTERACTIONS IN EMBRYONIC ZEBRAFISH.

R. L. Tanguay, M. T. Simonich, L. Truong and D. Mandrell. Oregon State University, Corvallis, OR.

At the foundation of the US National Academy of Sciences report, "Toxicity Testing in the 21st Century" is the goal to quantify the relationships between early molecular responses, following chemical exposure, to later toxicity. Once these relationships are defined, we will be in a stronger position to develop predictive in vivo approaches for risk assessment. A significant current challenge is that we lack sufficient whole animal phenotypic response data that is also coupled with early molecular response data. We propose that rapid whole animal toxicity testing is necessary to provide critical phenotypic anchoring to help identify the pathway perturbations that are necessary to produce whole animal toxicity. Developmental life stages are particularly susceptible to chemical perturbations because this dynamic period requires the full repertoire of molecular signaling to complete embryogenesis. Developmentally, therefore, many hazards that are HULIS, possess stable carbon centered radicals or are cytotoxic in vitro. Additional studies are required to confirm the presence of these insoluble nano-sized particles in cigarette smoke as well as their potential role in respiratory diseases.
the true strength of using the embryonic zebrafish model is the ability to rapidly de-
line the functional role of a gene product in producing toxicity by exploiting pow-
nerful genetic and transgenic approaches. This research was supported by NIH ES
ES019764, P42 ES016465, and P30 ES00210.

2726 MASHING THE DISEASEOME, TOXICOGENOMICVS,
AND FROZEN PIZZAS: NETWORK SYSTEMS BIOLOGY
AND ITS IMPACTS ON NEXGEN RISK ASSESSMENTS.

L. Burgoon. EPA, Research Triangle Park, NC.

One of the key hazard identification questions we face as we move towards the Next
Generation of Risk Assessment (NexGen) is how to integrate the relevant molecu-
lar (including omics data), biochemical, and high throughput assay data, convert it
to systems pathway knowledge, predict disease outcomes, and assess con-
tributive risk values. Under the hypothesis that a disease caused by any stressor will elic-
t the same biomarkers, we can use known and putative biomarkers linked to disease
to predict potential disease outcomes from chemical exposures. Generally, the next
step is to look for overlap between the disease and chemical datasets; however, the
problem is more complex than that, as sets of agglomerative biomarkers may be as-
sociated with multiple diseases at varying probabilities, and individual experiments
may lack the power or ability to detect all of the biomarkers. Using our network
topology based community-finding algorithms, frequent “itemset” mining meth-
ods commonly used in grocery store data mining, and Bayesian networks, we have
created a novel framework for mashing disease biomarkers with chemical exposure
biomarkers to create probabilistic predictions of chemical exposure-disease out-
comes. This talk will focus on the algorithms and the probabilistic disease predic-
tions we have made for 2,3,7,8-tetrachlorodibenzo-p-dioxin and selected phtha-
lates. This approach to disease outcome prediction will become more important as
we begin to graduate chemicals from our high throughput screening and prioritiza-
tion assays to classical and alternative species models allowing for a better under-
standing of chemical hazards as part of the NexGen Risk Assessment process.

2727 CHALLENGES AND OPPORTUNITIES IN EVALUATING
PROTEIN ALLERGENICITY ACROSS
BIOTECHNOLOGY INDUSTRIES.

N. J. Stagg1, G. Ladics2, K. Hastings3, R. Houze4 and S. Gendel5. 1Human
Health Assessment, Dow AgroSciences, Indianapolis, IN, 2DuPont Agricultural
Biotechnology, DuPont, Wilmington, DE, 3Corporate Regulatory Affairs, sanofi-
aventis, Bethesda, MD, 4DynPort Vaccine Company LLC, Frederick, MD and
5Center for Food Safety and Applied Nutrition, US FDA, College Park, MD.

Biotechnology is a field at the cutting-edge of science, using living cells and materi-
als produced by cells to prevent and fight disease, improve food production, and
benefit other industries as well, but there are increasing concerns over the aller-
genicity of biotechnology products that continue to receive increasing attention in
public and regulatory domains. These concerns range from the transfer of an exist-
ing allergen or cross-reactive protein into another crop or increasing endogenous
(existing) allergens in crops to accidental modification of therapeutic proteins re-
sulting in autoimmune reactions to endogenous molecules. This session will pro-
vide important information on allergenicity testing requirements and research in
the agricultural and biotech sector, pharmaceutical/biopharma sector, and vaccine
sector and includes speakers from industry and regulatory. An anticipated
outcome is discussion over why allergenicity is a concern, what tools are available to
evaluate allergenicity in the different areas of biotechnology, and any challenges
we face with this testing.

2728 EXISTING AND EMERGING METHODS AND
TECHNIQUES FOR ASSESSING ALLERGENICITY
OF GENETICALLY MODIFIED CROPS.

G. Ladics, DuPont Agricultural Biotechnology, DuPont, Wilmington, DE.

A rigorous safety assessment process exists for genetically modified (GM) crops de-

erived through Agricultural Biotechnology. It includes evaluation of the introduced
protein as well as the crop containing such protein with the goal of demonstrating the
GM crop is “as-safe-as” non-GM crops already in the food supply. A major issue for
GM crops is the assessment of the expressed protein for allergenic potential. The
objectives for assessing the potential allergenicity of GM crops are: (1) protect al-

ergic consumers from accidental exposure to allergens or cross-reactive proteins that
may trigger an adverse reaction in those already allergic to such proteins and (2)
protect the general population from risks associated with the introduction of genes
encoding proteins that may become food allergens. Currently, no single factor is
recognized as an identifier for protein allergenicity. Therefore, a weight-of-evidence
(WOE) approach is conducted (Codex Alimentarius Commission, 2009). This as-


2729 EVALUATING THE POTENTIAL ALLERGENICITY OF
VACCINES.

R. Houze. DynPort Vaccine Company LLC, Frederick, MD.

Probably more than any other medical product, vaccines face a high standard of
safety testing; this is due to their often large-scale use in otherwise healthy individ-
uals, including special populations such as children. Vaccines go through extensive
safety testing both before and during human testing, particularly regarding purity
and composition. As biological products, particularly ones that modify the immune
system therapeutically, they theoretically have the potential to exert immunotoxic
effects, including allergenicity. Although vaccines have traditionally not exhibited
significant allergenicity as a result of the vaccination process itself, great care
must be taken to ensure that each of the multiple components is evaluated for poten-
tial allergenicity, both individually and in conjunction with each other and with the
various other vaccine constituents. This presentation will describe the various com-
ponents of modern vaccines and how developers can evaluate the potential for these
components to induce unwanted immune reactions.

2730 FOOD REGULATORY PERSPECTIVE ON EVALUATION
OF ALLERGENICITY.

S. Gendel. Center for Food Safety and Applied Nutrition, US FDA, College Park,
MD. Sponsor: N. Stagg.

Consumers are exposed to a wide, and constantly changing, variety of proteins in
foods. These proteins are both fundamental constituents of the foods and important
sources of nutrition. While most consumers acquire immune tolerance to food pro-
teins, food allergic consumers develop immune responses that can lead to severe
life-threatening reactions when they are exposed to specific foods and food pro-
teins. Food allergies are more common in children than in adults, and there is evi-
dence that the prevalence of allergy is increasing. To protect the health of sensitive
consumers, regulatory agencies such as FDA need to consider potential allergenic-
ity in several different contexts. These include evaluating the potential for novel
foods and food proteins to become allergens, the possibility that novel foods or
novel food proteins will cause reactions in consumers sensitized to other foods
(cross-reactivity), and the effect of food processing on the allergenicity of food al-
lergens. In each case, the specific protein involved, the food matrix, and environ-
mental effects need to be considered. Because there are no validated animal or in-
vitro models that can be used to assess allergenicity, agencies rely on an approach
that integrates data from biochemical, biophysical, bioinformatic and exposure
analyses to assess potential public health risk. The past use of these approaches will
be reviewed and as will the potential for integrating new data sources in the aller-
genicity assessment process.

2731 ALLERGENICITY RESEARCH IN THE
PHARMACEUTICAL/BIOPHARMA SECTOR.

K. Hastings. Corporate Regulatory Affairs, sanofi-aventis, Bethesda, MD.

Drug allergy continues to be a serious concern in drug development. Although the
most dramatic forms are IgE-mediated, it is becoming clear that the majority of
drug allergic reactions are primarily T cell-mediated. The working assumption con-
cerning drug allergic reactions is that either the parent compound, or more likely a
reactive metabolite, covalently binds to proteins to form immunogenic (and potent-
entially allergenic) hapten(s) in situ. This model does not apply to biologic drugs, how-
ever. Almost certainly there are genetic susceptibility factors in drug allergy, but the
key to understanding adverse immune responses to biologic drugs appears to be the
danger hypothesis. All biologic drugs are inherently immunogenic, but a second
"danger" signal is needed to provoke drug allergy. Potential sources of "danger signal" include concurrent infections, concomitant exposure to more than one drug, or exposure to endogenous signals, such as those generated by inflammation or irritation. A complete pattern of possible factors is beginning to emerge concerning causation of drug allergy: genetic susceptibility, concurrent generation or exposure to danger signal(s), and induction of drug-specific immunity. Finally, the various types of pathology thought to be immune-mediated have expanded and the possible mechanisms appear more diverse.

2732 CHEMICAL STANDARDIZATION OF BOTANICAL MEDICINES FOR SAFE AND EFFECTIVE USE AS THERAPEUTIC AGENTS.
M. Soni and B. Mahadevan.

Botanical medicines have been used for millennia to cure ailments in traditional societies around the world. According to the World Health Organization (WHO), in Asian and African countries, over three fourths of the population rely on botanical medicines for their primary health care needs. The use of botanical medicines in the developed world is increasingly on the rise with individuals seeking alternate and/or complementary medicine options. The popular belief is that because botanical medicines are derived from natural sources, they are safe and pose no harm when used. However, within the scientific community, botanical medicines are under increased scrutiny due in part to concerns regarding their safety and clinical efficacy. Safety issues often arise from lack of controlled manufacturing conditions, unproven formulations, improper storage, and poor or ineffective quality control measures. In addition, a number of plant materials have been found to be contaminated with toxicological substances, including heavy metals and interferes with the actions of commonly prescribed medications, which has resulted in a number of adverse patient effects. Thus, use of botanical medicines is further confounded with considerable variations in chemical composition, inclusion of known toxic components during the processing, uncertain therapeutic potency, and potential safety issues when used alone or in combination with pharmaceutical agents. In an effort to broaden the understanding of the aforementioned issues pertaining to botanical medicines, the following key aspects will be addressed: the toxicity of botanical medicines; if it’s natural, it is necessarily safe; successful scientific strategies that are needed to ensure safe and effective use of botanical medicines; quality and safety issues of botanical medicines; chemical standardization of botanical medicines; and current state and federal regulations affecting this line of therapeutic agents.

2733 CHARACTERIZATION AND USE OF HERBAL PREPARATIONS AS TEST ARTICLES IN SAFETY ASSESSMENTS—ANALYTICAL CHALLENGES AND REASONABLE SOLUTIONS.
C. S. Smith. National Toxicology Program, National TeEnvironmental Health Sciences, Research Triangle Park, NC.

Herbal preparations are increasingly popular, yet they are not generally administered according to historical, "time tested" regimens. These facts drive the need for safety assessments conducted at much higher dose levels and with single herbal materials to evaluate toxicity in a modern context. However, herbal preparations are plant materials and as such, are very complex mixtures. Comprehensive analysis of the test article is a critical component to support the results of any safety assessment, but is challenging in the case of botanical preparations because of their complexity. In addition, formulation of test articles of botanical origin with vehicles compatible with animal models also presents challenges of stability and formulation analysis. The National Toxicology Program has undertaken the study of a range of botanicals, including test articles and in formulations, that meets the needs of those manufacturers in the botanical supplement market. This is often the limiting factor in the wide spread adoption of quality control for the purpose of efficacy and safety in the market. Also, characterization of a complex mixture is not an easy task. The overall need and successful scientific strategies required achieving consistency in composition and biological activity of botanical medicines essential for the safe and effective use of botanical products is important.

2734 PHARMACOPEIAL APPROACHES TO SETTING SPECIFICATIONS FOR ARTICLES OF BOTANICAL ORIGIN.

Complex mixtures derived from natural products are very popular as traditional medicines and dietary supplements in US. New regulations for dietary supplements allowed most of these products to be marketed as dietary supplements in the US. This has resulted in a strong public interest for updated state-of-the-art standards of identity purity, content, and absence of contaminants for these products. FDA has issued Botanical Drug Guidance and also new GMPs for dietary supplements requiring the manufacturers to set these specifications for the products they produce and the ingredients that they obtain from the purveyors. However, setting specifications for complex mixtures represent analytical challenges such as lack and expensiveness of purified substances for use as reference materials. Chromatographic profiles and spectrometric procedures as applied to characterize complex mixtures require the use of approaches that often depart from the customary use of these techniques to characterize purified small molecules, which is the subject of modern pharmacopeial monographs. US Pharmacopeia (USP) has taken on the challenge and has created a good number of new pharmacopeial monographs that represent adequate quality for human use for articles of botanical origin as dietary supplements. Public standards from USP help manufacturers, regulators and consumers by providing appropriate quality specifications for dietary supplements.

2735 INTEGRITY OF A PRODUCT: USE OF VALIDATED METHODS IN ASSESSING THE QUALITY AND SAFETY OF BOTANICALS.
I. Khan. School of Pharmacy’s National Center for Natural Products Research, University of Mississippi School of Pharmacy, Oxford, MS. Sponsor: M. Soni.

Authenticated raw material is the basic starting point for the development of a botanical product. However, harvesting, storing, processing and formulating methods may dramatically affect the quality and consistency of the final product by altering the desired market components or by increasing the possibility of unwanted contaminants. Thus, validated methods to ensure quality control in manufacturing and storage are required tools for optimal efficacy and safety of the products. These controls are also critical for the evaluation of pharmacological, toxicological and clinical studies of the botanical supplements. The need for new or improved approaches with modern detection modalities is a real need for the rigorous scientific evaluation of botanical products, whether applied to active constituents or to surrogate markers. Furthermore, this will require the availability of sufficient quantities of reference standards, preferably representing health-relevant principle(s), which have been isolated and structurally verified. Subsequently, gram or larger quantities of relevant principles will need to be prepared to meet the needs of the numerous manufacturers in the botanical supplement market. This is often the limiting factor in the widespread adoption of quality control for the purpose of efficacy and safety in the market. Also, characterization of a complex mixture is not an easy task. The overall need and successful scientific strategies required achieving consistency in composition and biological activity of botanical medicines essential for the safe and effective use of botanical products is important.

2736 STANDARDIZING SNOWFLAKES: USING TECHNOLOGY TO NORMALIZE NATURE.
C. Hopp, NIH/NCCAM, Bethesda, MD. Sponsor: M. Soni.

Much like no two snowflakes are identical, it has been shown that plants from the same species can produce very different chemical profiles. In fact, the same plant can display a variety of chemotypes depending on the growing conditions from season to season. In the face of this natural diversity, it can be extremely challenging to generate a "standardized" herbal preparation. Traditionally this has been accomplished by generating extracts with a defined amount of certain marker compounds. However, these typically represent only a fraction of all the metabolites produced by the plant. Furthermore, increasingly we are seeing that the marker compounds are chosen somewhat arbitrarily and are not responsible for the activity which draws this approach into question. There is a vast technological armamentarium available to the analytical chemist today which makes it possible to quantitatively, qualitatively, and universally capture the chemical diversity produced by the plant. Examples of such tools, such as metabolomics and principle component analysis, will be given with a discussion about how they can be applied to the field of botanical science.

2737 CHEMICAL STANDARDIZATION OF AN HERBAL FORMULATION AS SAFE AND EFFECTIVE THERAPEUTIC AGENT FOR PARKINSON’S DISEASE—A CASE STUDY.
B. Manyam. Department of Neurology, Penn State University, Hershey, PA. Sponsor: B. Mahadevan.

Mucuna pruriens is often touted as a remedy for Parkinson’s disease in the ancient Indian system of medicine- Ayurveda. In an attempt to develop this as a potential herbal drug, various components of the seeds were subjected to HPLC analysis to
identify and quantify the “lead” compound. By using a 6-Hydroxydopamine rat model and rotameter, the preclinical efficacy of Mucuna pruriens seed powder (MPSP) was then compared to the currently used Parkinson’s disease therapeutic, synthetic levodopa. Our results revealed that Mucuna pruriens was two to three times as effective as that of synthetic levodopa. A series of standard toxicity studies were conducted to assess the safety of the extract. In a multi-center clinical trial of MPSH (HP-200) performed in 60 patients with Parkinson’s disease and using a mean dose of 6±3 sachets (7.5 g each) per patient per day revealed that the drug was well tolerated. Consequently, HP-200 was approved as Investigational New Drug by the United States Food and Drug Administration. This case study shed light on the challenges and solutions of moving a botanical drug from discovery to development and human trials. Further, drug development from Ayurvedic source may be effective, safe and highly cost effective with a commendable success rate compared to that of synthetic source.
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