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Deadline for Proposals for SOT 2010 Annual Meeting Sessions: April 30, 2009

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

SESSION TYPES

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

2010 Thematic Approach
The Scientific Program Committee will continue the thematic approach for the 2010 Annual Meeting. All proposal submissions will be reviewed for their relevance under the following themes—Cell Signaling, Gene-Environment Interactions, Metabolic Disease, Mitochondrial Basis of Disease, Toxicity Testing in the 21st Century, and Translational Toxicology for the 2010 meeting. Please note that while we are actively soliciting proposals for the themes listed above, all proposal submissions will be reviewed under the current criteria for their timeliness and relevance to the field of toxicology.

Please refer to the SOT 2009 Program, Scientific Program Overview on the fold-out cover for a list of 2009 sessions highlighted under the thematic approach.

You can now submit your proposal on-line at www.toxicology.org

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the continuing education, symposia, workshop, roundtable, platform, and poster discussion sessions of the 48th Annual Meeting of the Society of Toxicology, held at the Baltimore Convention Center, March 15–19, 2009.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 469.

The issue also contains a Key Word Index (by subject or chemical) of all the presentations, beginning on page 487.

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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EXTENSION OF THE FRAMEWORK TO DROSE-RESPONSE ANALYSIS, AND THE EFFECT OF LIFESTAGE ON THE ANALYSIS. CASE STUDIES USED WILL SHOW HOW THE FRAMEWORK APPROACH IS USED TO ILLUSTRATE THE IMPORTANCE OF A SYSTHEMATIC EVALUATION OF THE AVAILABLE DATA, AND TO PROVIDE THE TOOLS TO BEGIN APPLYING THE MOA/HUMAN RELEVANCE FRAMEWORK.

TOPICS IN ETHICS: CONFLICT OF INTEREST - REAL OR IMAGINED? PBDEs AS A CASE STUDY.

S. Gilbert, P. Weston, M. Goozner, and M. Brown. INQDD, Seattle, WA; National Library of Medicine, Bethesda, MD; Center for Science in the Public Interest, Washington, DC, and Charles River Laboratories, East Rutherford, NJ.

Throughout their professional lives, most toxicologists will confront an array of issues beyond the strictly scientific ones they have trained for. These may range across topics such as animals in research, human subject research, investigational and reporting bias, and conflict of interest concerns. The interdisciplinary nature of toxicology, its sometimes tangled regulatory framework, and implications for public safety and health, may make policy considerations perhaps more relevant than they are for other sciences. Toxicologists, therefore, need to be brace for an array of ethical, legal, and social challenges, and to learn how to sensibly address allegations of conflict of interest or bias while following their science. This course will examine, through a case study related to polybrominated diphenyl ethers (PBDEs), the consequences of alleging conflict of interest or bias. In August, 2007 the EPA dismissed Deborah Rice from its PBDE review panel in compliance with a request from the American Chemistry Council, and expunged her comments from the official record. Dr. Rice had previously expressed her views about PBDE’s as part of work with the Marine government. The EPA’s rationale was “the perception of a potential conflict of interest.” This incident highlights the challenge of a scientist holding a scientifically credible opinion about an issue prior to review by an expert panel (on which he/she is serving) assigned to assess the same issue. Under what circumstance does a position become a conflict of interest or bias? The practical and ethical issues raised in staffing scientific review panels affects scientists and policy makers. Course time will be provided for a discussion of conflict of interest and an examination of related incidents. Students will be provided with a selected list of Web resources related to the ethical issues under discussion.

FREE RADICALS FOR TOXICOLOGISTS - FROM THE BASICS TO INFLAMMATION AND DISEASE.

L. J. Mannell and J. T. Zelekow. St John’s University College of Pharmacy, Queens, NY and New York University School of Medicine, Toronto, ON.

The production of reactive oxygen species/reactive nitrogen species (ROS/RNS) has been recognized to not only serve as a biomarker for oxidative stress, but also significantly contribute to the pathogenesis of various inflammatory tissue injuries and diseases. The emphasis of this course will be placed on an in-depth, state of the art review of the relationship among free radicals, immunologically-related inflammatory responses and environmental exposures and diseases. At the conclusion of this session, the participants will be able to describe the basic concepts of free radicals as they relate to immune-mediated events, better understand the production of reactive oxygen/nitrogen species (ROS/RNS) from various inflammatory responses and exposure to environmental toxins, better understand the impact of ROS/RNS on normal physiological responses and pathological processes.

CHARACTERIZING MODES OF ACTION AND THEIR RELEVANCE IN ASSESSING HUMAN HEALTH RISKS.

S. S. Olin and S. M. Cohen. ILSI Research Foundation, Washington, DC and University of Nebraska Medical Center, Omaha, NE.

Determining the mode(s) of action (MOA) of a toxicant is the goal of many toxicology studies, and these data are often used in risk assessment. A systematic approach to characterizing the mode(s) of action of toxicants is essential in the application of the framework for evaluating the relevance of an animal mode of action in assessing human risk. Frameworks for characterizing MOA data for carcinogens and analyzing its human relevance have been developed and cited in various national and international risk assessment guidelines. Recently, these concepts have been extended to non-cancer endpoints including reproductive, developmental, neurologic and other toxic effects. MOA evaluation is becoming a routine component of regulatory risk assessments thus an important consideration is always whether the MOA determined in animals can be assumed to be directly applicable for humans. The course will demonstrate the use of an MOA human relevance framework that asks first, is the weight of evidence sufficient to establish the MOA in animals? Application of modified Bradford Hill criteria to identify key events, including strength of evidence, leads to evaluation of the overall weight of evidence and discussion of possible alternative MOAs. Evaluation of the human relevance of the animal MOA includes assessing the weight of evidence both for qualitative and quantitative aspects. A brief history of and significance for risk assessment of MOA/human relevance analysis will lay the foundation providing the basic concepts involved in application of the framework. These applications will utilize selected case studies with both cancer and non-cancer endpoints. Case studies will examine issues such as multiple endpoints with shared or different MOAs, the

EVALUATION OF TOXICITY TO MALE AND FEMALE REPRODUCTIVE SYSTEMS: BIOLOGY, STUDY DESIGN AND DATA INTERPRETATION.

K. Hops and B. Melancon. Takeda Global Research & Development Center, Inc., Doverfield, IL, and Schoening, Poughkeepsie Institute, Summit, NJ.

The objectives this course are to provide the basic tools for toxicologists who desire a better understanding of how to assess toxicant-related effects on animal reproduction and the subsequent potential risk(s) to human reproduction. The anticipated audience includes toxicologists who work in regulated product development (e.g., pharmaceutical, chemical, and pesticide industries), as well as scientists who may be responsible for monitoring contracted reproductive toxicity studies so that they can understand the subject sufficiently to work with study directors (i.e., study design and interpretation of study results). Reproductive toxicity studies assess multiple interrelated endpoints in the male and female reproductive systems, respectively, as well as endocrine regulation of these systems. This course will include an overview of the study designs to evaluate toxicity to male and female reproductive systems based on current regulatory guidelines. The course will conclude with case studies of reproductive toxicity data, subsequent interpretation, and how these results are being used to assess potential risks to human reproduction. In summary, upon completion of this course, the attendee will have an appreciation for the key information required for the design of reproductive toxicity studies and interpretation of reproductive toxicity data and will be able to provide guidance for risk assessment in reproductive toxicity evaluation.

IMMUNOLOGY FOR TOXICOLOGISTS.

J. Kinin and R. Peters. University of Manchester, Manchester, United Kingdom and TRAS Utrecht University, Utrecht, Netherlands.

The adaptive immune response that is found in mammals comprises a dedicated interacting system of tissues, cells and molecules that work in concert to provide specific immune responses and host resistance to pathogenic microorganisms and transformed cells. Specific immunity is supplemented by, and works in harmony with, the phylogenetically more ancient innate immune system. Immunotoxicology describes the study of adverse health effects that may result from the interaction of xenobiotics with one or more components of the immune system. Such health effects may take a variety of forms. These include frank immunotoxicity where there is a functional impairment of the immune system. The concern here is that compromised immune function may translate into an increased susceptibility to infectious and/or malignant disease. A second potential consequence of the interaction of chemicals or proteins with the immune system is allergy: defined as the adverse health effects that may arise from the stimulation of a specific immune response. Allergic disease may take one of several forms, those of greatest significance for toxicologists being skin sensitization and allergic contact dermatitis, allergic sensitization of the respiratory tract, food allergy, and idiosyncratic drug reactions. Finally, xenobiotics have also been implicated in the induction or exacerbation of autoimmune responses and autoimmunity disease. This basic grade course will provide a firm grounding in fundamental and clinical aspects of immunology, and will describe the basic elements of immunotoxicology, allergy and autoimmunity in view of the interaction between innate and adaptive immunity. The objective is to deliver an accessible guide to the immune system and immunotoxicology for general toxicologists.

PRINCIPLES AND APPLICATIONS OF TOXICOKINETICS.

M. J. Bartels and C. Tischbach. Dow Chemical, Midland, MI and Battelle Pacific Northwest Laboratories, Richland, WA.

Toxicokinetic (TK) data play an important role in chemical risk assessments. Chemical risk assessments are increasingly incorporating consideration of the mode of action (MOA) of the chemically-induced toxicity. Increasing reliance on MOA in
such evaluations in turn requires increasingly detailed information regarding the active
chemical moiety (parent compound or metabolite) and relevant target tissue
metrics. This course will begin by providing background on the need for and
role of toxicokinetic data in risk assessments. This presentation will include a
discussion of the interaction between evaluation of MOA and toxicokinetic data
and the role of such data in both interspecies and high to low dose extrapolations in
risk assessment. We will go on to describe basic principles of pharmacokinetics from
both the classical and physiologically-based approaches. The presentation will pro-
vide a conceptual and mathematical basis for developing a better understanding
of pharmacokinetics and how pharmacokinetic analyses are conducted. In addition,
to these analyses we will address elements of the design of toxicokinetic studies.
This presentation will include discussion of the interaction of key chemical characteris-
tics with study design parameters and of the sampling and analysis considerations
that shape study design. Finally, the presenters will provide examples of the integra-
tion of toxicokinetic data into current risk assessments, including the incorporation
of human biomonitoring data in the evaluation of chemical exposures and risks.

**9 CURRENT APPROACHES IN MIXTURE RISK
ASSESSMENT.**

M. Mumra1, A. B. Lowry1, C. J. Borgert1 and K. Krishnan1, 1ATSDR, Atlanta,
Gainesville, FL and 4Université de Montréal, Montréal, QC, Canada.

Human exposure to combinations of chemicals and drugs is an everyday reality of
life. There is tremendous interest in scientific and regulatory tools for evaluating the
joint toxic action of chemicals and drugs in mixtures. This course will instruct par-
ticipants in the methods and tools reflective of the current state of knowledge in the
area of mixture risk assessment, as well as illustrative, real-life examples of their ap-
plication to risk assessment. The first talk will introduce the various approaches to
mixture risk assessment and illustrate the use of these methods to assess risks associ-
ated with human exposure to contaminants in selected hazardous waste sites.

The second talk will describe the process of cumulative risk assessment of pesticides,
highlighting the use of pharmacokinetic, pharmacodynamic and relative potency
factors in the process. The third presentation will describe the development and ap-
plication of relative potency factor approach to evaluate safety of mixtures of drugs.

The final talk will discuss the current approaches and tools for assessing the role of
interactions in mixture risk assessment, with particular emphasis on the use of
physiologically-based pharmacokinetic (PBPK) models. Included in this course will be
the evaluation strategies, data sets from real world examples, exercise results,
and discussion of uncertainty pertaining to the application of various mixture pro-
cedures. This course will be of interest to experimentalists, modelers, epidemiolo-
gists and risk assessors interested in the assessment of health risks associated with
human exposure to chemical and/or drug mixtures.

**10 HOW SIMILAR IS SIMILAR AND HOW RELEVANT IS
RELEVANT? CONSIDERATIONS IN THE DESIGN OF A
PREDICTIVE DEVELOPMENT PROGRAM FOR
BIOETHERAPEUTICS.**

J. Burns-Naas2, E. Geely1, J. Bussiere1, J. Cavagnaro1 and L. Andrews1, 1Pharmaceutical and Toxicology, Genzyme, Framingham, MA, 2Drug Safety Research
and Development, Pfizer, San Diego, CA, 3Pathology, Pfizer, Groton, CT, 4Amgen,
Thousand Oaks, CA and Access BHO, Boyce, VA.

Preclinical development programs that are designed to support the safe clinical use of
biopharmaceuticals have considerations that are very different from programs designed
to support the development of small molecule drugs. In particular, with more and
more targeted therapeutics being developed a traditional development program is
becoming more and more complex. While the ICH S6 guidance continues to
drive the program decisions more often than not a different approach is war-
ranted due to species specificity and paucity of relevant animal models. To design a
predictive non clinical program that will support not only first in human dosing but
also eventual approval of the therapeutic is becoming more and more complex. Assuring
safety in humans is the first and foremost task of a well designed program but as-
suring safety and application to specific patient populations is also essential to the
targeted and therapeutic products.

Topics to be addressed in this course will include general pathology and physiology
issues between species that might contribute to species selection/interpretation,
utility of tissue cross reactivity to determine relevant species, considerations into the
development of a homologous protein (from bench to beam), development and charac-
terization of animal models as relevant species (including KO animals and
models of disease), and additionally what to do if nothing is ‘relevant’. The course
attendee will learn key concepts in the considerations for designing a predictive
program for a biotherapeutic product.

**11 NEW FRONTIER IN METAL TOXICOLOGY: GENETIC
SUSCEPTIBILITY, EARLY DIAGNOSIS, AND RELATED BIOLOGICAL INDICES.**

C. D. Klaassen1, M. P. Waalkes2, D. A. Cory-Slechta3, W. Zheng4 and M. L.
McCabe5, 1Purdue University, West Lafayette, IN, 2University of Kansas, Kansas
City, KS, 3NCE at NIEHS, RTP, NC, and 5University of Rochester, Rochester, NY.

Physical and chemical properties of many toxic metals are common in their ten-
dency to donate electrons, their resistance to biotransformation and their similarity
in physical sizes and electrical charges. Yet human responses to metal insults are not

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**TRANSLATION OF SAFETY BIOMARKERS IN DRUG
DISCOVERY AND DEVELOPMENT.**

K. A. Crowell1, E. Evedes2, D. Bounous3, K. Crowell1, J. Colangelo4 and B. R.
Michael1, 1Drug Safety, Pfizer Global Research & Development, Groton, CT, 2Amgen
Pharmacotherapy, Southfield, WI, 3Bristol-Myers Squibb, Princeton, NJ and 4Michigan Technology & Research Institute, Ann Arbor, MI.

There are several major areas that prove problematic in translating animal data/biomarkers to humans. This 5 speaker session will focus on translational is-
"ues identified in hematology, clinical chemistry, coagulation, protein assays and
peptide assays. It will conclude with a risk assessment presentation that summarizes
the overall process in defining human relevance of safety and efficacy from preclini-
cal data. Preclinical data gathered in laboratory animals is required by regulatory
agencies to determine safety in humans prior to marketing of new drugs and ap-
plications. Species-specific differences in routine and more esoteric serum bio-
markers may make the relevance of findings in animals difficult to interpret.

Knowledge in this area is beneficial to the safe conduct of clinical trials and the
inclusion of relevant biomarkers as effective safety and efficacy endpoints during new
product development.

Research scientists, industry scientists, laboratory personnel, and pathologists inter-
ested in biomarker development, translation, execution and applications from pre-
clinical through clinical trials may be interested. This symposium will focus on the
difference between data obtained in preclinical and clinical circumstances.

Therefore, it may be of interest to anyone in a preclinical research setting through
those engaged in clinical trials.

After the session the participants will be able to 1) identify potential relevance or
non-relevance of animal-based hematologic and clinical chemistry biomarkers to
humans, 2) Assess the reliability of using coagulation biomarkers in preclinical species,
3) Identify methods of overcoming species-specific problems in protein and
peptide biomarkers to allow translation, and 4) understand the overall process re-
quired to determine human relevance of animal data and the impact of biomarker
utilization on speed and decision-making.

**CHARACTERIZING VARIABILITY AND UNCERTAINTY
WITH PHYSIOLOGICALLY BASED
PHARMACOKINETIC MODELS.**

H. A. Barton1, R. Serzer1, G. Iohannson1 and C. Tan2, 1ORD National Center for
Computational Toxicology, U.S. Environmental Protection Agency, Research Triangle
Park, NC, 2Institute of Environmental Medicine, Karolinska Institute, Stockholm,
Sweden and 3Center for Human Health Assessment, The Hamner Institutes for Health
Sciences, Research Triangle Park, NC.

As pharmacokinetic (PK) models are increasingly applied in risk and safety assess-
ments it is critical to improve the characterization of variability and uncertainty.
Variability can be described in terms of the relative differences among individuals arising from
 purely random factors, path, health status, genetics, and other factors that contribute to dif-
fences in internal exposures or tissue dosimetry. Absent perfect knowledge, there are uncertainties arising from a range of sources including experimental error, that can
impact confidence in model predictions. Physiologically based pharmacoki-
netic (PBPK) models provide a biologically motivated description of processes in-
fluencing the absorption, distribution, metabolism, and excretion of endogenous
compounds or xenobiotics. PBPK models rely on a wide range of in vitro and in
vivo data to estimate parameter values and demonstrate the predictive capabilities
of the models. This course will describe characterization of variability and uncer-
tainty using PBPK models from a number of perspectives. The range of PBPK
model structures and their applications in risk and safety assessment will be pre-
vented.

How to approach characterizing uncertainty in the presence of variability
will be described. Data from humans can be analyzed using PBPK models to char-
acterize PK variability. Finally, linking variations in external exposure pathways
with PK variability provides methods to characterize human dosimetry for use in
risk assessments or interpretation of biomonitoring data.

**HOW SIMILAR IS SIMILAR AND HOW RELEVANT IS
RELEVANT? CONSIDERATIONS IN THE DESIGN OF A
PREDICTIVE DEVELOPMENT PROGRAM FOR
BIOETHERAPEUTICS.**

J. Burns-Naas2, E. Geely1, J. Bussiere1, J. Cavagnaro1 and L. Andrews1, 1Pharmaceutical and Toxicology, Genzyme, Framingham, MA, 2Drug Safety Research
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Preclinical development programs that are designed to support the safe clinical use of
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**NEW FRONTIER IN METAL TOXICOLOGY: GENETIC
SUSCEPTIBILITY, EARLY DIAGNOSIS, AND RELATED BIOLOGICAL INDICES.**

C. D. Klaassen1, M. P. Waalkes2, D. A. Cory-Slechta3, W. Zheng4 and M. L.
McCabe5, 1Purdue University, West Lafayette, IN, 2University of Kansas, Kansas
City, KS, 3NCE at NIEHS, RTP, NC, and 5University of Rochester, Rochester, NY.

Physical and chemical properties of many toxic metals are common in their ten-
dency to donate electrons, their resistance to biotransformation and their similarity
in physical sizes and electrical charges. Yet human responses to metal insults are not
uniform at all such that metal-caused diseases may manifest in a particular population and spare in others. With a better understanding of gene-environmental interaction, it becomes clear that the genetic predisposition may exist in exposed individuals who have an inherited sensitivity to metal-induced disorders. Thus, the individual susceptibility must be taken into account when developing biomarkers for exposure and/or risk assessment. In many clinical cases, the signs and symptoms of metal intoxication are subtle, undetectable and imperceptible. Because of these, clinically well defined metal diseases, such as manganese-caused parkinsonism or lead-induced learning deficit, are usually diagnosed too late for an effective therapeutic intervention. Thus, a reliable biomarker of a particular type of metal disease, developed either based on injuries in biochemical and physiological functions or alterations in cellular signal pathways, bears a quintessential importance in metal toxicity research. This advanced course is intended to address the biological indices of metal toxicities from the angle of individual genetic susceptibility and population adaptability for early diagnosis, by providing cutting-edge knowledge on the concepts, theories, clinical outcome, and research methodologies in this area. The introduction will briefly review the unique physical, chemical and biological properties of metals which distinguishes them from organic chemicals. The first lecture will discuss the recent advancement in understanding the genetic susceptibility that contributes to metal-induced toxicitites. The second lecture will provide an overview on metal-related biomarkers that were established from animal and human studies and the application of these biomarkers in clinical diagnosis. The third lecture will use manganese as an example to address the methodological approaches to develop biomarkers and explore new ideas to combine exposure indices with biological outcomes. The final lecture will illustrate an innovative way to explore metal toxicity by targeting at metal interaction with the cellular signal pathways. Speakers will survey these new frontiers in metal toxicological research by providing details specific to “hot” metals, such as lead, manganese, arsenic and mercury. Speakers will also discuss pertinent concerns and controversies, including mechanistic-based risk assessments, related to risks to humans exposed to these metals. The course will serve the purpose for those who desire an advanced introduction to mechanisms of metal toxicities, an advanced knowledge on metal-gene interaction and risk assessment, and an advanced technical approach in developing a useful biomarker for metal intoxication. The course will be of interest to others engaged in wider aspects of metal toxicology, neurotoxicology, carcinogenesis, risk assessment, and occupational health.

12 STRESS AS A CONFOUNDING FACTOR IN TOXICOLOGY STUDIES.
1Toxicology, Argen, Seattle, WA; 2Pathology, Argen, Seattle, WA; 3North Carolina State University, Raleigh, NC; 4Schoring Research, Summit, NJ; 5Veterinary Pathobiology, Purdue University, West Lafayette, IN and 6ONH/MC/CDER, Food & Drug Administration, Silver Spring, MD.

Stress can confound the interpretation of toxicity studies. The biology of stress includes complex interrelationships between neurogenic and endocrine pathways. Stressors can have effects on in-life, clinical pathology, endocrine, and immune system parameters. Effects on any of these systems may be observed during a toxicity study. The challenge in toxicology is to differentiate between primary test article-related changes and secondary changes related to stress. This differentiation is crucial to the assessment of stress in the regulatory environment. Major systems impacted by stress and the types of responses seen will be discussed. Biomarkers that are suggested of a stress response will be discussed. Case studies will be presented to illustrate the challenges and best practices in characterization and interpretation of stress responses.

13 EAT WELL, BREATHE WELL: NUTRITIONAL DETERMINANTS OF SUSCEPTIBILITY TO AIRBORNE POLLUTANTS.
J. G. Wagner, Michigan State University, East Lansing, MI.

Susceptibility factors for adverse responses to air pollutant exposure include the host genome, exposure history, disease, age, and diet. In particular, the role of the underlying nutritional status has emerged as an important but understudied determinant of enhanced airway reactivity, inflammation, and immune responses that might be elicited or exacerbated by airborne toxins. For example deficiencies in certain micronutrients are associated with enhanced inflammation to ozone, and dietary supplementation with antioxidants can protect from ozone-induced deficits in airway function. Both clinical and animal studies demonstrate that ozone-induced airway reactivity is related to increased body mass index. Similar relationships of nutritional factors and adverse airway responses exist for diesel exhaust, particulate matter, and cigarette smoke. Specific cellular and airway defenses include small molecular weight antioxidants (e.g., ascorbate, glutathione), and end systems (e.g., catalase, SOD, Phase II enzymes) which are directly or transcriptionally affected by the diet. In addition, the type and amount of dietary lipids can predispose for either pro- or anti-inflammatory pathways. Based on our growing understanding of toxicological mechanisms that underlie the responses to many different types of inhaled pollutant exposure, it is now possible to test specific hypotheses for nutrient or lipid-based dietary interventions to protect from adverse airway inflammatory responses and their consequences in susceptible populations. To gain a clear understanding of these issues, it is important to address the current thinking and gaps in technical and clinical translational studies, approaches that merge basic toxicological principles and biochemical nutritional science, and actively study the comparison of nutrient deficiency, supplementation and energy imbalance on toxicological outcomes from air pollutant exposure.

14 FOOD FOR THE LUNG: HOW NUTRITION AFFECTS RESPIRATORY TOXICOLOGY.
L. Lajers, University of North Carolina, Chapel Hill, NC.

Nutrition has been well recognized as an important factor influencing a number of biological processes including immune responses and ageing, as well as diseases, such as cancer. However, whether and how the nutritional status of an individual could modify responsiveness to inhaled toxicants is not well understood. In the context of the ongoing obesity epidemic as well as the continually growing market for nutritional supplements, the importance of potential interactions between nutrition and effects elicited by inhaled toxicants become apparent. This overview will discuss the significance of the relationship between nutrition and adverse health effects induced by inhaled toxicants.

15 CIGARETTE SMOKE AND ANTIOXIDANT THERAPIES IN COPD.

Oxidative stress from cigarette smoke induces inflammation and is an important etiological factor in the pathogenesis of chronic obstructive pulmonary disease (COPD). Antioxidant agents such as thiol molecules (glutathione and molybdenum drugs, such as N-acetyl-L-cysteine and N-acetylcysteine), dietary polyphenols (curcumin, dillferuloylmethane, a principal compound of turmeric; resveratrol; a flavonoid found in red wine; green tea-epigallocatechin-3-galate; catechins/queretin), can control nuclear factor-kappaB (NF-kappab) activation, regulation of glutathione biosynthesis genes, chomatin remodeling, and hence airway inflammation. This presentation will discuss the role of oxidative stress in key signal transduction pathways and histone modifications involved in lung inflammation in response to cigarette smoke. Current results from studies using dietary polyphenols to modulate redox signaling and hence downregulation of pro-inflammatory mediators will also be emphasized.

16 PROTECTION FROM OZONE AND ENDOTOXIN-INDUCED AIRWAY INFLAMMATION BY GAMMA-TOCOPHEROL.
J. G. Wagner, Michigan State University, East Lansing, MI.

Vitamin E (alpha-, beta-, gamma-, and delta- tocopherol and tocotrienols), is best known as a lipid-soluble antioxidant, but is also capable of modulating cellular functions important in immunity and inflammation. However alpha-tocopherol, the Vitamin E isofrom for which an RDA has been established and is well-documented to limit lipid peroxidation pathways, has been ineffectual against ozone-induced inflammation in both human and animals. We will present data demonstrating novel antiinflammatory properties of gamma-tocopherol, a vitamin E isofrom common in diets of corn, walnuts and sunflower seeds, to inhibit eicosanoid and cytokine production in airways and to limit nitrosative stress, inflammatory cell recruitment and airway hyperreactivity. Experimental results from allergic and non-allergic animals exposed to ozone or endotoxin will be presented.

17 DIETARY INTERVENTION WITH SULFORAPHANE, A PHASE II ENZYME INDUCER TO PROTECT FROM AIRWAY INFLAMMATION.
D. Diaz-Sanchez, U.S. Environmental Protection Agency, Chapel Hill, NC. Sponsor: J. Wagner.

Cellular oxidative stress is thought to be the principle mechanism by which oxidant pollutants such as ozone and particulates mediate their pro-inflammatory effects. Intervention studies with exogenous antioxidants have met with mixed results. An
alternative strategy is the induction of endogenous antioxidants such as Phase II enzymes that lead to the production and metabolism of glutathione, among others. This presentation will focus on results from in vitro, animal, and human studies using sulforaphane, a potent inducer of Phase II enzymes that occurs naturally in cruciferous vegetables. We have recently conducted a clinical trial using broccoli sprouts that contain high levels of sulforaphane, to induce mucosal Phase II enzyme expression in the upper airway of human subjects. Results from this and ongoing human studies that investigate the effectiveness of this nutritional intervention to prevent inflammatory effects of oxidative stress induced by pollutants will be presented.

18 OBESITY ENHANCES AIRWAY INFLAMMATION AND REACTIVITY TO OZONE.

S. Shore. Harvard University, Boston, MA. Sponsor: L. Wagner.

Emerging data indicates that changes in pulmonary function induced by ozone are increased in obese individuals. Data from our lab indicate that obese mice also have augmented responses to acute ozone exposure. Mice obese because of genetic deficiencies in leptin (ob/ob mice), the leptin receptor (db/db mice), carboxypeptidase E (CPE/−/− mice), or in response to an ozone-induced increase in pulmonary resistance, airway responsiveness, and airway inflammation than lean mice. Data will be presented from these mouse models describing a role for adipokines including IL-6, TNFα, and adiponectin, in the relationship between obesity and responses to ozone.

19 COMPLEMENTARY AND ALTERNATIVE MEDICINE (CAM) IN PROTECTING AGAINST INHALED TOXICANTS.


Complementary and alternative medicine (CAM) therapies are an attractive approach to minimize airway responses to common environmental and occupational inducers of asthma and allergy exacerbation, such as ozone and endotoxin. Consequently, studies applying cutting-edge technologies to identify the underlying mechanisms and optimal use of anti-inflammatory CAM modalities are needed. We are currently translating in vitro and preclinical findings that used therapies predominately derived from food sources into clinical studies. Using established protocols, we are testing both the safety and efficacy of novel anti-inflammation and antioxidant CAM therapies to modify pollutant-induced exacerbation of airway inflammation in human volunteers. Stratification of the study group by polymorphisms in key inflammatory or antioxidant genes (i.e., TLRs, GSTs) will enhance our understanding of both the toxicological and therapeutic outcomes. A broad overview of how nutritional CAM agents might be used in clinical studies to modify pollutant-induced exacerbation of asthma will be discussed.

MicroRNAs IN BIOLOGY AND TOXICOLOGY.

M. E. Hahn1 and R. E. Novak1, 2. Biology, Woods Hole Oceanographic Institution, Woods Hole, MA and 1Wayne State University, Detroit, MI.

Gene expression is highly regulated at many levels and altered gene expression is an important part of many toxicological mechanisms. Very recently, a fundamentally new mechanism of gene regulation, involving small RNAs known as microRNAs (miRNAs), has been discovered. MicroRNAs are single-stranded RNA molecules of ~22 nucleotides that function to regulate the synthesis of proteins by inhibiting the translation of miRNAs and promoting their degradation, or in some cases by stimulating their translation. MicroRNAs are abundant and evolutionarily conserved in eumetazoan animals. The human genome encodes hundreds of miRNAs, and a similar number of miRNAs occur in the genomes of other animals. Each miRNA can target hundreds of different messenger RNAs for degradation; up to 20% of the genes in a given genome may be regulated by miRNAs. The biological functions of miRNAs are not fully understood. However, recent studies have demonstrated important roles for miRNAs in the regulation of transcription factor expression and in pre-mRNA splicing. Studies in zebrafish and mammals have shown that miRNAs have important roles during embryonic development and that disruption of miRNA synthesis in embryos can have dramatic consequences. Altered miRNA expression is seen in a variety of cancers and in some cases is involved in the mechanism of tumorigenesis. Furthermore, miRNAs are involved in several human diseases and appear to regulate cellular responses to a variety of physiological and environmental stressors, including diabetes, high blood pressure, nutrient stress, hypoxia, and environmental chemicals. Thus, miRNAs have critical biological functions and there is an emerging understanding of the important role of miRNAs in toxicology, development, metabolic disease, and carcinogenesis. MicroRNAs have not yet been widely studied in a toxicological context; however, it seems likely that these small RNAs may have significant roles in regulating the genomic, proteomic and functional response of cells and tissues to chemicals. [Supported by a WHOI Independent Study Award and by Walter A. and Hope Noyes Smith.]

21 MicroRNA REGULATION OF SIGNALING PATHWAYS DURING DEVELOPMENT.

J. G. Patton. Vanderbilt University, Nashville, TN. Sponsor: M. Hahn.

Recent genomic analyses have suggested that higher eukaryotic genomes are pervasively transcribed but that most RNA transcripts are noncoding. MicroRNAs are a family of gene regulatory small noncoding RNAs that inhibit translation when paired with elements in the 3' UTR of target mRNAs. Target pairing is imperfect enabling microRNAs to target multiple genes and a key remaining question is to identify the exact targets and functions of specific microRNAs. We have been using zebramouse as a model system to study microRNA function during early vertebrate development. Using gain of function and loss of function experiments, we have discovered multiple levels of microRNA regulation controlling the Hedgehog and Wnt signaling cascades during muscle development and fin regeneration. Additionally, we have discovered a regulatory role for miR-200b in controlling trafficking of transmembrane proteins during osmotic stress.

MicroRNAs IN HUMAN CANCERS AND CARCINOGENESIS.

C. M. Croce. College of Medicine, The Ohio State University, Columbus, OH. Sponsor: M. Hahn.

Expression profiling has revealed that microRNAs are dysregulated in a variety of human cancers, and microRNA profiles have proved useful in classifying tumors. In addition, some microRNA alterations are characteristic of multiple tumor types, suggesting an important role of microRNAs as tumor suppressors or oncogenes. This presentation will review the role of microRNAs in human cancers, focusing on two case studies. One involves the down-regulation of miR-15a and miR-16-1 in chronic lymphocytic leukemia (CLL), the most common adult human leukemia. Deletion of these microRNA genes is thought to be involved in a substantial fraction of CLL cases. miR-15a and miR-16-1 act as tumor suppressors, targeting the BCL2 oncogene. In contrast, members of the miR-17/92 cluster are overexpressed in a variety of human tumors and stimulate cell proliferation. The important roles of microRNAs in cancer points to their potential as therapeutic targets.

ABERRANT microRNA EXPRESSION IN HUMAN BREAST ONCOGENESIS.

A. A. Domnibiskovski1, R. Ranganathan1, D. Cukovic1, J. Zheng1, S. Gajda1, R. E. Novak1 and F. R. Miller2. 1Institute of Environmental Health Sciences, Wayne State University, Detroit, MI and 2Karmanos Cancer Institute, Detroit, MI.

Breast cancer is believed to develop in a multistep process, with premalignant lesions preceding invasive carcinoma. While recent work has implicated microRNAs in a variety of human diseases, including cancer, little is known about the role of microRNAs in preneoplastic events that precede malignant transformation. In this work we characterize miRNA expression associated with breast oncogenesis using a multistep cell line model comprised of MCF10A, AT, 3B, CA1h, and CA1a cell lines, and in associated mouse xenographs. This isogenic cell line series represents a progression from benign to premalignant cells to invasive carcinoma. Global miRNA microarrays were used to identify miRNAs that exhibit differential expression correlating with the progression of the disease. Principal components analysis (PCA) demonstrates that the cell lines are segregated along a trajectory of oncogenic potential based on the miRNA expression profiles. Among the microRNAs showing a marked increase of expression in the cell line progression are miRs 21, 200a, 200b, and 429. Several clusters of miRNAs were identified; each consisting of highly correlated expression profiles. Bioinformatics analysis of downstream targets using miR-AT, a computational tool developed in our laboratory, reveals gene ontology and biological pathways associated with the target genes. Our results indicate that preneoplastic changes in miRNA expression may portend events observed in invasive carcinoma. These premalignant alterations offer potential opportunities for risk assessment biomarkers and targets for early intervention. This research was supported by the Wayne State University President’s Research Enhancement Program, and microarray, bioinformatics, and cell culture work was facilitated by NIEHS P30 Center grant ES06669.
MicroRNA EXPRESSION IN HEPATIC AND NON-HEPATIC TISSUES OF DIOXIN-EXPOSED RODENTS.
A. B. Okey1 and P. A. Harper1,2. Pharmacology & Toxicology, University of Toronto, ON, Canada and Research Institute, The Hospital for Sick Children, Toronto, ON, Canada. Sponsor: M. Hahn.
Dioxins such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) are well-known to upregulate expression of hundreds of genes via the AhR (AHR) gene. Gene expression array studies in our laboratory and several other laboratories indicate that numerous genes are also downregulated by TCDD or by AHR status. Mechanisms of AHR-mediated upregulation (induction) by dioxin-like chemicals have been extensively studied but mechanisms of downregulation are obscure, as are the toxicologic consequences of downregulation. We hypothesized that downregulation of miRNA levels by TCDD was mediated by altered expression of microRNAs. Surprisingly, in livers of adult or fetal rodents TCDD has little effect on miRNA levels; thus microRNAs are not responsible for mRNA downregulation in liver nor are microRNAs likely to play a significant role in hepatic toxicity. In contrast to adult liver, TCDD causes significant changes in levels of several microRNAs in multiple non-hepatic tissues in fetal mice. MicroRNAs are particularly important in embryonic and fetal development. It is plausible that the potent teratogenic effects of TCDD may involve alteration of microRNA levels during critical developmental stages.

THE ROLE OF NUCLEAR RECEPTORS IN miRNA TRANSCRIPTION.
microRNAs are a family of non-coding RNAs that inhibit mRNA transcription and/or translation via imperfect base pairing to their target genes. Over 600 miRNAs have been identified in mammals and provide a critical level of gene regulation important in all aspects of cellular physiology. However, relatively little attention has been focused to date on the transcriptional regulation of microRNAs within a cell. Profiling analyses have shown that the nuclear receptor (NR) superfamily of liganded transcription factors are major regulators of miRNA biogenesis. Several lines of evidence point to transcriptional regulation as the main step in the control of miRNA expression. NR regulation of miRNA transcription provides authentic cis binding sites and promoter regions of miRNA genes and may offer a unique opportunity to modulate miRNA expression for potential therapeutic intervention.

SUPERANTIGENS, CYTOKINE STORM, AND TOXIC REACTIONS.
G. Gerberick1 and I. Kimber2. Procter & Gamble Co., Cincinnati, OH and University of Manchester, Manchester, United Kingdom.
The term superantigen (SAg) was coined by Marrack and Kapfer in 1990 to describe a large family of exotoxins secreted primarily by Staphylococcus aureus and Streptococcus pyogenes that are associated with adverse health effects that can range from relatively mild and transient symptoms to catastrophic shock and death. These toxins have been implicated in a number of diseases including scarlet fever, staphylococcal food poisoning and cases of both streptococcal and staphylococcal toxic shock syndrome, as well as being suspected of playing roles in autoimmune diseases. A unique feature of SAgS is that, unlike conventional antigens, they do not require processing by antigen presenting cells (APC) and they can interact with a large proportion of T cells. SAgS have been well characterized and have very specific binding sites on the α or β chain of MHC class II molecules expressed on APC, and on the T cell receptor (TCR) and T cell receptor forming a trimolecular complexes that bridge SAgS with T cells and APCs, thereby enhancing intracellular interactions. The polyclonal stimulation of T cells in juxtaposition with APC by SAgS leads to extensive cytokine production by both cell types including interleukin (IL)-2, interferon-γ and tumor necrosis factor-β by T cells, and IL-1β and tumor necrosis factor-β by APC. The collective action of these cytokines, known as cytokine storm, is the trigger for the clinical manifestations of superantigen immunotoxicity. Moreover, adverse health effects precipitated by cytokine storm are relevant also for considerations of drug safety. One illustrative example of the clinical picture of the TeGenero TGN1412 therapeutic monoclonal antibody was observed in the London UK 2006 trial. Finally, the structure of superantigens, the mechanisms through which they interact with the immune system, and the nature and clinical consequences of cytokine storm will be described and reviewed.

STRUCTURE AND FUNCTION OF SUPERANTIGENS.
I. Kimber. University of Manchester, Manchester, United Kingdom.
The first presentation will provide a background on what is known about the molecular structure and function of superantigens as immunotoxic agents that are produced by bacteria and viruses. In recent years, much knowledge has been gained on how these fascinating molecules interact with biological systems to cause adverse health effects. Superantigen molecules are approximately 20 to 25 kD in molecular weight and are bifunctional in that they utilize at least two types of receptors expressed on immune cells. Specifically, each superantigen has a unique immune fingerprint based on its specificity for T-cell receptor Vβ elements and MHC Class II gene products.

CLINICAL MANIFESTATIONS OF SUPERANTIGEN DISEASES.
Superantigen mediated diseases range from common food poisoning to streptococcal toxic shock syndrome (Besh-eating bacterial disease). Superantigens are suspected in association with autoimmune diseases such as Kawasaki disease and Rheumatoid arthritis. Each of these diseases presents with a constellation of unique clinical signs and symptoms. Treatment of these diseases many involve antibiotics that reduce toxin production in combination with i.v. immunoglobulin (IVIG) therapy.

IMMUNE RESPONSES TO SUPERANTIGENS.
Superantigens are unique in disease pathogenesis in that they form a trimolecular complex with the T-cell receptor and MHC Class II molecules on antigen presenting cells that lead to a cytokine storm. A unique feature of superantigens is that, unlike conventional antigens, they do not require processing by antigen-presenting cells and they can interact with a large number of T cells. Neutralizing antibodies are key to protecting the host from disease progression and newer research indicates that genetic susceptibility factors may be key in the intensity of the host response. For example, HLA alleles differ in their ability to present different SAgS and recent structural analyses has revealed that bound peptide may also play a role in modulating SAg binding to the MHC implying a linkage with susceptibility to SAg immunotoxicity. Specific human HLA haplotypes conferred strong protection from severe systemic disease caused by invasive streptococcal infection, whereas other haplotypes actually increased the risk of severe disease.

EXPERIMENTAL MODELS FOR EVALUATING BIOAVAILABILITY OF SUPERANTIGENS.
Human diseases caused by superantigens begin with their interaction with epithelial cells such as those of the skin, oral, nasal or vaginal mucosa. In the study of superantigen-mediated diseases, it has become important to understand mechanistically the exposure to superantigens, especially through mucosal tissues. The histological classification of epithelial tissues can have effect on the permeability of superantigens and is based on features such as the number of cell layers, shape of cells and specialization of cells as well as presence and composition of the lipid-based permeability barrier. It is known also that other toxins or disruption of epithelial barrier can have a profound impact on the permeability and eventual systemic bioavailability of superantigens.

TOXICOLOGICAL CONSIDERATIONS FOR SUPERANTIGEN-MEDIATED DISEASES.
G. Gerberick. Procter & Gamble Co., Cincinnati, OH.
Superantigens can be considered as immunotoxins that cause disease through their extreme activation of the immune system. However, the amount and dose metric for superantigen-mediated diseases is not understood well. In some instances, bacteremia is not a feature of the disease. For example, TSST-1 mediated menstrual toxic shock syndrome is believed not to involve any systemic infection of Staphylococcus aureus. Systemic penetration of the toxin through a mucosal barrier is a presumptive prerequisite for the development of the acute illness. It is reported that superantigens can stimulate T-cells in the picomole range.
S 32 ZINC, INFLAMMATION, AND DIABETES: INTRODUCTION.

L. Cai, Medicine & Radiation Oncology, University of Louisville, Louisville, KY.

Diabetes is a serious public issue due to its risk for chronic cardiovascular complications. However, mechanisms by which diabetes causes cardiovascular complications remain incompletely understood. Zinc (Zn) is one of the most abundant metals in the human body and therefore essential for the structure and activity of more than 300 enzymes and proteins. Zn deficiency was found to be associated with diabetes, but the direct role of Zn in diabetic etiology and its mechanisms are under-explored. Recent studies have shown that chronic inflammation is not only related to the onset of Type I and Type II diabetes, but also related to the development of diabetic complications. Zn deficiency may cause systemic inflammation that in turn becomes the initial factor for the onset of diabetes and the development of diabetic complications. The current understanding of the roles of Zn homeostasis in the insulin signaling, inflammatory system, inflammation, diabetes and diabetic complications will be explored, as will the public awareness of proper intake of Zn-containing food in daily life. A brief overview highlighting the association of Zn with inflammation, diabetes and diabetic complications will be presented. Finally, the evidence of Zn to protect ischimic and the diabetic heart will be presented.

S 33 PATHOPHYSIOLOGY OF CELLULAR ZINC AND REDOX BUFFERING MECHANISMS IN DIABETES.

W. Maret. The University of Texas Medical Branch, Galveston, TX. Sponsor: L. Cai.

Interference with the homeostatic control of cellular zinc metabolism can compromise many functions and cause cellular injury. Homeostatic control includes regulation as well as storage. The intracellular zinc buffering capacity is limited. Furthermore, the cellular zinc and redox buffering capacities overlap, because cellular thiols have dual functions in redox reactions and in zinc binding. When these thiols react with environmental toxins or reactive species, their redox state changes. Conditions of excess zinc or reactive species or their testing picomolar concentrations and elicit a cytotoxic response (pro-antioxidant) in a redox-active zinc metalloprotein, in which thiol reactivity and zinc binding are linked. These fundamental principles are important for cellular signaling, because zinc ions are potent effectors of proteins. For example, zinc is involved in insulin storage and secretion in beta-cells and in the modulation of insulin signaling in target organs. The insulin-enhancing actions of zinc ions are due to zinc inhibition of protein tyrosine phosphatase 1B, the major phosphatase regulating the phosphorylation state of the insulin receptor. Reactive carbonyls formed during aberrant glucose metabolism, reactive species generated during inflammation, nutritional deficiencies, and environmental exposures to toxins all affect cellular physiology by interfering with zinc homeostatic mechanisms.

S 34 ZN REGULATION OF SYSTEMIC INFLAMMATION AND INSULIN RESISTANCE IN OBESITY.

J. Ye, Molecular Biology, Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA. Sponsor: L. Cai.

Inflammation is elevated in obesity, and involved in the pathogenesis of many obesity-associated complications, such as type 2 diabetes and cardiovascular diseases. Inflammation increases the risk of type 2 diabetes through impairment of insulin action. It reduces body’s response to insulin, and leads to so called “insulin resistance”. The molecular mechanism is related to inhibition of signal transduction of the insulin receptor pathway. Through this mechanism, inflammation is able to convert transient physiological “insulin resistance” into permanent pathological “insulin resistance”, which is the earliest event of type 2 diabetes. In the inflammatory response, the NF-kB pathway is activated, and required for expression of inflammatory cytokines. In obesity, this signaling pathway is activated by fatty acids and their derivatives (such as DAG or Ceramide). Activation of toll-like receptor 4 (TLR4) and protein kinase C (PKC) are the downstream events of the bioactive fatty acids. Addition of a hypoxia response also plays an important role in the activation of NF-kB. In the adipose tissue, macrophage infiltration is a marker of chronic inflammation and serves to promote inflammatory response. Inhibition of NF-kB activity in macrophages is able to prevent inflammation, and protect mice from insulin resistance. Zn has dual activities in the regulation of NF-kB activity. It is required for DNA-binding of NF-kB, and also involved in activation of the negative feedback loop of the NF-kB pathway. At a high concentration, Zn may inhibit inflammation through the negative feedback mechanism. These possibilities suggest that administration of Zn in obese condition may reduce inflammation and provide beneficial effects in the prevention of inflammation, insulin resistance and type 2 diabetes.

S 35 METALLOTHIONEIN GENE POLYMORPHISMS AND ZINC HOMEOSTASIS IN THE SUSCEPTIBILITY OF DIABETES MELLITUS AND CARDIOVASCULAR COMPLICATIONS.

R. Giaccon6,1, M. Malavol61, B. Bonfigli2, R. Tezz2, A. Lattanzio61 and E. Mochegeani61.

Metallothioneins (MT) are metal-binding proteins which regulate zinc homeostasis and counterbalance oxidative stress induced by the diabetic condition. Recently, several single nucleotide polymorphisms (SNPs) in MT genes have been involved in diabetes mellitus (DM2) predisposition. An association between +209 A/G MT2A polymorphism, hyperglycemia, and the prevalence of ischaemic cardiomyopathy in old atherosclerotic patients with DM2 has been found. +647 Asp/Thr MT-1A polymorphism affects zinc homeostasis and is associated with longevity in Italian cohort. Thr/Thr genotype (named C+ genotype) is less frequent in centenarians and associated with lower intracellular zinc release (iZnR) induced by NO donors, increased MT levels and IL-6 plasma concentrations. We have found an association of +647 MT1A polymorphism with DM2. The study included 694 old Italian subjects: 242 healthy controls, 217 DM2 patients without CVD clinical evidence (DNC) and 235 diabetic patients with CVD diagnosis (DCVD). C+ carriers displayed higher glycemia and glycosylated hemoglobin than C- ones in DCVD group. A modulation of MT levels and iZnR in PBMcs were observed in DCVD cohort when related to +647 Asp/Thr MT1A polymorphism. In summary, we demonstrate an association of +647 Asp/Thr MT1A polymorphism with DM2 and its influence on glycemic control, iZnR and MT production, in presence of diabetic cardiovascular complications. These results enable us to further investigate the MT genetic background and zinc homeostasis in the prevention and treatment of DM2 and its complications.
and T. Dosage and dosing regimen for developmental and reproductive studies for biotherapeutics. Emphasis will be placed on choosing a high dose for toxicity signs will be covered, in addition to addressing some of the challenges with respect to replications as well as challenges that may be encountered with reproductive study design. Challenges continue to exist in the appropriate study design for reproductive evaluation of biologics and the utility of such studies to the safety package. These considerations that go into selection of the highest doses to be used in a study, use of recovery groups, and the impact of immunogenicity on the outcome and interpretation of the study. Traditional approaches that rely upon a maximum tolerated dose for high dose selection may not be relevant or even feasible when considering biotherapeutic products. Establishing a range of toxicity endpoints in a study using a classical approach is often not feasible or practical with biotherapeutic products due to challenges that might involve formulation, concentration, stability and volume issues. In addition, the use of recovery groups needs to be considered and the utility of the information gained from the use of additional animals added to a study. Aspects of full recovery evaluation and the impact of immunogenicity on the outcome and interpretation of the study design will be discussed. Challenges continue to exist in the appropriate study design for reproductive evaluation of biologics and the utility of such studies to the safety package. These considerations as well as challenges that may be encountered with reproductive study designs will be covered, in addition to addressing some of the challenges with respect to dose selection and study design considerations, including reproductive study design for biotherapeutics. Emphasis will be placed on choosing a high dose for toxicology studies and how does that relate to clinical trial dosing strategies; the impact of immunogenicity on the dosing strategy and the potential to dose higher; the use of information to be gathered from recovery animals, and the selection of doses and dosing regimen for developmental and reproductive studies for biotherapeutics.

DOSE SELECTION AND DESIGN CONSIDERATIONS IN SAFETY STUDIES FOR BIOThERAPEUTICS

L. Andrews and T. MacLachlan, Pharmacology and Toxicology, Genzyme, Framingham, MA.

With many biotherapeutics being evaluated on a case-by-case basis there is often significant consideration that goes into selection of the highest doses to be used in a study, use of recovery groups, and the impact of immunogenicity on the outcome and interpretation of the study. Traditional approaches that rely upon a maximum tolerated dose for high dose selection may not be relevant or even feasible when considering biotherapeutic products. Establishing a range of toxicity endpoints in a study using a classical approach is often not feasible or practical with biotherapeutic products due to challenges that might involve formulation, concentration, stability and volume issues. In addition, the use of recovery groups needs to be considered and the utility of the information gained from the use of additional animals added to a study. Aspects of full recovery evaluation and the impact of immunogenicity on the outcome and interpretation of the study design will be discussed. Challenges continue to exist in the appropriate study design for reproductive evaluation of biologics and the utility of such studies to the safety package. These considerations as well as challenges that may be encountered with reproductive study designs will be covered, in addition to addressing some of the challenges with respect to dose selection and study design considerations, including reproductive study design for biotherapeutics. Emphasis will be placed on choosing a high dose for toxicology studies and how does that relate to clinical trial dosing strategies; the impact of immunogenicity on the dosing strategy and the potential to dose higher; the use of information to be gathered from recovery animals, and the selection of doses and dosing regimen for developmental and reproductive studies for biotherapeutics.

HOW HIGH IS HIGH ENOUGH WHEN SELECTING TOP DOSES FOR GENERAL TOXICOLOGY STUDIES?


In the practical art of applied nonclinical toxicology most of the recent debate on dose selection has focused on the method used for establishing the first-in-human dose (FIHD). Several methods have been proposed for this purpose, based on calculation of the no adverse effect (dose) level (NOAEL), pharmacologically active dose (PAD) or the minimum anticipated biologically effective (dose) level (MABEL). For many products, these methods will utilize pharmacology and/or toxicology information gathered from the low- or mid-dose groups, rather than the high-dose groups. Historically, high doses have been selected with the intention of demonstrating toxicity, often resulting in identification of the maximum tolerated dose (MTD) or dose-limiting toxicity (DLT). However, demonstration of toxicity or adverse effects, even if attributable to intended pharmacologic properties (super pharmacology), can be difficult for some products, especially biologics. For such products, there is a lack of consensus on the justification for selection of the highest dose in toxicology studies intended to support clinical dosing. Possible approaches might include a maximum feasible dose (MFD), multiple of human dose (MHD), multiple of human exposure (MHE), or other empirical selections (e.g., 100 mg/kg for monoclonal antibodies). Even if the highest dose is not identified as an MTD, it may provide significant safety margins over the lowest and highest planned human doses. However, even if the high dose exceeds the maximum pharmacologic effect (MPED), failure to establish an MTD may be seen by some regulatory reviewers as a reason to delay clinical development. This presentation will attempt to provide rationales for selecting the top dose for general toxicology studies.

IMPACT OF IMMUNOGENICITY ON HIGH DOSE SELECTION FOR CHRONIC STUDIES – SHOULD YOU DOSE HIGHER? PROS AND CONS.

D. Wierda, Biopharmaceutical Immunotoxicology, Eli Lilly & Co, Indianapolis, IN.

The formation of neutralizing anti-drug antibodies in non-clinical studies with human biopharmaceuticals may effectively reduce the pharmacodynamics of the drug as well as the duration of the toxicity studies. One strategy that has been recommended to mitigate against this risk is to dose through the formation of neutralizing antibodies by increasing either the frequency of dosing, or the administered dose level of drug, sufficiently to overcome any neutralizing or clearing effects of anti-drug antibody. A potential advantage of this strategy is that high doses of soluble antigens tend to induce immunological tolerization. On the other hand, a significant disadvantage is the possible loss of dose-response information required for estimating the safe clinical starting dose of the therapeutic. This presentation will provide an overview of factors and limitations that must be considered when deciding whether or not to implement a dosing through strategy.

HIGH DOSE SELECTION FOR DART STUDIES.

L. Couch, Pharmacology/Toxicology, Genzyme, Framingham, MA.

The guidelines for assessment of reproductive toxicity (ICH S5A & S5B) include recommendations for: selection of dose, dosing intervals, and duration of dosing of medicinal products. In the case of biologics, these recommendations may not always be appropriate as biologics have widely varied pharmacokinetic and pharmacodynamic parameters, clinical dosing regimens, and potential for immunogenicity. Selection of dose and dosing regimen for reproductive toxicology studies should therefore be considered on a case by case approach, including consideration of product-specific biology rather than based strictly on ICH S5A & S5B guidelines. Examples of preclinical development programs implementing case-based and clinically relevant approaches to the design of reproductive toxicology studies for biologics will be discussed.

WORKSHOP INTRODUCTION: SYSTEMS BIOLOGY & BIOLOGICAL MODELS.

S. Edwards1 and C. Timchalk2. INHEERL, U.S. EPA, Research Triangle Park, NC, and Biological Science, FNEL, Richland, WA.

As we consider the future of toxicity testing, the importance of applying biological models to this problem is clear. Modeling efforts exist along a continuum with respect to the level of organization (e.g. cell, tissue, organism) linked to the resolution
of the model. Generally, a tradeoff is made whereby higher levels of organization are models with lower resolution. Consideration will be given to modeling efforts across the full range of this spectrum. First, a model will be described for intracellular signaling including important information on how the choice of cell type for in vitro studies affect the signaling seen. Next, two systems approaches will be high-lighted which model genome-wide molecular changes (i.e. ‘omics) within and between tissues and the application to pharmaceutical target discovery and toxicity testing. Finally, biologically-based dose-response models for risk assessment and data requirements associated with these models will be covered following a progress report on the development of detailed tissue models and integration of such models with molecular and cellular changes. If incorporated into future toxicology testing, these approaches should greatly facilitate the definition and quantitative modeling of mode of action for important environmental stressors. The systems approaches also have the potential to greatly improve interspecies extrapolation. In addition, the models described will facilitate the use of in vitro data for risk assessment thereby increasing the number of chemicals that can be evaluated each year. In contrast to the single tissue modeling approaches, we hope to lay the groundwork for a better understanding of the future opportunities and challenges we face as we strive for increasingly higher resolution models at greater levels of organization. [This abstract does not reflect EPA policy.]

**44 QUANTITATIVE SYSTEMS ANALYSIS OF HEPATOCELLULAR RESPONSES TO INFLAMMATORY CYTOKINES AND PHARMACOLOGICAL AGENTS.**


We are pursuing a systematic approach to quantitatively assess relationships between multiple combinations of inflammatory cytokines and pharmacological agents in inducing hepatotoxicity in a set of in vitro systems including primary rat hepatocytes, primary human hepatocytes, and a transformed hepatic cell line (HepG2). Our goal is to gain insights concerning how physiologically-relevant cytokines might facilitate development of lethal (apoptotic and necrotic cell death) and/or sub-lethal (loss of differentiated function) hepatotoxicities by idiosyncratic hepatotoxic drugs. In the work we report here, combinatorial data sets of cytokine- and drug-induced lethal hepatotoxicity phenotypes in each cellular system were used to identify multivariate relationships between underlying drug and cytokine treatment variables and to compare these relationships between cellular systems. Further studies combining cytokine treatment with non-idiosyncratic and idiosyn-cratic drug treatments indicate that drug synergy exists at physiologically-relevant concentrations and are informative in distinguishing idiosyncratic hepatotoxic drugs from non-hepatotoxic, non-idiosyncratic analogs. Because biological function is context sensitive and the structures, dynamics, and outputs of canonical signaling pathways vary from cell to cell type to cell type and between normal and diseased cells, effective application of pathways maps to the study of disease and therapy requires context-specific information on specific cells and tissues in normal and abnormal states. Thus, we further analyzed key discrepancies between the human primary hepatocytes and HepG2 cell. The most striking differences were alterations in pathways involved in innate immune responses and NF-kB function, associated with substantive changes in consequent cytokine secretion. We believe that our experiment and computer modeling approach could find useful application to stress and environmental health applications.

**INFORMATIC AND STATISTICAL INTEGRATION OF ‘OMICS DATA TO MODEL CELLULAR RESPONSE TO STRESS.**


The advent of the genomic era has brought the promise of “systems science” methods to advance toxicological research and improve human and environmental health. However, development of interpretive and analytic computational tools has not kept pace with advances in the collection of data, both in terms of breadth (vast amounts of data) and depth (proteomics, metabolomics). The single greatest challenge for the implementation of the NRC vision for Toxicity Testing in the 21st Century is the abstraction of cell response networks from ‘omics data which accurately reflect the circuitry needed for predictive mathematical modeling. The use of integrated genomic, transcriptomic, proteomic, and metabolomic data is essential to fully capture multiple levels of molecular hierarchy and modes of regulation necessary to reconstruct the global response of a cell to computational tools capable of generating quantitative cellular responses to toxic stimuli. For example, we have found that microarray data are sufficient to distinguish between antigenic- and particle-induced inflammation, where both the cell receptor interaction and toxicity mechanism are unique. However, integration of proteomic data allows discrimination between different classes of nanomaterials, such as amorphous silica, carbon nanotubes and crystalline silica, highlighting different pathways of cell activation. We also find that integrated data for RNA levels, protein abundance and phosphorylation status synergistically enhance network reconstruction beyond any single data type alone. Furthermore, identification of toxicity pathways and their distinction from adaptive/protective processes through temporal and dose-dependent measurements from in vivo and in vitro systems to account for inherent differences in exposure. Conserved response pathways between alveolar epithelial cells, macrophage cells and whole lung tissue provide cell-specific response measurements necessary to cover these gaps. The linkage of computational and experimental approaches for cell response modeling will be illustrated. This work was funded by NIEHS through RO1 ES016122 and U54-ES016105 and by the Environmental Biomarkers Initiative at PNNL.

**46 GENE NETWORKS REFLECTING TISSUE-TISSUE INTERACTIONS HIGHLIGHT NOVEL CAUSAL PATTERNS OF ASSOCIATION WITH HUMAN DISEASE.**


Network approaches to understanding complex system behaviors have largely ignored tissue-to-tissue communications that are critical to living systems manifesting complex behaviors like disease. To decipher the communication between tissues at the molecular level, we examine interactions among gene expression traits in blood, adipose, muscle, pancreas, liver, and brain tissues from human and experimental mice across populations using integrative genomics approaches previously applied to single tissues. Gene-gene relationships specific to interactions between two given tissues are observed to give rise to coherent subnetworks involved in important functional traits like circadian rhythm and energy balance that are independent of subnetworks detected from single tissue analyses, highlighting novel associations with disease that have previously escaped notice. Further, not only do the tissue-tissue networks highlight genes in one tissue that respond to changes in genes in a second tissue, but they elucidate entire subnetworks in one tissue that influence subnetworks in a second tissue. Our modeling approach provides direct support for cross-tissue processes influencing a diversity of disease traits related to obesity, diabetes, atherosclerosis, and Alzheimer’s, and suggests hypotheses on how biological processes observed in one tissue as driving a given disease (e.g., obesity) may influence processes observed in a different tissue as driving a related disease (e.g., diabetes). Many of the specific causal relationships we detect via the tissue-tissue network would be difficult or impossible to detect via single-tissue analyses. Our analyses provide further support that complex traits like obesity, diabetes, and atherosclerosis are emergent properties of complex interactions among molecular networks in different tissues that are modulated by complex genetic loci and environmental factors.

**47 BIOLOGICALLY BASED DOSE-RESPONSE MODELING. THE POTENTIAL FOR ACCURATE DESCRIPTION OF THE LINKAGES IN THE APPLIED DOSE - TISSUE DOSE - HEALTH EFFECT CONTINUUM.**


In the game of chess, computers have become stronger players than the strongest grandmasters. Computer simulations are now used to study events, such as the evolution of the universe shortly after the Big Bang, that cannot be studied in the laboratory. In biology, the ‘omics’ technologies have stimulated development of computational tools that probe the biological structures that gave rise to the data. All of these developments are reflections of Moore’s Law, which states that computing power doubles about every two years. It is difficult to see with any clarity where computational technologies will lead us in the coming years. What is not difficult to predict is that computation will be integral to scientific research in biology, toxicology, and human health risk assessment. Biology has until recently been a largely intuitive discipline but the future will require formulation of quantitative hypotheses amenable to evaluation in the laboratory and in silico. There is no point in arguing about whether or not this should happen. The only relevant questions are exactly how it will happen, and how best to manage and adapt to it. In this presentation these questions will be pursued in the context of dose-response analysis for toxic chemicals. Given knowledge of exposure, the shape of the dose response curve is the key to predicting health risk, which in turn determines allowable levels of exposure and the associated economic costs of compliance. The public health and billions of dollars are at stake, so it is well worth doing a good job! Specific issues that will be addressed include (1) the state-of-the-art in dosimetry modeling, (2) strategies for computational tools to support dose-response modeling, and (3) application of computational modeling of data collected in vitro to provide estimates of risk in vivo. This work has been reviewed by the EPA and approved for publication but does not necessarily reflect the views or policies of the Agency.
VIRTUAL ORGAN MODELS FOR MULTI-SCALE INTEGRATION OF DOSE-RESPONSE RELATIONSHIPS.

R. A. Corless1, K. R. Minard2, R. E. Jacob1, C. Timchalk1, A. P. Kuprash1, J. P. Lamanna1, S. Kalluri1, C. Frevert1, M. Eaves1, Pacific Northwest National Laboratory, Richland, WA. 2University of Washington, Seattle, WA and 3University of Alabama, Birmingham, AL.

Organs consist of a spatial organization of specialized cells that together carry out higher order functions. Spatial organization has largely been ignored in dose-response models due to computational and data limitations. New imaging and computational approaches are now enabling the development and functional validation of comprehensive virtual organs that capture 3D tissue architecture and cellular distributions. For example, multi-dimensional models of the respiratory and cardiovascular system and their associated imaging and computational technologies are improving our understanding of environment-disease interactions for human exposures to gases, vapors and particulates, as well as enhancing drug delivery or surgical interventions. Likewise, the ‘omics’ revolution significantly enhances the development of cellular response models as discussed by others in this symposium. To truly be effective for dose-response modeling, atlases must be constructed that map the spatial distribution of specialized cells with their functional attributes to the 3D organ models to provide an integrated dose-response framework. Similar approaches have recently been developed for the Allen Brain Atlas, a 3D interactive atlas of cell distribution and gene expression in the mouse brain. However, respiratory and cardiovascular atlases must extend beyond gene expression in normal tissue to include spatial and temporal changes in genes, proteins, and metabolic function in specialized cells. This presentation will focus on the current status of multi-scale virtual organ modeling using examples primarily from the respiratory system but also from the heart, vascular system, brain and liver-to provide a perspective on where these models may impact human health research and risk assessment over the next 10 years. Funding from NIH (HL073598, ES011617, EB008192, VA and University of Alabama, Birmingham, AL) will be forthcoming for development of these models.

DATA INTEGRATION STRATEGIES REVEAL POTENTIAL LIABILITIES IN EARLY LEAD OPTIMIZATION.

M. Cooper, Discovery & Investigative Safety Group, Boehringer Ingelheim, Baltimore, MD.

Drug-induced liver injury (DILI) is the leading cause of drug safety attritions. By applying systems biology and integrating mining, modeling, manipulation and measurements, we have assessed a panel of in vitro assays with good predictivity to enhanced risk assessment, and improve biomarker development. Technical challenges and use cases will also be discussed.

ORGAN SLICES PROVIDE MECHANISTIC INSIGHT INTO DRUG-INDUCED ORGAN INJURY AND FACILITATE COMPARISONS OF ANIMAL AND HUMAN TISSUE.


Drug-induced liver injury (DILI) is the leading cause of drug safety attritions. By applying systems biology and integrating mining, modeling, manipulation and measurements, we have assessed a panel of in vitro assays with good predictivity to clinical DILI including a significant number of type II or idiosyncratic hepatotoxicity. Using drugs as test agents and measuring a panel of cellular phenotypes that are directly linked to key mechanisms of hepatotoxicity, we have developed an in vitro testing strategy that is predictive of many clinical outcomes of DILI. Mitochondrial damage, oxidative stress, and intracellular glutathione, all measured by high content cellular imaging in primary human hepatocyte cultures, are the three most important features contributing to the hepatotoxicity prediction. When
applied to over 300 drugs and chemicals including many that caused rare and idio-
syncratic liver toxicity in humans, our testing strategy has a true-positive rate of 50-
60% and an exceptionally low false positive rate of 0-5%. These in vitro predictions
can augment the performance of the combined traditional preclinical animal tests
by identifying idiosyncratic human hepatotoxicants such as telithromycin, nefazodone,
trogloxazone, tetracycline, sulindac, zileuton, labetalol, diclofenac, chloroxazone,
dantrolene, and many others. Our findings provide insight to key DILI mechanisms,
and suggest a new approach in hepatotoxicity testing of pharmaceuticals.

W

54 IN VITRO MICROARRAY ANALYSIS OF HEPATOCYTES FOR LEAD COMPOUND SELECTION.

Investigative Toxicology and Pathology, Lilly Research Laboratories, Greenfield, IN.

Early identification of toxicologic concerns can improve drug candidate quality and
reduce cycle times. This presentation will focus on the use of a contextual genomics
database, DrugMatrixTM, in early compound screening. In a rat primary hepatocyte
model, sparse linear programming-derived gene signatures are leveraged to predict in vivo
toxicity. Key to successful application of the technology is demonstration of its predictive power. We have tested in the hepatocyte model more than 100 proprietary compounds with known outcomes for hepatic pathology in short term rat safety assessment studies. The analysis demonstrated acceptable performance metrics for prediction of in vivo outcomes. In addition, a specific example will be presented which illustrates the use of in vivo gene signatures to associate phos-
pholipidosis from target organ toxicity within a series of highly structurally related
compounds. Overall, the data indicate that a robust contextual chemogenomics database can leverage in vivo gene signatures to direct project teams towards an SAR(s) that is most likely to yield a drug candidate with a favorable in vivo safety profile.

PL

55 AUTOMATED DOSE-RESPONSE ANALYSIS OF THE RELATIVE HEPATIC GENE EXPRESSION POTENCY OF TCDF IN C57BL/6 MICE.

1Biochemistry & Molecular Biology, Michigan State University, East Lansing, MI. 2National Food Safety & Toxicology Center, Michigan State University, East Lansing, MI. 3Center for Integrative Toxicology, Michigan State University, East Lansing, MI. 4The Dow Chemical Company, Midland, MI and 5Toxicogenomics Informatics and Solutions, LLC, Lansing, MI.

Toxic equivalency factors (TEFs) are assigned to dioxins and dioxin-like chemicals based on relative potency (REP) values of all individual adaptive and toxic re-
sponses compared to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). We used Agilent 4x44k oligonucleotide microarrays to examine the hepatic gene expression potency of 2,3,7,8-tetrachlorodibenzo furan (TCDF), relative to TCDD. Immature ovariectomized C57BL/6 mice were gavaged with either 0.03, 0.1, 0.3, 1, 3, 10, 30, or 100 µg/kg TCDD, the WHO TEF adjusted doses (10 x TCDD dose) TCDF or sesame oil vehicle, and sacrificed at 72hrs. 2,288 TCDD and 1,347 TCDF differenti-
ately expressed genes were identified. Automated dose response modeling (ToxResponse Modeler) identified 1,078 genes with 610 genes exhibiting a TCDD
like response. Of these, REPs were calculated for the 83 genes that exhibited similar
responses compared to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). We used
ToxResponse Modeler to identify idiosyncratic human hepatotoxicants such as telithromycin, nefazodone, troglloxazone, tetracycline, sulindac, zileuton, labetalol, diclofenac, chloroxazone, dantrolene, and many others. Our findings provide insight to key DILI mechanisms, and suggest a new approach in hepatotoxicity testing of pharmaceuticals.

PL

56 METABOLOMICS: A NOVEL TOOL FOR UNDERSTANDING THE EARLY-STAGE MECHANISTIC UNDERPINNINGS OF DRUG ACTION AND SAFETY.


Traditionally, drug action and safety has involved monitoring gross physiological changes to animals dosed with drug. With increasing numbers of drug candidates and potential targets, earlier information on potential drug safety issues is required
before deciding which compounds to take into the clinic. Metabolomics, the global profiling of biochemicals, provides unparalleled insight into the mechanistic action of drugs. Many of these biochemical changes are seen within hours of dosing, providing early-stage indication of drug safety issues. This presentation will demonstrate the use of metabolomics in drug safety research through two case studies done in collaboration with leading pharmaceutical companies. Valproate (VPA) is a drug widely used to treat epilepsy, but it has serious adverse effects including hepatotoxicity, teratogenicity and antifolate activity. Rats were treated with VPA (500 mg/kg) or saline (vehicle) once daily for 1, 5 or 28 days. Urine and kidney tissue samples were collected from the vehicle group at days 1, 3, 5 and 28. Full data curation of kidney tissue samples yielded 547 metabolites, and urine yielded 657 metabolites. Analysis of the metabolic changes showed Valproate stim-
ulated fatty acid omega-oxidation, likely to compensate for a defective beta-oxidiza-
tion. Additionally bile acids were identified as potential markers for VPA-induced liver toxicity. Fenofibrate (Fen) and Phenobarbital (Pb) are two known rodent carcinogens. To better understand their mechanism of toxicity, rats were dosed with fen (500 mg/kg/day), or Pb (50 mg/kg/day) or vehicle. Plasma and 24-hour urine samples from rats were collected at day 2 and day 14. A metabolomics analysis yielded 496 biochemicals in plasma and 974 compounds in urine. Potential cell proliferation biomarkers in plasma and urine were identified. Their biochemical and physiological roles were related with liver and kidney malfunctions such as hyper-
plasia and cell proliferation.

PL

57 GENE EXPRESSION PROFILING IN PRIMARY MOUSE HEPATOCYTES TO SCREEN COMPOUNDS FOR CARCINOGENIC PROPERTIES.

1Health Risk Analysis and Toxicology, Maastricht University, Maastricht, Netherlands and 2Netherlands Toxicogenomics Center, Maastricht University, Maastricht, Netherlands. Sponsor: H. van Loveren.

Sandwich-cultured mouse primary hepatocytes are an attractive model for toxi-
cogenomics-based screening of chemicals to predict their toxicity. This is, because they are metabolically competent, the liver is frequently a target organ and mouse models gain in usage due to the development of transgenic models. Furthermore, mouse primary hepatocytes are favoured over rat hepatocytes, which are relatively
unstable, as well as human hepatocytes, which are limited in availability. Therefore, we aim to develop in sandwich-cultured mouse primary hepatocytes transcripti-
omics-based prediction tools for the toxic properties of compounds, with the ini-
tial focus on specific classes of genotoxins and carcinogens. Compared to rat hepa-
tocytes, the metabolic system of mouse hepatocytes showed to be more stable
during several days, as shown by gene expression of phase I and II enzymes and ac-
tivity of CYP450 enzymes. Time- and dose-dependent transcriptomics of benzo[a]pyrene exposed cells indicate that time has the biggest effect on the pro-
files, and that the modulation continuously increases over time (12-48h).
Transcriptomics-based discrimination of genotoxic from non-genotoxic carci-
gen was developed, showing that this was possible at various time points at 24h and
48h, but not at 12h, by Nearest Shrunken Centroids Analysis. Finally, the dis-
rimination of true genotoxins from false-positive genotoxins was addressed at 24h
and 48h. Correct classification was achieved for 9 out of the 10 compounds, at both time points. Currently, improvements are sought in the application of other
supervised clustering algorithms and a mechanistic approach.

PL

58 MULTI-OmICS CHARACTERIZATION OF THE ANTI-
PARKINSON'S DRUG TOLCAPONE AND ITS
POTENTIAL IN INDUCING IDIOSYNCRATIC LIVER
TOXICITY - COMPARED TO ENTACAPONE.

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The Liver Toxicity Biomarker Study (LTBS) is a public/private consortium created
to address the unmet need of predicting clinical idiosyncratic drug-induced liver injury (DILI) in the preclinical setting. The basic study design is to select multiple pairs of drugs with similar mechanisms of action where one contributed to cases of idiosyncratic liver injury in the clinical setting and the other has not — with both drugs being free of signs of liver toxicity in the preclinical environment. Drug pairs are evaluated in a 3-day and 28-day Sprague-Dawley rat model with multiple cross-
sectional modalities. The first paired compound analysis has been completed and the results will be presented — Entacapone (DILI) versus Entacapone (clean). The combined gene expression, proteomic, and metabolomic data analysis ap-
proach indicated induction of inflammation specific events triggered through LPS-
BP and IL-1 induced cascades. These LBP/IL-1 mediated effects result in induction
of nuclear hormone receptors for both Tolcapone and Entacapone. The complex downstream effects induce select CYP450 genes indicative of Tolcapone being a preferential PXR/ CAR activator (induction of Cyp3A4 [Tolcapone] > Entacapone) and OATP-2 distinct from Entacapone. In addition, Tolcapone down-regulated the expression of critical CYP450 genes, the products of which are required for metabolism and clearance of the compound. Thus, Tolcapone may exacerbate its own metabolism and clearance of the compound. These described mechanisms are consistent with idiosyncratic toxicity observed in the clinical trials of Tolcapone, but not for Entacapone. Interestingly, these overlapping and distinct drug characteristics were identified across all different omics’ profiles.

### 59 PROTEOME ANALYSIS OF LYMPH NODES IN A MOUSE MODEL OF CHEMICAL-INDUCED ASTHMA.
S. Haenen1,2, E. Clynen2, V. De Vooght1, P.H. Hoog1, L. Schoofs1 and J.A. VanOirbeek1.


Asthma is one of the most prevalent chronic airway diseases, characterized by reversible airway obstruction, airway inflammation, and non-specific airway hyperreactivity. Among up to 15% of all asthma cases in adults due to chemical exposures at the workplace. The early identification of biomarkers of sensitization could help to move diagnosis to an earlier stage. Using a mouse model of chemical-induced asthma we explore the process of sensitization towards chemicals by analyzing the protein level. On days 1 and 8, mice are treated on both ears with 0.3% toluene-2,4-diisocyante (TDI, 20μl), or the vehicle (2.3, acetone-oil). On day 15, mice are oropharyngeally challenged with 0.01% TDI or vehicle. On day 16, airway hyperreactivity to methacholine and BAL cell count are carried out. Two-dimensional differential gel electrophoresis (2D-DIGE) was used to analyze the differential expression of proteins in auricular lymph nodes of TDI-treated mice and vehicle-treated mice. Sensitized mice exhibit enhanced airway reactivity to methacholine (2.6 fold increase) compared to vehicle-treated mice, accompanied by a neutrophil infiltration (50%). Proteome analysis of the auricular lymph nodes illustrate differential expression of 52 proteins comparing TDI and vehicle-treated (control) mice (p<0.01). 36 proteins are found to be significantly upregulated in sensitized mice. 21 proteins could already be identified by peptide mass fingerprinting. Among the identified proteins structural proteins such as vimentin and immune related proteins, such as lymphocyte specific protein-1 involved in neutrophil activation, were found. This study demonstrates the use of 2D-DIGE in the search for (early) biomarkers of chemical-induced asthma. Further studies will have to point out whether (some of) these proteins could act as valuable biomarkers. Therefore, more samples (bronchoalveolar lavage fluid, serum,……) will be analyzed.

### 60 APPLICATION OF FTICR NON-TARGETED METABOLOMICS TO ASSESS THE PHARMACOLOGIC AND TOXICOLOGIC PROPERTIES OF PPAR ALPHA/Delta DUAL AGONIST, GSK610742X.
S. Ritchie1, W. Casey1 and E. Brigham2.

Safety Assessment, GlaxoSmithKline, Durham, NC and Phenomenex Discoveries Inc, Saskatoon, SK, Canada. Sponsor: P. Kwanuyen.

Being able to measure the metabolic and phenotypic effects of drugs is critical to understanding their pharmacological and toxicological modes of action. Recent advances in global metabolic profiling represent new opportunities to characterize the pharmacological effects of drugs on a system-wide basis. Since no prior knowledge about the effect of a compound is required, the data can point to metabolic pathways directly affected by the treatment, which can then be used to build a hypothesis about the mechanism of toxicological or pharmacological activity. This poster highlights the sensitivity of a global metabolic profiling technology based on accurate mass Fourier Transform Ion Cyclotron Resonance (FTICR) mass spectrometry for identifying tissue-specific metabolic responses of animals treated with PPAR alpha/delta dual agonist GSK610742X. High doses of GSK610742X have been associated with skeletal myopathy in rodents. The analysis of serum, adipose tissue, skeletal muscle, whole blood and liver revealed substantial changes in several different classes of lipids, including peroxisomally-derived vinyl-ether lipids, and early changes in bile acids. A dramatic increase in lipid peroxides, indicative of oxidative stress, was also observed. Although the liver and skeletal muscle were the sites anticipated to have the largest metabolic effects, changes in serum lipid composition was the most dramatically affected. It was also possible to track the metabolism of the drug in vivo without the need for isotope labelling. The structures of drug metabolites were identifiable due to the high accurate mass of the FTMS, making it possible to determine the relative tissue-specific deposition of the drug and its metabolites. In summary, the findings of this project provide new insight into tissue-specific aspects of PPAR physiology, as well as data supporting a mechanistic understanding of PPAR-mediated skeletal myopathy.

### 61 GENOMIC ANALYSIS OF SKELETAL MUSCLE INJURY INDUCED IN THE RAT BY CHRONIC EXPOSURE TO CHLOROQUINE.

DAPR, CIDER, FDA, Silver Spring, MD.

Phospholipidosis (PL) is a common finding in preclinical studies of pharmaceuticals but is of uncertain toxicological significance, which can delay drug development and regulatory review. Studies were initiated to examine the relationship between PL and toxicity using drugs from different therapeutic classes at doses that induce toxicity in rats. Chloroquine (CQ) is a drug commonly used with clinical myopathy during long-term therapy, was administered in the feed at 5 dose levels (0.03 to 0.15%) for 4 weeks to male Sprague Dawley rats. The incidence of PL in tissues was assessed by Nile Red fluorescence above 630 nm. CHQ induced a dose and time-dependent induction of PL in spleen and skeletal muscle. In type I skeletal muscle, CHQ-induced PL was seen primarily in samples that also displayed mild to moderate levels of necrosis and inflammation. Type II skeletal muscle was less sensitive to CHQ. Functional analysis of Affymetrix microarray data for soleus muscle revealed signs of all asthma cases of stress-induced genes and transcript levels for lysosomal and extracellular matrix proteins, while gene transcript levels for proteins involved in mitochondrial energy production were downregulated by CHQ treatment. Although CHQ did not limit body weight gain when administered at levels above 0.03% in the feed, the observed changes in expression in soleus muscle were of limited comparability to a gene profile associated with skeletal muscle atrophy caused by weight loss. The transcriptional response in soleus muscle to CHQ treatment is consistent with reports that CHQ induces an increase in lytic vesicles in skeletal muscle, which is thought to promote its effect on phospholipid accumulation.

### 62 MICROARRAY ANALYSIS OF CALCINEURIN INHIBITOR IMMUNOSUPPRESSANT-MEDIATED NEPHROTOXICITY IN THE RAT.
Y. Cai1, J. Auman1, Q. Huang2, S. Iyad1 and B.S. Pauls1.

UNC Eshelman School of Pharmacy, Chapel Hill, NC and Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT.

The use of calcineurin inhibitor (CI) immunosuppressants has revolutionized the clinical practice of organ transplantation. However, chronic nephrotoxicity limits its long-term utility. In order to understand the changes in gene expression that underlie the development of CI immunosuppressant-mediated nephrotoxicity, male SD rats were dosed daily with cyclosporine A (CsA) (2.5 and 25 mg/kg/day i.p.) or FK506 (0.6 and 6 mg/kg/day i.p.) for 1 to 28 days. Rats were also dosed with rapamycin (1 and 10 mg/kg/day i.p.), an immunosuppressant that does not elicit nephrotoxicity at therapeutic levels. Gene expression profiling was performed on RNA isolated from kidneys and blood 24 hours after 1, 7, 14 or 28 daily doses using the Affymetrix GeneChip Rat Genome 230 2.0 array. Our results indicate that repeated administration (14 and 28 days) of CsA (high dose) and FK506 affects similar biological processes in kidney. These processes are correlated with changes in kidney histopathology and kidney function. A gene expression signature, quantitatively correlated with kidney damage was identified and consists of 10 genes, including Calb1, Egl and Scl12a3. Interestingly, the transcriptional change of this signature has an earlier onset than the kidney injury biomarkers KiM1 and Clusterin, and is CI-specific. The down-regulation of Scl12a3 (thiazide-sensitive sodium-chloride cotransporter) in distal convoluted tubules was also verified by immunohistochemistry staining. Since mutation of SLC12A3 can cause salt wasting disease in humans, the inhibition of this gene may play an important role in the CI immunosuppressant-mediated nephrotoxicity. Furthermore, a gene expression signature in blood was also identified, which can separate animals with tubule necrosis caused by CsA from the non-injured animals. This suggests that blood transcription profiling may be useful as an indicator of kidney injury.

### 63 UTILIZING FUNCTIONAL GENOMICS IN YEAST TO DISCOVER NOVEL BIOMARKERS OF BENZENE TOXICITY IN HUMANS.
M. North1, A. Logainov2, V. Tandon2, B. Ko and C.D. Vinoge3.

Nutritional Sciences and Toxicology, The University of California Berkeley, Berkeley, CA.

The carcinogenic organic compound benzene is used extensively in industrial processes such as plastic production, and is also present in cigarette smoke and gasoline. In addition, there are thousands of benzene-contaminated sites in the...
USA alone. With a large workforce exposed to the compound, and the ever-increasing identification of benzene exposure-associated diseases, there is a mounting effort to identify biomarkers for susceptibility. Sarcocystis cerevisiae is the best-characterized simple eukaryotic organism, and has hundreds of tools available for genetic study. This includes a complete set of non-essential gene deletions, genetically tagged so that individual strains can be identified in competitive growth experiments. Using this deletion set, parallel systematic analysis can be completed to identify mutant strains that are susceptible to toxicant treatments. We have used this technique to establish the cellular processes targeted by three of the most well-studied benzene metabolites: hydroquinone, catechol, and 1,2,4-benzenetriol. Comparing the global deletion profiles of the metabolites revealed that deletion of certain genes renders sensitivity to all three compounds, for example the epimerase RPE1. This particular result indicates a requirement for NADH in metabolite detoxification; unsurprising since NADH is required for the function of oxidoreductases, which reduce the more toxic quinone forms of these metabolites (produced through autoxidation). Conversely, we also found that some mutations cause sensitivity to only one or two of the metabolites, for example int2, a negative regulator of Ras. Several of the genes identified have direct human homologues with conserved biological function, and many more have functional orthologs, supporting the notion that the mechanisms of toxicity identified are relevant to human disease. This study highlights S. cerevisiae as a simple but valuable model for identifying biomarkers for genetic susceptibility to toxicant-related disease.

**66 CERIUM OXIDE NANOPARTICLES INDUCE MAST CELL ACTIVATION, ALTER VASCULAR RELAXATION AND ARE ASSOCIATED WITH PRO-INFLAMMATORY CYTOKINE PRODUCTION.**

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Due to their unique physical and chemical characteristics, the use of nanoparticles has increased dramatically in recent years leading to a growing need for research examining the potential impact of nanoparticles on the environment and human health. Cerium oxide (CeO2) represents an important nanomaterial with wide-ranging applications; however, toxicity data for CeO2 is limited. Mast cells have long been recognized for their prominent role in allergic inflammations, asthma, and cardiovascular disease. These cells reside within tissues including the respiratory tract where they can interface with environmental particles. The aim of this study was to examine the activation of mast cells and the vascular response following exposure to CeO2. We examined the ability of CeO2 to activate human and murine mast cells as measured by degranulation, cytokine production and quantitative PCR arrays. Additionally, we assessed vascular reactivity and cytokine profiles in a mouse model instilled with CeO2. Mast cells were exposed to CeO2 (0-300 μg/mL) for 6-48 hrs and both IgE and non-IgE mediated mast cell activation was examined. CeO2 exposure resulted in minimal cytotoxicity as measured by LDH release and did not significantly affect mast cell degranulation. However, mast cell production of IL-8 and osteopontin was dose dependently increased following exposure to CeO2. Mice exposed to CeO2 exhibited reduced vascular relaxation to acetylcholine which was associated with an elevation in the cytokines IL-9 and IL-2. These findings demonstrate that CeO2 can direct mast cell production of pro-inflammatory mediators which could potentially contribute to impaired vascular relaxation and adverse health effects in vivo. This work supported in part by grant from Philip Morris USA/International to CJW.

**65 RESVERATROL INHIBITS IL-2-INDUCED TOXICITY WHILE MAINTAINING EFFECTIVE TREATMENT OF MELANOMA BY DIFFERENTIALLY MODULATING THE SUPPRESSIVE FUNCTIONS OF MYELOID-DERIVED SUPPRESSOR CELLS.**

H. Guan, P. S. Nagaraj and M. Nagaraj. Pathology, Microbiology and Immunology, University of South Carolina School of Medicine, Columbia, SC.

High-dose IL-2 therapy is an effective mode of treatment against melanomas. However, its use is limited by the occurrence of severe life-threatening toxicity including vascular leak syndrome (VLS). The precise mechanisms of VLS are unclear and there is no specific therapy to block VLS and enhance IL-2-based immunotherapy. In this study, we report that administration of resveratrol into IL-2-treated melanoma-bearing mice dramatically inhibited IL-2-induced VLS and enhanced IL-2 effectiveness. Resveratrol-treated mice showed decreased levels of metastatized tumor leading to increased survival when compared to the controls. Resveratrol administration prevented endothelial cell apoptosis and maintained endothelial integrity in interestingly, resveratrol did not affect the emigration of lymphocytes across the endothelium and into tumor site. We noticed that production of TNF-alpha in the serum dramatically decreased with resveratrol treatment. Furthermore, we found that resveratrol could induce proliferation of myeloid-derived suppressor cells (MDSCs). Amount of MDSC dramatically increased in the spleen, lung and liver in resveratrol-treated mice. Adoptive transfer of MDSC could prevent and block IL-2-induced VLS. We found the resveratrol-educated MDSC played a differential role on cytolytic killing of IL-2-activated killer T cells against endothelium and melanoma, which could be through the synchronized signaling from both cytokine and oxygen receptor. Our results demonstrate for the first time that resveratrol provides protection from IL-2-induced toxicity while maintaining IL-2 effectiveness by enhancing the suppressive function of myeloid-derived regulatory cells. (This research was supported in part by grants: NIH/A1053703, ES09098, A1058300, DA016545, HL058641 and P01AT003961.)
**PL 68** HIGH PHYSIOLOGICAL LEVELS OF THE STRESS HORMONE, CORTICOSTERONE (CORT), EXACERBATE BRAIN INFLAMMATORY RESPONSES TO LIPOPOLYSACCHARIDE (LPS) AND TO THE NERVE AGENT, DFP: ROLE IN GULF WAR ILLNESS?

J. P. O’Callaghan1, D. B. Miller2 and S. M. Lasley3, 2 "CDC-NIOSH, Morgantown, WV and 3University of Illinois College of Medicine, Peoria, IL.

Gulf War Illness (GWI) is a multi-symptom disorder with features characteristic of “sickness” behavior including, cognitive impairment, fatigue, depression, sleep disruption, GI and renal abnormalities, and hypertension. Typically, sickness behavior is an acute, maladaptive response to stress, with a return to homeostasis. However, in the environment of the Gulf War theater, agent exposures unique to this war, and a re-activation of neutrophils that secrete the serine protease, neutrophil elastase (EL). HX and EL resulted in plasma DON concentration of 20 ppm for 8 wk reduced both circulating IGF1 (12.5 mg/kg bw) upregulated in muscle, spleen and liver of DON-exposed mice. Additionally, EL treatment caused dose-dependent activation of caspase 3/7 that was not affected by HX coexposure. The injury caused by HX/EL was explored further in HepG2 cells. Intracellular reactive oxygen species production was significantly increased within 15 min exposure to HX/EL but required 180 min by HX alone. Phosphorylation of p38 (p-p38) was examined by immunocytochemistry. HX and EL each increased the number of p-p38 positive cells. No further increase was observed with cotreatment; however, there was selective nuclear translocation of p-p38 in HX/EL-exposed cells. These results indicate that HX/EL treatment is cytotoxic to HPCs and HepG2 cells and selectively increases early oxidative stress and p-p38 nuclear translocation. (Supported by RO1-ES004159 and T32-ES007255).

**PL 71** ROLE OF NO2S IN BLEOMYCIN-MEDIATED LUNG INJURY.

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Recent evidence has shown that NO2S, the inducible isomorph of nitric oxide synthase, is a critical mediator of acute pulmonary inflammation. Here, we examine the effect of NO2S inhibition upon inflammation resulting from intratracheally administered bleomycin. Mice were sacrificed 8 days post administration of 8 U/kg of bleomycin sulfate (bleo) or saline (control). Lungs, bronchoalveolar lavage (BAL), and cellular infiltrates were analyzed. One week prior to bleomycin administration, a mini-Airtrap was placed subcutaneously containing either saline (control) or 1400W, a potent specific inhibitor of NO2S. In bleo mice there was a significant increase in nitrites within the BAL, indicating increased NO2S activity. 1400W treatment abrogated this increase, showing that NO2S is responsible for the increase in nitrites. Bleomycin administration induced pulmonary inflammation evidenced by increased protein content and macrophage numbers within the BAL and the tissue inflammatory score. 1400W significantly reduced all of these indicators showing the importance of NO2S. As a measure of inflammatory signaling, we examined the ability of BAL from treated mice to induce chemotaxis in RAW cells. Bleo treated BAL produced a significant increase in chemotaxis over that from control. This increase was lost by treatment with 1400W. In addition, nitrosylated surfactant protein-D, another marker of inflammation, was increased in bleo treated BAL. This increase was inhibited by 1400W. We examined the level of chemokine expression by RT-PCR of BAL cells from bleo and control mice. GM-CSF was unaffected by bleo administration while IL-8 was induced, demonstrating the importance of neutrophil attraction in this model. Interestingly 1400W treatment significantly reduced IL-8 levels to below control. In contrast, TNF-α expression was increased in 1400W inhibited bleo treated mice. These observations indicate that NO2S inhibition reduces lung injury in response to bleomycin and appears to accelerate repair.

**PL 72** GENOMIC PROFILE OF RAT PLACENTA AFTER PHTHALATE EXPOSURE.

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Assessing potential endocrine disrupting effects of human phthalate exposure requires development of appropriate biomarkers. With the long-term goal of developing surrogate tissue phthalate biomarkers, the aim of this research was to identify phthalate-induced genomic changes in rat placenta. Sprague-Dawley rats (n = 5) were fed control diet or diet containing 1750 ppm (165 mg/kg/day) dibutyl-phthalate (DBP) for gestational days (GD) 1 through 21. At GD21, placental gene expression was analyzed using Illumina RatRef 12 expression chips. In the target tissue (fetal testis), DBP exposure significantly increased formation of multinucleated germ cells. DBP did not significantly affect litter size, pup weight, or pup anogenital distance. 492 placental genes showed a >2 fold change. Similar to testis, DBP exposure decreased placental steroidogenic acute regulatory protein (Star) gene expression (-1.18 fold; -2.39 z ratio), and Ingenuity-based pathway analysis of the placental gene set revealed a significant change in functional pathways associated with lipid metabolism, including metabolism of eicosanoids and cholesterol transport. However, transcript levels in DBP treated animals were similar to control. Although preliminary, these data demonstrate that phthalate exposure alters gene expression within rat placenta and suggest similar functional pathways are affected in both the phthalate target tissue (testis) and a potential phthalate surrogate biomarker tissue (placenta). Future experiments will...
Hedgehog (Hh) signaling plays an important role in prostate development and prostate cancer. The major goal of our research is to elucidate the molecular mechanisms by which Hh signaling regulates prostate growth. Here, we used both in vivo and in vitro methods to determine the paracrine and autocrine Hh effect on epithelial growth during prostate development. An immortalized mesenchymal cell line-U937-2 was transfected with activated form of Hh signaling intermediates Smoothened (Smo) and Gli2 to simulate an activated ligand independent Hh paracrine pathway. U937 cells isolated from both prenatatal and postnatal stages were co-cultured with U937-2 cells in explant culture plates for one week. Immunostaining was performed for BrdU and Pan-cytokeratin on the paraffin sections. The results showed that the paracrine Hh signals promote epithelial budding and proliferation at the stage of fetal initiation, while inhibit epithelial proliferation on the initiated buds. By co-culture strategy, we also achieved conditioned activated and conditioned null Hh signaling in mesenchyme layer. IHC staining on prostates from both prenatatal and postnatal stages showed consistent results in vitro explant co-culture experiment data. This suggests stage-specific Hh responsiveness during normal prostate development. For autocrine Hh signaling, we first detect its expression by X-gal staining using Ptc/LacZ and Gli1/LacZ mice. Then we also generated a mouse model that has activated or null autocrine Hh signaling by cre-lox mating system to observe its effect on progenitor cell proliferation. Our findings will introduce a novel paradigm into our efforts to understand normal growth regulation and offers a common mechanism both in vivo and in vitro.

**PL 73** TRADITIONAL CHINESE MEDICINE ON MALE GENITAL SYSTEM IN MICE EXPOSED TO DIESEL EXHAUST PARTICLES.

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Background: Spermatogenic development and activity has been reported to be affected by air pollution exposure. However, the mechanisms remain unclear. We hypothesize that exposure to diesel exhaust particles (DEP) affected sperm count and motility, and Radix Astragali (RA), a traditional Chinese herbal medicine, had protective effects on sperms in mice. Methods: Male Institute for Cancer Research, Swiss-derived (ICR) mice at age of 5 weeks were randomized into DEP (360 μg), DEP + RA, or control groups (vehicle, n = 6) via intratracheal exposure twice a week for 4 weeks. RA (20mg/kg) was delivered intraperitoneally daily coupled with DEP exposure. Epididymis, sperm count, motility and weights were analyzed. The ability to induce acrosome reaction in sperm of mice was compared to their ability to fertilize in vitro oocytes from counterpart females (non-exposed). The levels of IL-1β and IL-1β in the sera were also examined by specific ELISA and real time PCR. Results: Epididymis weight and its ratio (epididymis weight/ body weight) were significantly increased in the DEP group than in the control group. Sperm count, motility, and in vitro fertilization rate were significantly decreased in the DEP group compared with the control group. Testicular IL-1β and IL-1β levels were significantly higher in the exposed mice compared with the control mice. There were no significant changes about epididymis, sperm count and in vitro acrosome reaction in the RA treated mice compared with the control group. Testis weight and ratio (testis weight/body weight) showed no significant difference among the three groups.

Conclusion: Short-term exposure to diesel air pollution decreases sperm count and motility, and significantly reduced the capacity of the sperms to fertilize females. A traditional Chinese medicinal herb, Radix Astragali, has protective effects on sperm.

**PL 74** HEDGEHOG SIGNALING REGULATES PROSTATE DEVELOPMENT: INTERPLAY OF PARACRINE AND AUTOCRINE MECHANISM.

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Hedgehog (Hh) signaling plays an important role in prostate development and prostate cancer. The major goal of our research is to elucidate the molecular mechanisms by which Hh signaling regulates prostate growth. Here, we used both in vivo and in vitro methods to determine the paracrine and autocrine Hh effect on epithelium growth during prostate development. An immortalized mesenchymal cell line-U937-2 was transfected with activated form of Hh signaling intermediates Smoothened (Smo) and Gli2 to simulate an activated ligand independent Hh paracrine pathway. U937 cells isolated from both prenatatal and postnatal stages were co-cultured with U937-2 cells in explant culture plates for one week. Immunostaining was performed for BrdU and Pan-cytokeratin on the paraffin sections. The results showed that the paracrine Hh signals promote epithelial budding and proliferation at the stage of fetal initiation, while inhibit epithelial proliferation on the initiated buds. By co-culture strategy, we also achieved conditioned activated and conditioned null Hh signaling in mesenchyme layer. IHC staining on prostates from both prenatatal and postnatal stages showed consistent results in vitro explant co-culture experiment data. This suggests stage-specific Hh responsiveness during normal prostate development. For autocrine Hh signaling, we first detect its expression by X-gal staining using Ptc/LacZ and Gli1/LacZ mice. Then we also generated a mouse model that has activated or null autocrine Hh signaling by cre-lox mating system to observe its effect on progenitor cell proliferation. Our findings will introduce a novel paradigm into our efforts to understand normal growth regulation and offers important insights into the role of Hh signaling in prostate cancer.

**PL 75** MIXTURE OF IPRIDONE AND VINCOLOZIN DELAYS THE ONSET OF PUBERTY IN THE MALE RAT IN A CUMULATIVE MANNER.

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Vincolozin and iprodione are dicarboximide fungicides that display anti-androgenic effects in the male rat, which suggests co-exposure to these fungicides would lead to cumulative effects on androgen-sensitive endpoints. In order to test for cumulative effects by iprodione and vincolozin, we examined the effects of a mixture of these fungicides on pubertal development in the male rats. The mixture combined a dose of iprodione that does not significantly affect androgen-dependent morphological endpoints, with vincolozin (a 5×2 factorial design). Sprague-Dawley rats were dosed by gavage with effective vincolozin doses (0, 10, 30, 60, and 100 mg/kg/d) with and without 50 mg iprodione/kg/d from postnatal day (PND) 23 to 55-57 (n = 8/group). Onset of puberty (progression of prepubal separation (PPS)) was measured starting on PND 37. Organ weights, serum hormones, and ex vivo testis steroid hormone production under stimulation with chorionic gonadotropin (hCG) and unstimulated (hCG) conditions were measured at necropsy. Vincolozin alone delayed pubertal progression, reduced androgen sensitive organ weights, and increased serum testosterone levels as previously reported. The addition of 50 mg iprodione enhanced the vincolozin-induced delay in PPS (PND 47.5 vs 49.1; 2-way ANOVA: iprodione main effect p = 0.0002) and reduction of several reproductive organ weights. Furthermore, the increase in serum testosterone by vincolozin, due to its AR antagonism, was reduced by the addition of iprodione (a steroidogenic inhibitor) in vivo, but not ex vivo. These results demonstrate these fungicides interact when co-administered to delay pubertal development (i.e. PPS) in the male rat in a cumulative, antiandrogenic manner. CRD and CVR were supported through the USEPA-NIEHS Cooperative Training Agreement. This abstract does not necessarily reflect US EPA policy.

**PL 76** CYCLOPHOSPHAMIDE AND ITS ACTIVE METABOLITE PHOSPHORAMIDE MUSTARD CAUSE OVARIAN FOLLICLE LOSS AND DNA DAMAGE IN VIVO AND IN VITRO.

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Cyclophosphamide (CPA) is an ovotoxic drug frequently used to treat multiple types of cancers. It is activated by cytochrome P450 enzymes to form phosphoramidate mustard (PM) in vivo. PM is the active antitumor agent, but it also causes ovarian follicle destruction. We have shown that exposure of cultured rodent neonatal ovaries to PM in vitro cause concentration-dependent ovarian follicle loss and DNA damage at 24h. The goal of this study is to compare the effects of CPA in vivo with the in vitro effects of PM on mammalian oocytes. Single exposures to CPA or PM were carried out in vivo in adult mice and in a post-natal day 4 (PND4) ovarian culture system, respectively, to determine if oocytes suffer DNA double-strand breaks (DSBs). CPA ovaries from CD-1 mice were exposed after 4 days of PM (0.3-μM). Following 8 days of culture, ovaries were fixed for follicle counts or for fluorescent immunohistochemistry using a phosphorylated histone H2AX (γ-H2AX), a marker of DNA DSBs. In culture, PM caused significant loss of primordial (PM) and small primary follicles (PM1.5M), relative to control. Under the same conditions, numbers of follicles with a5 γ-H2AX foci/oocyte were increased. CD-1 mice injected with a single dose of CPA (75-250μg/kg) were sacrificed 1 or 8 days later. Compared to control, kidney and body weight did not change, whereas spleen weight decreased significantly at day 1 and increased at 8 days (250μg/kg), and liver weight significantly increased at 8 days after CPA exposures (250μg/kg). Primordial follicles were significantly decreased in CPA-exposed mice at both time points, while antral follicles were significantly decreased only at 24h (250μg/kg). γ-H2AX staining was observed in apopotic granulosa cells of antral follicles and certain oocytes of small follicles, but individual foci were difficult to identify. Data indicate that DSBs can persist for at least 8 days in oocytes in cultured ovaries, and that such damage also occurs in vivo, suggesting a common mechanism both in vivo and in vitro.

**PL 77** DIET-INDUCED OBESITY IN MALE MICE ASSOCIATED WITH REDUCED FERTILITY AND POTENTIATION OF ACRYLAMIDE-INDUCED GERM CELL TOXICITY.

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Over the past 20 years obesity trends have aggressively increased within the US with 60% of the states reporting prevalence of obesity ≥ 25%. Associated with elevated BMI, human risks for the development of chronic diseases: type 2 diabetes, infertility, cardiovascular diseases and cancer have also risen. Recently studies have suggested an association between obesity and male infertility. Current studies were designed to address the hypothesis that diet-induced obesity is associated with increased male infertility and obese male mice are more susceptible to reproductive toxicity of environmental chemicals such as acrylamide (AA). Genetically intact male C57Bl/6j mice were fed either a control or high fat diet (60% kcal fat) from 5 to 30 weeks of age. At age 30 weeks, mice fed high fat diet exhibited significantly increased levels of plasma testosterone, body weight, percent body fat, testicular volume, epididymal weight and decreased percent sperm motility, and significantly reduced the capacity of the sperms to fertilize females. A traditional Chinese medicinal herb, Radix Astragali, has protective effects on sperm.
higher body weight and % body fat. Fertility of oocyte and control mice was compared by mating with untreated female mice. In late gestation females were sacrificed and uterine content examined. Significant increase in the number of placentas and pregnancies were evident in females mated to oocytes vs. controls. To assess the reproductive effects of AA, 30 week oocyte or control mice were gavaged treated with tap water or 25 mg AA/kg/day for 5 days and mated to untreated females. AA exposure exacerbated the decrease in number of placentas and pregnancies in females mated to oocyte or control mice. The effect of AA was more striking in females mated to oocytes, as % of live fetuses decreased and resorptions (%) further increased. In conclusion, diet-induced obesity led to significant reduction in male fertility and exacerbated AA-induced germ cell mutagenicity. This model proved to be valuable in studying the impact of obesity on the susceptibility to environmental chemicals and may be used to study the link between obesity and chronic disorders in humans.

**80 IN UTERO TREATMENT WITH THE HERBICIDE LINURON PRODUCES MALE RAT REPRODUCTIVE MALFORMATIONS SIMILAR TO THE PHTHALATE ESTERS BUT THROUGH A DIFFERENT MECHANISM OF ACTION.**


Although linuron has been reported to act as an androgen receptor (AR) antagonist, the suite of malformations observed in male rat offspring after in utero exposure differs from that of other AR antagonists and more closely resembles that produced by phthalate esters (PE) such as di-2-ethylhexyl phthalate and diethylhexyl phthalate. However, only the PEs induce malformations of the gubernacular ligament. Many of the malformations in male rat offspring produced by PEs have been attributed to decreased fetal testosterone synthesis due to lowered steroidogenic gene expression and decreased 3α gene expression from the fetal Leydig cell; 3α being critical for proper gubernaculal ligament development. The current study investigated the dose response effects of in utero linuron treatment on fetal tesis gene expression and testosterone production. Pregnant Sprague Dawley rats were administered corn oil or 12.5, 25, 50 or 75 mg linuron/kg/day orally from gestation day (G)13-18. Fetal testes were collected on G18 for ex vivo testosterone (T) production and gene expression. Ex vivo T, but not progesterone production, was significantly decreased by 50 and 75 mg linuron/kg/day. Unlike the phthalate esters, linuron treated controls did not affect 3α, cyp17α, cyp11β and SAB mRNA expression. In a second study, control GD18 fetal testes were incubated with increasing concentrations of linuron (1 to 500 μM) to evaluate if linuron inhibited T production in vitro. T production was significantly reduced at 30 μM and above whereas progesterone production was not reduced at any concentration indicating that linuron directly inhibited testosterone production in the absence of cytotoxicity. Taken together, these data demonstrate that even though the profile of malformations produced by linuron resembles, in part, that of phthalate esters, the mechanisms of action of linuron clearly differs from the phthalate esters. Disclaimer: Does not necessarily reflect US EPA policy.

**78 THE ABILITY OF THE ARYL HYDROCARBON RECEPTOR TO REGULATE OVARIAN FOLLICLE GROWTH AND OVULATION MAY DEPEND ON SEXUAL MATURITY IN MICE.**

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The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor known for mediating the toxicity of polychlorinated biphenyls and dioxins. However, the endogenous roles of the AHR are still under investigation. We previously have shown that young Ahr-deficient mice (AHRKO) have decreased numbers of antral follicles and corpora lutea (CL) compared to young wild-type mice (WT) on postnatal day (PD) 54. In addition, AHRKO cultured follicles have decreased follicle growth and estradiol (E2) production compared to WT cultured follicles on PD 32. These previous data suggest that the AHR may regulate follicle growth and ovulation in young mice. The current study evaluated the role of the AHR in follicle growth, steroidogenic capacity and ovulation in cycling adult mice. Ovaries were collected from WT and AHRKO adult mice (PD 90) and subjected to morphological evaluation of antral follicle and CL numbers. Growth of antral follicles (PD 90) was evaluated in culture by measuring follicle diameters at 168 h. Media samples from cultured follicles were collected at 72 h and 168 h, and subjected to measurements of androstenedione (ASD) and E2 levels. Interestingly, WT and AHRKO ovaries contained similar numbers of antral follicles (WT=167±9.3; AHRKO=160±9.25; p=0.56) and CL. (WT=49±9.9; AHRKO=74±2.7; p=0.35). Further, isolated antral follicles from WT and AHRKO ovaries grew to similar sizes by 168 h of culture (WT=531±17.2 μm; AHRKO=530±18.4 μm; n=3 separate cultures; p=0.09). E2 levels produced by WT and AHRKO follicles were similar at 72 h and at 168 h. ASD levels produced by WT and AHRKO follicles were similar at 72 h, but AHRKO follicles produced 1.5-fold higher levels of ASD compared to WT follicles at 168 h of culture (WT=1.9±0.2 mg/ml; AHRKO=2.9±0.2 mg/ml; p=0.04). Collectively, these data suggest that the reduced follicle numbers and ovulations present in young AHRKO mice are restored to normal levels in cycling adult AHRKO mice, possibly by compensation from increased levels of theca cell-derived hormones. Support: NIH HD047275.
82  TRANSCRIPT DIVERSITY OF ARYL HYDROCARBON RECEPTOR 2 (AHR2) IN SHARKS.


Aryl hydrocarbon receptors (AHR) are intracellular proteins that bind environmental chemicals, including 2,3,7,8-tetrachlorodibenzo-p-dioxin and related structures. Upon activation, conformational changes promote AHR nuclear translocation and subsequent transcriptional regulation of genes involved in cell physiology. *Squalus acanthias* (spiny dogfish shark) express more than 3 distinct AHR genes encoding well-conserved amino acid sequence in the bHLH and PAS domains and divergent sequence in the carboxyl-terminal half of the protein. Shark AHR2 is unique because it contains an octapeptide repeat motif (OPM) domain in the carboxyl-terminal region, which is not present in other vertebrate AHR2 orthologues. To investigate the structure and function of the OPM domain, we used RT-PCR to amplify the entire OPM domain from multiple tissues of three sharks, and sequenced the OPM domain of genomic DNA and cDNA clones containing *S. acanthias* AHR2. We identified at least 6 AHR2 isoforms from cDNA and additional sequence diversity in the genome. The number of octapeptide repeats in the OPM varies from 27 to 55 and three or more isoforms are expressed in individual sharks; however, there is no clear tissue-specific expression pattern. AHR2s are activated by ligand and supports expression of a dioxin response element-regulated luciferase reporter in transient transfection experiments. We are currently evaluating ligand activation and transcriptional activity of the other AHR2 isoforms. While it is unclear whether these AHR2 isoforms represent different genes, alternative splicing of a single AHR2 gene, or another mechanism promoting genetic variability, our studies will clarify the role for AHR2s in responses to environmental chemicals at cellular and genetic levels in cartilaginous fishes and provide additional information about the endogenous function of the AHR signaling pathway in vertebrates. Research supported by RI-INBRE Grant # P20 RR-016457 and ME-INBRE Grant # P20 RR-016463 from NCRR, NIH, and NIEHS Center for Membrane Toxicity Studies Grant # P30 ES058328-20 at the Mount Desert Island Biological Laboratory.

83  TOXICITY OF TCDD IN NOVEL MOUSE LINES EXPRESSING RAT AHR RECEPTOR VARIANTS.

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Han/Wistar (Kuopio) rats are exceptionally resistant to the acute toxicity of TCDD (LDD50 >10 mg/kg). Their resistance has been shown to be mainly due to a species difference in the AHR receptor (AHR) resulting in the presence of two AHR proteins in these animals, a deletion (DEL) and an insertion (INS) variant (the predominant form), both of which are restructured at the transcriptional domain in the C-terminal end of the AHR. Recently, novel mouse lines were generated on the C57BL/6 background which express globally rat wild-type (rWT), DEL variant or INS variant AHR (manuscript submitted). They proved to have no major phenotypic changes apart from increased testis size, and their AHRs were shown to be functional both physiologically and as mediators for TCDD. The lines exhibited clear sensitivity differences to acute toxicity of TCDD with rWT mice being highly sensitive, DEL mice moderately resistant and INS mice highly resistant. Here, the lines were further characterized by subjecting them to a time-course study (1, 3 and 6 days after 500 μg/kg TCDD, females) and to a gene-response study (12% and 100% TCDD; 4 days; males) with TCDD. In both studies, rWT mice displayed substantial body weight loss (14 or >20% by 6 or 4 days for females and males, respectively). In DEL mice, body weight decline was more moderate (4% and 10%). Strikingly, INS mice gained weight (6 and 1%) with the rate of change being significantly higher than in INS mice. The same was true of liver enlargement which reached significance already on day-1 in rWT mice but only on day-6 in INS mice. Histologically, liver was much more severely affected in rWT than in INS mice. These mouse lines thus represent a promising new tool for dioxin and AHR research.

84  XENOBIOTICS-MEDIATED TRANSLOCATION OF ADENOVIRUS-DRIVEN CONSTITUTIVE ANDROSTANE RECEPTOR IN HUMAN PRIMARY HEPATOCYTES.

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The constitutive androstane receptor (CAR; NR1H3) is an important xenobiotic-sensing transcription factor that controls the expression of numerous hepatic genes involved in the detoxification of xenobiotics. In primary hepatocytes and intact liver, CAR resides in the cytoplasm under basal conditions and translocates to the nucleus upon exposure to inducers. However, human (h) CAR spontaneously accumulates in the nucleus of immortalized cell lines and exhibits constitutive activation in the absence of activators, which make the identification of CAR activators extremely challenging. Here, we have established an adenovirus-driven fluorescent protein-tagged hCAR expression system (Ad/EYFP-hCAR) in human primary hepatocytes (HPPH). Our results demonstrated that Ad/EYFP-hCAR infected HPH with high efficiency, and the majority of the Ad/EYFP-hCAR (>90%) expressed in the cytoplasm of HPH without treatment, while translocated to the nucleus in response to chemical activators. Furthermore, 22 compounds including known hCAR activators, deactivators, typical activators of other nuclear receptors, as well as rodent CYP2B inducers lack of CAR activation information were evaluated in this system. In general, xenobiotics-mediated hCAR translocation in HPH significantly correlated with hCAR activation and target gene induction. Comparing with cell-based reporter assay in immortalized cells and nuclear translocation of hCAR in Car-/- mice, the established Ad/EYFP-hCAR translocation assay in HPH exhibits obvious advantages such as responding to both direct and indirect activators, and the potential for a high-throughput manner. Thus, nuclear translocation of Ad/EYFP-hCAR in HPH represents an efficient means for in vivo prediction of xenobiota-mediated human CAR activation.

85  CELL CYCLE WITHDRAWAL INCREASES LIGAND-MEDIATED CYP1A1 EXPRESSION IN HUMAN KERATINOCYTES.

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Ligand-mediated induction of genes regulated by the aryl hydrocarbon receptor (AHR) reaches a maximum in confluent keratinocytes and is associated with the differentiation process. An early event in keratinocyte differentiation is cell-cycle withdrawal. Studies were carried out to determine if cell cycle withdrawal, independent of differentiation, triggered the increased level of AHR-regulated genes. Preconfluent normal human keratinocytes were pretreated with chemicals that induce cell cycle withdrawal at G0/G1, followed by treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and CYP1A1 mRNA analysis. mRNA levels of an early marker of differentiation, keratin 1, did not increase, indicating that cell cycle withdrawal occurred independent of differentiation. Pretreatment with TGFβ1, rapamycin or 8-Cl-adenosine increased TCDD-mediated CYP1A1 mRNA induction 2-, 3- and 20-fold, respectively, over the control samples (TCDD treatment only) demonstrating that cell cycle withdrawal increases AHR activation in human keratinocytes. Lower 8-Cl-adenosine concentrations that did not result in cell cycle withdrawal did not induce CYP1A1 mRNA, further supporting this association. Although cell cycle withdrawal increased TCDD-mediated induction of CYP1A1, the levels were still lower than TCDD-mediated induction in cells allowed to grow to confluence, indicating that additional density/differentiation-related signal are necessary for maximal AHR activation. As the chemicals used in this study have multiple effects (i.e. TGFβ is known to modulate AHR levels in some cell types), ongoing work includes measuring AHR levels and inducing cell cycle withdrawal through overexpression of cyclin-dependent kinase inhibitors.

86  ROLE OF MU-OPIOID RECEPTOR IN ANTINOICEPTION OF GABAPENTIN AND TRAMADOL.

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Rational combination of antinociceptive agents with different mechanisms of action can improve efficacy and reduce toxicity. Tramadol is an atypical opioid agonist, which has been proposed to produce antinociception via both opioid and non-opioid pathways, and gabapentin is an antinociceptive adjuvant with unknown mechanism of action. Previously, we demonstrated that gabapentin enhances antinociceptive action of and reduces tolerance to tramadol. The mechanism responsible for the synergy of gabapentin and tramadol remains unclear. The μ-OR receptor(μ-OR) plays a key role in the control of nociceptive pathways in the nervous system and is a major target for antinociceptive drugs. The present study is to determine whether the μ-OR is implicated in the antinociceptive synergy of gabapentin and tramadol. Experiments were conducted in adult male wild-type and μ-OR knockout mice. Gabapentin (75 mg/kg) and tramadol (60 mg/kg), alone or in combination, were administered to mice by gavage and intraperitoneal injection, respectively. The tail-flick response of mice was tested 60 min after drug administration. Antinociceptive responses in the tail-flick are expressed as the percentage of maximum possible effect (% MPE) at the time point at which the greatest antinociceptive responses were observed. The MPE values were 0% in wild-type...
mice and 16% in μ-OR knockout mice after gabapentin administration (P<0.05). Following tramadol injection, the MPE values were 46% and 5% in wild-type and μ-OR knockout mice, respectively (P<0.05). The MPE values were 70% in wild-type mice and 13% in μ-OR knockout mice with administration of the two drugs in combination (P<0.05). The result indicated that μ-OR is a major target for tramadol but not for gabapentin. The μ-OR is also implicated in the antinociceptive synergy of gabapentin and tramadol.

**87 DEVELOPMENT AND APPLICATION OF A RECOMBINANT HUMAN HEPG2 CELL BIOASSAY FOR IDENTIFYING AHR LIGANDS.**

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The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor that not only binds 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin) and related halogenated aromatic hydrocarbons, but also many structurally unrelated chemicals, and it is responsible for many of the toxic and biological effects of these compounds. Stable transfection of an AhR-responsive luciferase reporter gene into mouse, rat and guinea pig cell lines allowed development of recombinant cell lines for use in the detection and characterization of novel AhR ligands (agonists and antagonists). However, a human CALUX cell bioassay would be useful for comparative studies and would provide a more relevant assay to examine the activation of the human AhR by human therapeutic drugs. Accordingly, we have developed, characterized, and validated such a recombinant human hepatoma (HePG2) cell line (HG2L6.1c1). Luciferase induction in these cells occurred in a dose-, chemical-, and time-dependent manner with an EC50 for TCDD of 1.29 nM. Optimal temperature and time for induction of luciferase activity in HG2L6.1c1 cells were found to be 33°C and 48 hours. This bioassay was used to screen 147 commercially available human therapeutic chemicals and other compounds, and the results compared to data using the mouse, rat, and guinea pig cell lines. The induction profiles of the pharmaceutical drugs revealed that although HG2L6.1c1 cells were somewhat less sensitive than that of other species, they could be used to identify several known and unknown human AhR agonists. Interestingly, some chemicals, such as the anthracyclins (epirubicin, daunorubicin), cadmium chloride and sodium arsenite, did not stimulate AhR DNA binding, but induced AhR-dependent gene expression. The availability of the human CALUX cell line provides an avenue in which to identify and characterize novel human AhR agonists. Supported by NIEHS grants ES04699 and ES012498.

**88 TRPA1 IS A MAJOR OXIDANT SENSOR IN AIRWAY SENSORY NEURONS.**

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Sensory neurons in the airways are finely tuned to respond to reactive chemicals important in regulation of gene expression. Originally identified as the receptor for the aryl hydrocarbon receptor (AhR) is a basic helix-loop-helix transcription factor that not only binds 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin) and related halogenated aromatic hydrocarbons, but also many structurally unrelated chemicals, and it is responsible for many of the toxic and biological effects of these compounds. Stable transfection of an AhR-responsive luciferase reporter gene into mouse, rat and guinea pig cell lines allowed development of recombinant cell lines for use in the detection and characterization of novel AhR ligands (agonists and antagonists). However, a human CALUX cell bioassay would be useful for comparative studies and would provide a more relevant assay to examine the activation of the human AhR by human therapeutic drugs. Accordingly, we have developed, characterized, and validated such a recombinant human hepatoma (HePG2) cell line (HG2L6.1c1). Luciferase induction in these cells occurred in a dose-, chemical-, and time-dependent manner with an EC50 for TCDD of 1.29 nM. Optimal temperature and time for induction of luciferase activity in HG2L6.1c1 cells were found to be 33°C and 48 hours. This bioassay was used to screen 147 commercially available human therapeutic chemicals and other compounds, and the results compared to data using the mouse, rat, and guinea pig cell lines. The induction profiles of the pharmaceutical drugs revealed that although HG2L6.1c1 cells were somewhat less sensitive than that of other species, they could be used to identify several known and unknown human AhR agonists. Interestingly, some chemicals, such as the anthracyclins (epirubicin, daunorubicin), cadmium chloride and sodium arsenite, did not stimulate AhR DNA binding, but induced AhR-dependent gene expression. The availability of the human CALUX cell line provides an avenue in which to identify and characterize novel human AhR agonists. Supported by NIEHS grants ES04699 and ES012498.

**89 THE ARYL HYDROCARBON RECEPTOR (AHR) SIGNALING PATHWAY INTERACTIONS INFLUENCES AHR-MEDIATED CHANGES IN MATRIX METALLOPROTEINASE GENE EXPRESSION.**

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The aryl hydrocarbon receptor (AhR) is a basic helix-loop-helix transcription factor involved in regulation of gene expression. Originally identified as the receptor for environmental contaminants, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), it is now known to be activated by a variety of exogenous and endogenous compounds. Despite this, the endogenous function of this pathway remains unclear. We hypothesize that the AhR is a stress-activated pathway that functions to modulate normal cellular homeostasis involving the cellular decision to proliferate, undergo apoptosis, or migrate. Our laboratory has focused on this pathway's ability to alter cellular migration via regulation of matrix metalloproteinase (MMP) expression. MMPs are essential for tissue remodeling and cellular migration, and are inappropriately expressed in a variety of pathologies, such as rheumatoid arthritis and tumor metastasis. Our data show that AhR activation alters expression and activity of MMPs in human melanoma cells and in normal human keratinocytes, and that such changes in MMP expression are mediated through distinct mechanisms. We suggest that cell-type variations in the AhR response are influenced by three factors: expression of AhR, expression of AhR repressor (AhRR), and cross-talk with other signaling pathways. In support of this, we have found a correlation between the AhR/AhRR ratio with melanoma aggressiveness and invasiveness. We also show that interactions between the AhR and Ras/Raf signaling pathway are critical for AhR-mediated induction of MMPs in melanoma cells. Both chemical inhibition of Ras/Raf and siRNA knockdown of Braf in human A2058 melanoma cells inhibits TCDD-induced expression of MMP-1. Conversely, inhibition of AhR also decreases ERK phosphorylation. Taken together, these data imply that cross-talk between the Ras/Raf and AhR signaling pathways to alter MMP-1 production in A2058 melanoma cells.

**90 BOTH AHR AND UNLIGANDED ERα ATTENUATE CYTOKINE-MEDIATED INDUCTION OF IL-8.**

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Recent investigations have increasingly highlighted a role for the AhR in mediation of inflammatory responses. For example, we have recently shown that IL-1β signaling combined with activated AhR/ARNT heterodimer to synergistically induce IL-6 transcription in ER-positive ECC-1 endocervical cancer cells. Concurrently, it is well known that various nuclear receptors undergo crosstalk, with AhR and ERα being one example. Recently, we have found that crosstalk between unliganded ERα and AhR modulates IL-8 induction via a possible cytoplasmic interaction in ECC-1 cells. In the presence of the ER agonist estradiol, quantitative real time PCR shows that IL-1β induces a 500-fold increase of IL-8 mRNA over treatment with vehicle control. Removal of estradiol results in a 50% decrease in IL-8 transcription, while the presence or absence of the AhR agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has no effect on the IL-8 repression. Knockdown of either ERα or AhR through siRNA transfection rescues maximal IL-8 induction levels, even in the absence of estradiol. Interestingly, removal of either receptor also results in an approximate 6-fold increase of basal IL-8 transcription. siRNA knockdown of ARNT, which leads to a near complete ablation of TCDD-induced CYP1A1 induction, fails to prevent repression of IL-8 induction in the absence of estradiol; thus pointing to the involvement of AhR in a non-classical manner. Likewise, siRNA knockdown of ERβ also fails to prevent repression of IL-8. These results, combined with research having shown protein-protein interactions between ERα and AhR, suggest the presence of a multi-protein complex in which the AhR and unliganded ERα interact with transcription factors in the cytoplasm to modulate inflammatory responses.

**91 ANALYSIS OF AHR PROTEIN STABILITY IN A YEAST EXPRESSION MODEL.**

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The aryl hydrocarbon receptor (AHR) and the aryl hydrocarbon receptor nuclear translocator (ARNT) are well-characterized bHLH-PAS transcription factors involved in the response of organisms to certain environmental contaminants such as 2,3,6,8-tetrachlorodibenzo-p-dioxin (TCDD). Previous studies have demonstrated that the AHR is degraded following TCDD binding. To generate a model system useful to screen for proteins involved in TCDD-mediated AHR degradation, the cDNAs for mouse AHR and ARNT were stably integrated in the yeast genome. Initial studies of recombinant strains have shown that 1) both AHR and ARNT proteins can be detected by Western blotting, 2) treatment of the recombinant yeast strain with ligand induces a TCDD-responsive reporter gene and 3) the AHR protein is rapidly degraded in the yeast cells in a ligand-independent manner. Thus, the yeast has a functional AHR signaling system, but the rapid loss of the AHR in the absence of ligand has confounded the analysis TCDD-mediated degradation. To address whether the lack of AHR stability is due to improper folding of the AHR by the yeast chaperone proteins, the cDNA for mouse heat shock protein 90 was ligated into an expression vector immediately downstream of a constitutive promoter. The Hsp90 expression plasmid was then transformed into the recombinant strain and the stability of the AHR was directly evaluated by Western blotting and
immunotopicization. Another approach to assess protein turnover is to inhibit specific degradation pathways with chemical inhibitors. However, the yeast cell wall is impermeable to these compounds. To overcome this problem, the gene encoding the Erg9 cell wall protein was disrupted in the recombinant strain. This new strain was evaluated for AHR stability following treatment with various protease inhibitors in order to identify the pathway involved in the ligand-independent degradation of the AHR. Results suggest that folding of the AHR in yeast may be a critical step in the ligand-mediated degradation in this model system. Supported by NIEHS NIEHS515481.

**THE ARYL HYDROCARBON RECEPTOR (AHR) IS NOT REQUIRED FOR ESTROGEN-DEPENDENT TUMOR FORMATION BY MCF-7 BREAST CANCER CELLS.**

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The AHR was initially identified and characterized as a ligand-activated transcription factor mediating toxic effects of chlorinated dioxins and other halogenated and non-halogenated aromatic hydrocarbons. In recent publications, evidence supporting involvement of the AHR in cell-cycle regulation and tumorigenesis has been presented. To further define the roles of the AHR in cancer, we investigated the effects of AHR expression on cell proliferation, migration, and tumorigenicity of MCF-7 human breast cancer cells. In these studies, the properties of MCF-7 cells and two MCF-7-derived sublines, AHR100 cells, which express minimal AHR, and AHR2 cells, which overexpress AHR, were compared. Real-time PCR, Western immunoblots, experiments with CYP1A1- and CYP1B1-promoter-luciferase reporter constructs, and ethoxyresorufin O-deethylase assays showed the lack of AHR expression and AHR-regulated CYP1 expression in AHR100 cells, and the enhanced AHR expression and AHR-regulated CYP1 expression in AHR2 cells. In the presence of 1 μM estradiol, the proliferation rates of MCF-7, AHR100, and AHR2 cells were similar. However, AHR100 cells showed elevated rates of migration and invasion in chamber assays with 1 μM estradiol as the chemoattractant and enhanced colony formation in soft agar compared with those of MCF-7 and AHR2 cells. Furthermore, AHR 100 cells retained the ability to form tumors in severe combined immunodeficient mice when supplemented with estradiol, producing mean tumor volumes comparable to those from MCF-7 cells. In xenograft assays with AHR2 cells, decreased tumorigenesis, as evidenced by decreased tumor volumes in estrogen-supplemented mice compared with those from MCF-7, was observed. These studies indicate that invasion, migration, anchorage-independent growth, and estrogen-stimulated tumor formation of MCF-7 cells do not require the AHR, and tumor formation is inhibited by its overexpression. Supported by NIH grant CA081243.

**FUNCTIONAL ROLE OF VON-HIPPEL LINDAU TUMOR SUPPRESSOR GENE MUTATIONS IN ARNT-MEDIATED TRANSCRIPTIONAL REGULATION OF DRUG METABOLISM.**

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The von-Hippel Lindau (VHL) tumor suppressor gene is commonly mutated in sporadic renal cell carcinoma, and while it plays a critical role in regulating the cellular response to hypoxia, little is known about the functional consequences of VHL gene mutations in the context of susceptibility to chemical insult. Mutations in VHL can disrupt its targeting of hypoxia-inducible factor 1alpha (HIF1alpha) for degradation during normoxia. HIF1alpha and the arylhydrocarbon receptor (AhR) are both conditionally regulated basic helix-loop-helix transcription factors that share Per-Arnt-Sim homology domain for dimerization with the arylhydrocarbon nuclear translocator (ARNT). Consequently, we hypothesize that VHL status may determine susceptibility to chemical insult by altering xenobiotic induction, metabolism and ultimately detoxification. To investigate this we have generated Vhl +/-, Vhl +/-, Vhl +/-embryonic stem (ES) cells derived from C57BL/6j x BALB/c F1 blastocysts. In these studies, the properties of MCF-7 cells and two MCF-7-derived sublines, AHR100 cells, which express minimal AHR, and AHR2 cells, which overexpress AHR, were compared. Real-time PCR, Western immunoblots, experiments with CYP1A1- and CYP1B1-promoter-luciferase reporter constructs, and ethoxyresorufin O-deethylase assays showed the lack of AHR expression and AHR-regulated CYP1 expression in AHR100 cells, and the enhanced AHR expression and AHR-regulated CYP1 expression in AHR2 cells. In the presence of 1 μM estradiol, the proliferation rates of MCF-7, AHR100, and AHR2 cells were similar. However, AHR100 cells showed elevated rates of migration and invasion in chamber assays with 1 μM estradiol as the chemoattractant and enhanced colony formation in soft agar compared with those of MCF-7 and AHR2 cells. Furthermore, AHR 100 cells retained the ability to form tumors in severe combined immunodeficient mice when supplemented with estradiol, producing mean tumor volumes comparable to those from MCF-7 cells. In xenograft assays with AHR2 cells, decreased tumorigenesis, as evidenced by decreased tumor volumes in estrogen-supplemented mice compared with those from MCF-7, was observed. These studies indicate that invasion, migration, anchorage-independent growth, and estrogen-stimulated tumor formation of MCF-7 cells do not require the AHR, and tumor formation is inhibited by its overexpression. Supported by NIH grant CA081243.

**AMELIORATING EFFECT OF NOOTROPIC DRUG AMPAKINE CX-717 ON PRENATAL NICOTINE-INDUCED IMPAIRMENT IN SYNAPTIC AMPA RECEPTOR FUNCTION.**

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Maternal smoking causes severe cognitive dysfunction in younger children. Prenatal nicotine exposure impairs hippocampal dependent memory forms. Most of the excitatory neurotransmission in mammalian brain is mediated by glutamatergic AMPA receptors (AMPAR). We showed potent modulatory effect of nootropic drug Ampakine CX-717 on synaptosomal AMPAR activity. Therefore we hypothesized that AMPAR function and expression may be impaired in prenatal nicotine exposed rats and resulted impaired long-term potentiation and can be ameliorated by ampanke CX-717 treatment. Pregnant rats were treated with Nicotine from the day 3 of pregnancy until pups were born, via osmotic mini pumps implanted subcutaneously throughout gestation. Prenatal exposed animals showed spatial memory impairment in Y maze task, impaired basal synaptic transmission, significantly reduced LTD; reduced amplitude of AMPAR mediated whole cell synaptic currents and reduced activity of synaptosomal AMPAR single channel activity. Synaptosomes isolated from three groups of rats were exposed to 1 nM estradiol as the chemoattractant and enhanced colony formation in soft agar compared with those of MCF-7 and AHR2 cells. Therefore, CX-717 may be a potential therapeutic option for cognitive impairment associated with prenatal nicotine exposure that impairs AMPAR function.

**INVESTIGATION OF TRPV1 AND ER STRESS IN LUNG CELL DEATH IN VITRO AND ACUTE LUNG INJURY IN VIVO.**

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The capsaicin receptor, TRPV1, is activated by both exogenous (e.g., capsaicin) and endogenous (e.g., anandamide, H2S, LTB4) ligands. In human lung cells, plasma membrane and endoplasmic reticulum (ER) TRPV1 differentially regulate inflammatory cytokine gene expression and cell death after TRPV1 activation. Calcium efflux from the ER initiates the unfolded protein/ER stress response and cell death via EIF2alpha activation and increased expression of pro-apoptotic DNA damage-inducible transcript (3) (DDIT3, CHOP or GADD153). Structural modifications to the 3-MeO-4-OH-benzylamide pharmacophore of capsaicinoids decreased TRPV1 activation and ER stress-induced cell death in lung cells. The endogenous TRPV1 agonist anandamide also induced ER stress and lung cell death in vitro. Endovanilloids including anandamide damage the lung during inflammation. Treatment of mice i.p. with LPS induced systemic inflammation and endovanilloid synthesis and caused lung injury in mice in a TRPV1- and ER stress-dependent manner. TRPV1 knockout mice and mice treated with TRPV1 antagonist LJO-328 were protected from lung injury and increased expression of GADD153 and IL-6 were not observed. Because TRPV1 contributes to lung injury during experimental endotoxemia, TRPV1 may be a novel target for prevention of lung injury during pathological systemic inflammation due to sepsis. This work was supported by NIH grant (HL069813).

**GH-MEDIATED REGULATION OF STEAROYL-COA DESATURASE 1.**

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3,5,7-tetrachlorodibenzo-p-dioxin (TCDD) is a potent, environmentally persistent contaminant that modulates gene expression through aryl-hydrocarbon receptor (AhR) mediated interactions with dioxin response elements (DREs) in the reg-
ulotary regions of genes. Recently, we identified species-specific gene expression changes associated with lipid metabolism that may be mediated through species-specific DREs in ovariectomized Sprague Dawley rats and C57BL/6 mice gavaged with 10 or 30 µg/kg TCDD, respectively. Rats experienced specific decreases in body weight gain while mice exhibited steatosis and steatohepatitis at 72 and 168 h, respectively. Gas chromatography/mass spectrometry (GC-MS) hepatic lipid profiling revealed a significant increase in monounsaturated fatty acids (MUFA) in treated versus control mice. Of particular interest was the increase in oleate (C18:1 n-9; 1.5 x 24 h, 2.0 x at 72 h), the MUFAs Stearoyl-Co-A desaturase (Scd1) converts palmitate (16:0) and stearate (18:0) to the MUFAs palmitoleate (16:1 n-7) and oleate (18:1 n-9) respectively. Published Scd1 null mice studies implicate Scd1 in obesity, non-alcoholic fatty liver disease and the metabolic syndrome. qRT-PCR confirmed species-specific induction of Scd1 in rat liver, but not in mouse liver. Non-conserved putative DREs in the regulatory region of the human, mouse and rat Scd1 gene were computationally identified using a position weight matrix. Reporter gene assays containing regions with these putative DREs indicated that the inducible promoter (AhRR) and histone deacetylases (HDACs) are reported to play a significant role in suppressing the transcriptional activity of AhR. In this study, we investigated the mechanism of tissue-specific modulation of AhR-dependent gene expression by focusing on the functions of AhRAR and HDACs. The level of CYP1A1 induction was about 600-fold larger in the liver than in the spleen. On the other hand, the level of CYP1A1 induction was about 600-fold larger in the liver than in the spleen. Thus, the level of CYP1A1 induced by the same amount of TCDD was about 12-fold larger in the liver than in the spleen. Time-course study showed that CYP1A1 was markedly induced at 6 h in the liver and increased until 48 h. In the spleen, however, it was weakly induced at 6 h, peaked at 12 h, and decreased thereafter. In consistent with the weak induction of CYP1A1 in the spleen, AhR was not induced at 6 h in the spleen and did not decrease until 48 h. The liver showed only a weak AhR induction. Chromatin immunoprecipitation assay showed that HDAC3 was recruited to the CYP1A1 promoter in the liver upon TCDD exposure. However, the binding was decreased immediately after 12 h. These results indicate that AhR is strongly induced in the spleen and attributes to the weak induction of CYP1A1 in the spleen compared to the liver.

98 ACTIVATED ARYL HYDROCARBON RECEPTOR MODULATES THE GENOMIC BINDING PROFILE OF ESTROGEN RECEPTOR ALPHA.

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In this study, chromatin immunoprecipitation followed by microarray technology (ChIP-chip) was used to compare the genomic binding profiles of the aryl hydrocarbon receptor (AhR) and estrogen receptor alpha (ERα). Chromatin was isolated from asynchronous 1-T47D human breast cancer cells treated with 10 nM 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) for 1h. Protein-chromatin complexes were isolated using specific antibodies against AhR and ERα. Immunoprecipitated DNA was linearly amplified and hybridized to Affymetrix® human promoter 1.0R tiling arrays that contain 25,500 promoter regions. Enriched regions were identified using CisGenome® and TileMap software set at a 1% false detection rate. Of the 905 ERα identified regions 214 overlapped (>50 % sequence identity) with the 1191 AhR identified regions following TCDD treatment. Conventional ChIPs confirmed the recruitment of AhR and ERα to 36 of the identified regions. Sequential ChIPs showed that both AhR and ERα are recruited at the same time to all of the identified regions we examined. The regions were mapped to the closest genes and qPCR was used to determine TCDD-dependent changes in mRNA levels. We identified mRNAs that were increased, decreased or unchanged following TCDD treatment. Computational analysis of the shared regions (bound by both AhR and ERα) revealed that there was an over-representation of AhRs and not ERE suggesting that AhR drives the recruitment of ERα. mRNA-mediated knockdown of AhR confirmed its requirement for the recruitment of ERα to the shared regions. However, knockdown of ERα did not affect the recruitment of AhR to the regions tested. Overall, AhR modulates the genomic binding patterns of ERα following the addition of the AhR selective agonist TCDD. Our findings confirm the strong functional overlap between ERα and AhR signaling pathways and that activation of AhR influences ERα activity.

99 DIFFERENTIAL GENE REGULATION BY THE HUMAN AHRR IN TRANSGENIC HUMANIZED MOUSE HEPATOCYTES.

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The human and mouse aryl hydrocarbon receptor (hAhRR and mAhRR) share limited (58%) transcriptional activation domain (TAD) sequence identity and display distinct ligand affinity with the hAhR having 10-fold lower affinity compared to prototypical AhR ligands such as 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD). In addition the hAhR and mAhR also display functional differences. Previous studies have shown that the mAhR and hAhR can differentially recruit LXXLL co-activator-motif proteins and utilize different TAD subdomains in gene transactivation. We decided to investigate whether the hAhR and mAhR differentially regulate gene expression in response to TCDD. Hepatocytes were isolated from wild type C57BL/6j and hepatocyte-specific AhR transgenic mice (B6.Cg-AhRtm3.Bta Tg (Alb-cre, Trp5-H2R(1Gip)) and treated with 10nM TCDD or vehicle for 6h. Microarray and quantitative PCR analysis of TCDD-treated hepatocytes revealed that a number of classical AhR target genes such as Cyp1a1, Cyp1b1, Ahrr and Nqo1 are significantly activated by both receptors in response to TCDD. Hepatocytes were isolated from wild type C57BL/6j and hepatocyte-specific AhR transgenic mice (B6.Cg-AhRtm3.Bta Tg (Alb-cre, Trp5-H2R(1Gip)) and treated with 10nM TCDD or vehicle for 6h. Microarray and quantitative PCR analysis of TCDD-treated hepatocytes revealed that a number of classical AhR target genes such as Cyp1a1, Cyp1b1, Ahrr and Nqo1 are significantly activated by both receptors in response to TCDD. Conversely of the 782 and 1103 genes significantly repressed in hAhR and mAhR hepatocytes respectively, only 463 (<25%) genes were significantly repressed by both receptors in response to TCDD treatment. Genes identified as differentially expressed are known to be involved in a number of biological pathways, including cell proliferation (e.g. Bcl2, Pkrar2B, Cav2), and inflammatory response (e.g. Il-10, Ifil). Limited overlap in the genes regulated by the mAhR and hAhR suggests that the hAhR may differentially modulate gene expression. Furthermore this discovery suggests that compared to the mAhR, the hAhR may play a contrasting role in TCDD toxicity and thus highlights the limitations of traditional TCDD toxicity studies in rodent models and how these studies relate to human risk. (Supported by grants from NIH ES04869 and Dow Chemical Company).

100 TCDD DOWNREGULATES SOX9B mRNA EXPRESSION IN ZEBRAFISH JAW EXPLANTS.

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) causes craniofacial malformation in developing zebrafish embryos that includes dysmorphology of the upper and lower jaw. Overt TCDD toxicity that includes craniofacial malformation is mediated by AhR2 signaling in zebrafish embryos. Reduced sox9b expression is tantamount to TCDD-induced impairment of jaw development (Xiong KM et al 2008). Sox9b encodes a transcription factor essential for chondrogenesis. However, it is unclear how sox9b expression is regulated. We hypothesize that TCDD reduces sox9b mRNA expression by AhR2-mediated inhibition of a sox9b activator or induction of a sox9b repressor. To test this hypothesis we developed a larval zebrafish jaw explant model. The jaw was microdissected from 5 dpf larva and grown in organ culture containing 10 nM TCDD or vehicle for 15, 30, and 45 min. Immunofluorescence microscopy of explanted jaws showed a decrease in BrdU staining with 10 nM TCDD compared to control. The decrease in BrdU staining was accompanied by a decrease in sox9b expression. These results indicate that AhR is strongly induced in the spleen and attributes to the weak induction of CYP1A1 in the spleen compared to the liver.
Importantly, reduced sox9b mRNA expression was identified in TCDD-treated jaw explants. The reduction of sox9b mRNA in jaw explants is similar to the magnitude of reduction in jaws from in vivo TCDD-treated larvae. Experiments using this jaw explant system to identify cis regulatory elements in the sox9b gene are described.

**101 MECHANISMS OF INTESTINAL BCRP INDUCTION BY 3MC: EVIDENCE FOR AHR SIGNALING PATHWAY.**

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The gut and small intestine are the primary sites of absorption for nutrients and orally-administered drugs. Breast cancer resistance protein (BCRP, ABCG2), a novel efflux transporter, is responsible for the efflux of endobiotics and xenobiotics and regulates the bioavailability of substrate drugs. Initially identified as the mediator of chemotherapeutic resistance in breast cancer tumors, BCRP expression has since been detected in normal tissues of the colon, small intestine, kidney, liver, and barrier structures such as the placenta and the blood-brain barrier. In the small intestine, several xenobiotic receptors including AhR have been postulated as regulators of xenobiotic-induced BCRP expression. However, the underlying molecular mechanisms of this induction are largely unexplored. In the human colorectal carcinoma cell line LS174T, quantitative real-time PCR data demonstrated regulation of BCRP expression by hCAR and AhR following treatment with phenobarbital (PB) and 3-methylcholanthrene (3MC), respectively. PB showed an 11-fold induction, while 3MC caused a 27-fold induction of BCRP expression. Additional AhR agonists demonstrated their ability to upregulate BCRP—the pharmacological, reoviral with 3.6-fold induction and the proton pump inhibitor, omeprazole with 5.9-fold. Computer analysis of the BCRP promoter demonstrated the presence of several putative AhR response elements (AhRE) that may contribute to the observed induction in small intestine. Two promoter constructs, 1.2kb with 3 putative AhREs and 3.8kb with 5 putative AhREs, were treated with 3MC and were activated 2.5-fold and 5.9-fold, respectively. A greater response from the 3.8kb region having more AhREs suggests the involvement of more than one element for maximal AhR-mediated induction. Site-directed mutagenesis and EMA studies are underway to confirm these findings. AhR agonists are found in natural products and commonly-used drugs presenting the potential for unexpected drug-drug interactions with BCRP substrate drugs.

**103 BENZO[a]PYRENE INCREASES REACTIVE OXYGEN SPECIES AND REGULATES THE METALLOTHIONEIN, CYTOCHROME P450 AND ALDO-KETO REDUCTASES MESSENGER RNA LEVELS IN HUMAN LUNG ADENOCARCINOMA A549 CELLS.**

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The effects of benzo[a]pyrene (BaP), a polycyclic aromatic hydrocarbon and an environmental contaminant, on cytochrome P450 1A1 (CYP1A1), aldo-keto reductases 1C1 and 1C3 (AKR1C1 and AKR1C3) and metallothionein 1A and 2A (MT1A and MT2A) mRNA levels, and ROS mechanisms by which most develop cytotoxicity in cells. Cell cultures were treated with 1, 5, 10, 20, 30 and 50 μM BaP for 1, 3, 6, 12 and 24 h, kept at 37°C and CO2, 5%. Cell viability was measured with propidium iodide assay. NO and H2O2 intracellular production were evaluated with dihydroethidium (DHE) fluorescence and 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA) assay, respectively. CYP1AI, AKRI, AKR1C3, MT1A and MT2A mRNA levels were assessed by quantitative polymerase chain reaction (QPCR). Results show that BaP does not affect cell viability at any treatment, and ROS production was increased in a concentration- and time- dependent manner. CYP1AI, AKR1C1, AKR1C3, MT1A and MT2A expression were up-regulated by BaP at different times and BaP concentrations. These results suggest that the pleiotropic effect of BaP on CYP1AI, AKRI, AKR1C3, MT1A and MT2A expression happen through different pathways. The extent by which CYP1AI, AKRI, AKR1C3, MT1A and MT2A are regulated by BaP itself, BaP metabolites and ROS requires further investigation. (Supported by grant CONACYT-SEMARNAT, FOSEMARNAT-2004-01-293, Mexico).

**104 MICRORNAS IN DEVELOPMENTAL TOXICOLOGY: EFFECTS OF TCDD ON MICRORNA EXPRESSION IN EMBRYOS.**


Many drugs and environmental chemicals are teratogenic, and fetal exposure is associated with human birth defects. Embryonal exposure to xenobiotics can alter miRNA expression and contribute to the mechanisms by which environmental chemicals disrupt embryonic development. To test this hypothesis for one well-known teratogen, we exposed zebrafish embryos to DMSO (0.1%) or TCDD (5 nM) for 1 hr at 30 hours post fertilization (hpf). Three biological replicates of 200 pooled embryos were sampled at 36 and 60 hpf. TCDD caused strong induction of CYP1A at 36 hpf (62-fold) and 60 hpf (135-fold) as determined by qPCR. MiRNA profiling was performed using miRCURY LNA arrays (Exiqon). Samples were compared to a universal RNA reference made from equal amounts of all RNAs. Expression data were compared between sampling times and treatments. Eighty-two of the 223 miRNAs on the array were differentially expressed between 36 and 60 hpf (DMSO-treated embryos; fold-change: -4.01 to +5.41). Comparison of DMSO and TCDD-treated embryos showed 8 miRNAs with differential expression (1 induced; 7 repressed) at 36 hpf, 22 miRNAs with differential expression at 60 hpf. Comparison of DMSO and TCDD-treated embryos showed 8 miRNAs with differential expression (1 induced; 7 repressed) at 36 hpf, 22 miRNAs with differential expression at 60 hpf. Changes were modest, but possibly of biological significance. For example, the down-regulation of miR-144 and miR-451 by TCDD at 60 hpf is intriguing because these miRNAs have been shown to be required for erythropoiesis and TCDD is known to inhibit definitive erythropoiesis in zebrafish embryos. Future directions include array profiling with a variety known teratogens and identification of miRNA target genes involved in developmental toxicity. [Support: Mellon Foundation and Smith Chair]

**105 3-METHYLCHOLANTHRENE INDUCED GENOMIC WIDE BINDING PROFILES OF ARYL HYDROCARBON RECEPTOR AND ESTROGEN RECEPTOR ALPHA.**

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In this study, ChIP-chip was used to determine the 3-methylcholanthrene (3MC)-induced genomic footprints of aryl hydrocarbon receptor (AhR) and estrogen receptor (ERα). T-47D human breast cancer cells were treated with 1 μM of 3MC.
2,106 LOW-DOSE REPRESSION OF ARYL HYDROCARBON RECEPTOR SIGNALING BY RESVERATROL IS MEDIATED BY ESTROGEN RECEPTORS IN HUMAN CANCER CELL LINES.

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Resveratrol, a red wine phenolic, has been thought to have intrinsic cardiac and chemoprotective effects. It is found in abundance in many foodstuffs such as grapes, berries and peanuts. At high doses (micromolar) it acts as a pharmacological antagonist of the aryl hydrocarbon receptor, a weak agonist for estrogen receptors and binds sirtuins. However, these levels are not attainable through regular diet and not likely to account for the protective effects attributed to resveratrol. We have observed that low, nanomolar doses of resveratrol are capable of significantly repressing dioxin inducible CYP1A1 transcription in human MCF-7 breast and Caco-2 colonic cancer cell lines. These effects are dose-dependent and lead to a concomitant decrease in CYP1A1 protein and activity levels. While 100 nanomolar resveratrol repressed dioxin inducible CYP1A1 transcription by approximately 50%, it did not prevent nuclear translocation of the activated receptor or binding of the receptor to the regulatory regions of the CYP1A1 gene, nor did it displace 2-azido-3-[125I]iodo-7,8-dibromodibenzo-p-dioxin from the aryl hydrocarbon receptor. While low doses of resveratrol failed to activate estrogen receptor target gene transcription, repression of estrogen receptors’ levels with siRNA’s abrogated the repression effect of resveratrol on dioxin inducible gene transcription. Therefore, this mechanism may account for some of the putative chemo-protective effects of dietary resveratrol. (Supported by grants from NIH ES04869 and the Natural Sciences and Engineering Research Council [NSERC] of Canada).

107 DEVELOPING TOOLS FOR RISK ASSESSMENT IN PROTECTED SPECIES. RELATIVE POTENCIES INFERRED FROM COMPETITIVE BINDING OF HALOGENATED AROMATIC HYDROCARBONS TO ARYL HYDROCARBON RECEPTORS FROM BELUGA (DELPHINAPTERUS LEUCAS).

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Persistent organic pollutants such as halogenated aromatic hydrocarbons (HAHs) are biomagnified in food webs and accumulate to high concentrations in top predators, including odontocete cetaceans (toothed whales). The most toxic HAHs are the 2,3,7,8-substituted chlorinated dibenz-p-dioxins and non-ortho-substituted polychlorinated biphenyls, which exert effects through the aryl hydrocarbon receptor (AHR). Understanding the impact of HAHs in wildlife is limited by the lack of taxon-specific information about the relative toxicities of toxicologically important congeners. To assess whether Toxic Equivalency Factors (TEFs) determined in rodents are predictive of HAH relative potencies in a cetacean, we used beluga and mouse AHRs expressed in vitro from cloned cDNAs to measure the relative AHR-binding affinities of ten HAHs from five different structural classes. The rank order of mean IC50s for competitive binding to beluga AHR was: TCDD>TCD5>TCD6>PCB-126>PCB-169>PCB-77>PCB-81>PCB-84>PCB-156>PCB-118. The rank order of mean IC50s for binding to the mouse AHR was: TCDD>TCD5>TCD6>PCB-126>PCB-169>PCB-77>PCB-81>PCB-84>PCB-156>PCB-118. Calculated Ki values for binding of HAHs to beluga and mouse AHRs were highly correlated (r2 = 0.9614). Comparison of Ki values indicated that the beluga AHR had a higher affinity than the mouse AHR for most of the HAHs tested, consistent with the 2-fold higher [SH]TCDD-binding affinity determined previously. These results support the use of the WHO mammalian (rodent) TEFs for non- and mono-ortho PCB congeners in beluga and, by inference, other cetaceans. The overall higher HAH binding affinity of the beluga AHR as compared to a high-affinity AHR from a dioxin-responsive mouse strain indicates that beluga, and perhaps cetaceans in general, may be particularly sensitive to the toxic effects of AHR agonists.

108 2, 3, 7, 8-TETRAChLORODIBENZO-P-DIOXIN INCREASES OF MATRIX METALLOPROTEINASE-9 EXPRESSION THROUGH ACTIVATION OF ETS-1 IN HUMAN FIBROSARCOMA HT-1080 CELLS.

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), a prototype of many halogenated aromatic hydrocarbons, is a ubiquitous, persistent environmental contaminant and the most powerful tumor promoter in rodent bioassays. In the present study, we investigated the effect of TCDD on the expression of matrix metalloproteinase-9 (MMP-9) in human fibrosarcoma HT-1080 cells. TCDD dose dependently increased MMP-9 mRNA and stimulated MMP-9 activity using reverse transcription-polymerase chain reaction and zymography assay, respectively. TCDD enhanced MMP-9 gene promoter activity; however, there was no response to TCDD in DNA constructs with an ETS-1 site mutation. Electrophoretic mobility shift assay revealed that TCDD induced the activation of the ETS-1 to a form capable of binding specifically to the ETS-1 site sequence of the MMP-9 gene promoter. Furthermore, TCDD enhanced migration and invasion in human fibrosarcoma HT-1080 cells. Taken together, these results demonstrate that TCDD can stimulate the production of MMP-9 through activation of ETS-1 in human fibrosarcoma HT-1080 cells, which play an important cancer-promoting role.

109 MODULATION OF GENE EXPRESSION NETWORKS BY 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) DURING VERTEBRATE DEVELOPMENT.

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The aryl hydrocarbon receptor (AHR) mediates toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). TCDD is the archetype of a family of related polychlorinated compounds found ubiquitously in the environment. Carcinogenic and endocrine activities of TCDD are well documented, however, little is understood about the developmental effects or molecular mechanisms of action of this compound. Among other effects, developmental exposure to TCDD results in craniofacial abnormalities and diseases similar to those associated with developmentally exposed to retinoic acid and estrogen, respectively. Significant data suggest that there is cross-talk between receptors for TCDD, retinoids and estrogens. Using zebrafish (Danio rerio) as a vertebrate model, this project explores mechanisms underlying TCDD-mediated craniofacial abnormalities. Microarray technology was used to identify significantly differentially expressed genes in zebrafish embryos exposed to control conditions or environmentally relevant levels of TCDD (1 nM). These results were evaluated in combination with curated data from the publicly available Comparative Toxicogenomics Database (CTD; http://ced.miblb.org) to identify gene networks targeted developmentally by TCDD. Effects of TCDD on specific members of a network were confirmed using quantitative RT-PCR. Results from this project may provide novel mechanisms for understanding specific developmental effects of TCDD.

110 BODY-ONLY EXPOSURE SYSTEM FOR DETERMINING DERMAL ABSORPTION AND TOXICITY OF CHEMICAL VAPORS IN RATS.


Assessment of the dermal absorption and toxicity of chemical vapors is necessary for improving the design criteria for personal protective equipment. The design of a practical in vivo test system was needed to evaluate percutaneous vapor exposure in
Laboratory animals without inclusion of inhalation exposure. An in vivo system is beneficial because the metabolism/nervous/hormonal responses of the skin are intact. The body-only exposure system described herein is more simplistic and less labor intensive than previous work in this field. In the current system, rats are contained during the exposure in specialized restraint modules fitted into an exposure chamber. The restrainers are oriented in the faceplate such that only the bodies of the rats (shaved) are located inside the exposure chamber and exposed to the vaporized test atmosphere. Pulmonary exposure by the rats to the test vapor is minimized by the orientation of the head of the rats outside the exposure chamber, a flexible barrier fitted around their necks, and maintenance of a slight negative pressure inside the chamber. The system was validated by exposing 6 rats, body-only, for 4 hours to a 8000 ppm vapor atmosphere of toluene. Blood samples were collected from each rat at 0, 1, 4, and 24 hour timepoints. The mean blood concentrations of toluene at the 1-, and 4-hour timepoints were 11 ± 5.1 and 9.9 ± 2.0 μg/ml, respectively, which are similar values to data contained in a previously published study utilizing a body-only exposure system. A nose-only inhalation exposure was also conducted with the same generation system and general study design as the whole-body exposure. The mean blood concentration of toluene for exposing 6 rats, nose-only, for 4 hours to a 7800 ppm vapor atmosphere of toluene at the 1- and 4-hour timepoints were 230 ± 11 and 200 ± 22 μg/ml, respectively. The current body-only exposure system appears to be an accurate and practical method for assessment of dermal absorption and toxicity of various chemical vapors in rats.

**111** A METHOD DEVELOPMENT STUDY TO ASSESS THE EFFECTIVENESS OF MEASURES TO PREVENT CROSS CONTAMINATION DURING TOPICAL APPLICATION OF A TEST ARTICLE TO THE GÖTTINGEN MINIPIG.


Although the rabbit is commonly used for the assessment of primary dermal irritation, pigs have generally been considered to be a better model for the more sophisticated study of dermal permeability and toxicity. Many studies have shown the close resemblance of human and minipig skin in terms of architectural structure/morphology, histology and physiological characteristics. As a model in toxicology, the minipig has been used extensively in dermal/topical application studies. One of the most significant issues for dermal studies is the extensive measures required to prevent cross-contamination of blood and tissue samples, taken to monitor local and systemic exposure to the test article. This is more difficult with the minipig simply because of its size and temperament. ITR has completed a method development topical application study utilizing the Göttingen minipig. The study objective was to assess the effectiveness of control processes designed to preclude contamination of Control animals/samples with test substance. In the study, test animals were treated by non-occluded topical application of Lidocaine 2.5% cream (EMLA®) for 4 hours/day over 7 consecutive days. Control animals were similarly treated with Glycerin USP. Specific standard procedures were identified in the study protocol to highlight the areas of concern for the technical groups. This included those working with the animals, processing blood samples to plasma and the analytical department. Blood samples were collected at various timepoints during the treatment phase, processed and analyzed. Results from the analysis of blood samples collected during the study revealed that there were no measurable levels of drug in the Control animals at any time point. Treated animals, however, showed appropriate levels of drug at all timepoints. It can be concluded therefore, that the measures employed on the study were effective in preventing contamination of Control animals and samples with drug.

**112** DETERMINATION OF THE OPERATIONAL BARRIER PH OF PORCINE SKIN USING A DERMAL ABSORPTION MODEL.

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The prediction of drug and chemical absorption across the skin is largely based on mathematical models that assume passive Fickian diffusion as the primary mechanism for transdermal delivery. This assumption implies that only non-charged neutral chemicals pass through the stratum corneum that comprises the epidermal penetration barrier. The purpose of the present analysis was to determine the effective pH of the skin barrier relative to dermal penetration by using a QSPr model on an existing database of dermal permeability constants (log Dp) consisting of 12 marker compounds in 24 diverse chemical mixture combinations obtained in porcine skin. Model fit was assessed as a function of penetrant’s log Dp at different pH. Log Dp, the log octanol/water partition coefficient used in many QSPr models which assumes a pH of 7.4. All analyses converged on a value for effective stratum corneum barrier pH of 6, a number within the range of measured stratum corneum pH. These results suggest that when such QSPr models are used to estimate dermal absorption, risk assessment models should employ this acidic pH as the operational value for barrier penetration. (Supported by NICHD OH-07555)

**113** DERMAL UPTAKE OF INDUSTRIAL CHEMICALS - SHOULD EVAPORATION FROM SKIN BE INCLUDED IN THE BASIS FOR SKIN NOTATION?

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Skin permeating chemicals are highly volatile and evaporation may significantly decrease the amount available for absorption following e.g. an accidental spill on the skin. The impact of evaporation in relation to dermal absorption was investigated for 83 substances, whereof 75 should have a skin notation according to the ECETOC criterion (Strategy for assigning a skin notation. ECETOC, Brussels, 31/1993). Evaporation rates were calculated from molar masses, vapor pressures, the universal gas constant, the temperature (set to 30°C=303K) and mass transfer coefficients in air. The latter depends mainly on molar volume, molecular weight and air speed over the exposed surface and was calculated with the Spary method for an air speed of 0.6 m/s. Dermal absorption rates of neat chemical were calculated from published data on skin permeability coefficients. The time to complete elimination from the skin surface, either by evaporation or absorption, was calculated for an arbitrary initial thickness of 20 μm. The evaporation rate of the 73 chemicals varies from 10^-2 (vinyl chloride) to 10^-5 (hydroquinone) on a relative scale (n-butyraldehyde). Similarly, the time to total elimination (absorption-evaporation) varies from 0.2 seconds to 4 days and x of the 73 chemicals are eliminated in less than 1 hour. The absorbed fraction (absorbed/eliminated) varies from 0.997 (pentachlorophenol) to 10^-5 (tetrahydrofuran). For most substances (57 of 73), less than 50% is absorbed. In conclusion, the extent of evaporation varies widely between chemicals, ranging from practically negligible to the completely dominating source of elimination from the skin surface. Furthermore, the huge variability in time to elimination, ranging from seconds to days, suggests that the assumption of a uniform dermal exposure duration (1 hour in the ECETOC criterion) and infinite dose, often used in the decision basis for skin notation, may be misleading. The study was supported by the Swedish Council for Working Life and Social Research (FAS).

**114** POLYCYCLIC AROMATIC HYDROCARBONS IN BLACK TATTOO INKS AND SINGLET OXYGEN GENERATION.


In recent years, the number of people with tattoos increased enormously. Black tattoo inks are based on Carbon Black, which is known to act as a significant strong sorptive phase for polycyclic aromatic hydrocarbons (PAHs). The US Environmental Protection Agency US- EPA created a Priority Pollutant list for 16 PAHs, because many of PAHs are toxic or mutagenic. In addition, many PAHs are suspected to generate reactive oxygen species (ROS) under UVA irradiation, such as singlet oxygen that affects DNA integrity. Since black tattoos inks are placed in the skin at high concentrations, the goal of our investigation was the determination of PAHs concentration in these tattoo inks. We developed an ultrasonic-assisted extraction method using a mixture of benzene/acetone, heat and centrifugation. We quantitatively extracted PAHs from 17 commercially available black tattoo inks using the Internal Standard method by HPLC Diode Array Technology and mass spectrometry. The tattoo inks contained different amounts of PAHs according to the US- EPA list. The total amount of PAHs was up to 226 ug per mL tattoo suspension of black inks. In addition, we found a high amounts of phenol with up to 430 ug per mL black tattoo ink. Then, we determined the quantum yield of singlet oxygen generation for each PAH using luminescence spectroscopy. The quantum yield of the different PAHs ranged from 0.46 to 0.82, which is higher than for photo-sensitizers used in photodynamic tumor therapy. Human keratinocytes were incubated with PAHs that have been extracted form 1mL Black Tattoo Ink. After irradiation with broadband UVA, MTT-assay showed significantly decreasing cell viability, which clearly depended on the UVA light dose and the respective black ink extract. In conclusion, black tattoo inks contain high amounts of PAHs and phenol. The PAHs generate singlet oxygen, which in turn affects cell viability. The work is supported by a grant of the Deutsche Forschungsgemeinschaft (DFG, BA7145/1-2).
115 EVALUATION OF THE SENSITIZING POTENTIAL OF DIPHOTERINE® IN ADULT VOLUNTEERS WITH NORMAL SKIN.

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Diphoterine®, a decontamination solution for chemical splashes, contains an amphoteric compound. It is non-irritating, non-cytotoxic, non-mutagenic and non-toxic by dermal and oral routes in rodents, as sensitizing capacity has been previously evaluated in the Guinea pig without any observed reaction. The objective was to assess the allergenic potential of Diphoterine® in humans when applied pure under an occlusive patch with dermatological control following the Marzulli-Maibach method. 164 normal volunteers were divided into 3 groups. A sample of 25 μl (on a paper filter disk) was applied on the homological (induction phase) and contralateral scapular area under an occlusive dressing. Another dressing without Diphoterine® was applied in the same conditions as a blank application. During the induction phase, the application of the product was renewed 5 times per week for 48H each time. There were 8 or 9 applications. The induction, latency and elicitation phases ended after 3 weeks, 2 weeks, and 1 week respectively. Neither the homolateral area during the Induction Phase nor the contralateral area during the challenge phase were flared. 161 volunteers completed the study. Clinical examinations during the induction phase allowed continuing Diphoterine® and blank applications throughout this period and during the elicitation phase. During the elicitation phase, no reaction was observed. An index of average irritation (I.M.M) was calculated at each examination. The I.M.M at day 22 for the 111 normal volunteers who had 9 successive Diphoterine® applications, was 0.09. Diphoterine® remains non-irritating during the first 5 applications, then an increase in irritation (I.I.M) was calculated at each examination. The I.M.M at day 22, for the 111 normal volunteers who had 9 successive Diphoterine® applications, Diphoterine® was not a skin irritant. To conclude, the risks of skin sensitization were minimal during the induction and challenge phase. Diphoterine® remains non-irritating during the first 5 applications, then an increase in irritation was calculated at each examination. The I.M.M at day 22, for the 111 normal volunteers who had 9 successive Diphoterine® applications, Diphoterine® was not a skin irritant. To conclude, the risks of skin sensitization from dermal contact with Diphterine® are negligible. Thus Diphoterine® may be classified as hypoallergenic.

116 CYTOKINES IN RAT SKIN AFTER A ONE-HOUR EXPOSURE OF JET PROPELLION FUEL-8.

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Jet Propulsion fuel-8 (JP-8) may cause inflammation upon skin contact. To develop therapeutic or prophylactic measures, we have to understand the trigger of inflammation induced by JP-8. One of the early events in inflammation is the expression of inflammatory cytokines. In this study, we have investigated the expression of inflammatory cytokines, interleukin (IL) -1 alpha, IL-1 beta, IL-6, macrophage inflammatory protein-2 (CXCCL2) and tumor necrosis factor (TNF) alpha at different times after a 1-h exposure of rat skin to JP-8. A patch containing five hundred microliters of JP-8 or an empty patch was placed on the fur-clipped back of male Fisher rats. At 0, 1, 2, 3, 4, and 8h post exposure, we humanely killed the rats using an overdose of CO2 and excised the skin. We isolated protein, to quantify cytokines, by pulverizing and homogenizing the skin in extraction buffer with protease inhibitors. The cytokines were estimated using a sandwich ELISA utilizing capture and detection antibodies from several commercial sources (T Cell Systems, MN). IL-1 alpha decreased at 3h and CXCCL2 increased at 1h post exposure. IL-1 beta, IL-6 and TNF alpha did not significantly change at any time point up to 8h. IL-1 alpha is thought to be performed and stored in skin. The reduction in the level of IL-1 alpha may be due to its release in response to JP-8 exposure. Released cytokine binds to the receptor and may be internalized for degradation. CXCCL2, a chemokine involved in the attraction of polymorphonuclear granulocytes, was detected in low amounts compared to the other cytokines. We expected to see a significant change in the signaling protein levels based on mRNA expression studies in our lab. In this study, we show that only two cytokines among the five studied have a change in protein levels. The decrease in IL-1 alpha and increase in CXCCL2 levels may be some of the earliest protein events in the inflammatory cascade induced by JP-8. (Sponsored by Air Force Office of Scientific Research)

117 UV EFFECTS AND CHEMICALS SKIN TOLERANCE ASSESSMENT USING THE IN VITRO RECONSTRUCTED HUMAN FULL THICKNESS SKIN MODEL REALSKIN.

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In vitro Reconstructed human skin models are of growing interest for regulatory purposes in the framework of alternatives to animal testing, and for safety or efficacy studies.

The reconstructed human full thickness skin model RealSkin is an organotypic culture composed of a living dermal equivalent (human fibroblast-contracted collagen gel) and a well-stratified and fully differentiated epidermis (human keratinocytes). In the present study, we investigated dermis and epidermis compartments responses after exposure to different stress agents. Thus, tissues were subjected to UV’s exposure as well as topical treatment with known potentially cytotoxic and non cytoxic chemicals with available historical in vivo skin tolerance data. For UV effects, skin tissues were exposed to increasing doses of UVA or UVSRR (UVA+B) from 0 to 55 J/cm2. For skin tolerance studies, chemical were applied onto the skin. Following UV or product treatments, tissues were incubated overnight at 37°C. 5% CO2 with fresh maintenance medium. Cell viability assessment (MTT conversion test), pro-inflammatory mediator measurements and histological studies were performed. Our results showed that 1- UVAt, damages predominantly affected the dermal compartment whereas UVa+B exposure simultaneously induced damages in both dermal and epidermal compartments. 2- The skin model with adapted protocols was able to discriminate between cytotoxic and non cytotoxic chemicals in relation with their irritant potency. 3- damages depth can be documented. These preliminary results suggested that RealSkin is a promising tool for understanding in vitro skin tolerance effects of products and for studies involving UV effects. They may serve as a basis for further larger scale experiments.

118 EFFECT OF METAL WORKING FORMULATIONS ON BIOCIDE ABSORPTION IN A PDMS MEMBRANE COATED FIBER.

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Biocides and preservatives are commonly used in metal working industry applications as additives to extend the shelf life of commercially manufactured aqueous metal working fluid (MWF) formulations. While little is known of the dermal absorption and disposition of these and other MWF formulation components, it is hypothesized that the combined administration of topical and systemic glutathione will reduce the severity of cutaneous radiation injury and accelerate healing. Radiation has been shown to deplete the function of glutathione reductase and a decrease in glutathione can occur systemically or locally in affected tissues. Thus it is hypothesized that the combined administration of topical and systemic glutathione will reduce the severity of cutaneous radiation injury and accelerate healing. A stable liposomal encapsulation of glutathione that can be orally and topically administered has recently become available and has been evaluated for efficacy in the treatment of chemotherapeutic or prophylactic measures, we have to understand the trigger of inflammation induced by JP-8. This work was supported by NIOSH grant R01-01-03669.

119 CUTANEOUS RADIATION INJURY: LIPOSOMAL GLUTATHIONE TREATMENT AND MONITORING BY OPTICAL REFLECTANCE SPECTROSCOPY.

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Exposure to ionizing radiation from radiological or nuclear weapons and terrorist devices will likely cause life threatening radiation cutaneous injuries. To date, mechanistic and health effects of cutaneous radiation injury have been investigated only to a limited extent, and no specific countermeasures are available for treatment. Radiation has been shown to deplete the function of glutathione reductase and a decrease in glutathione can occur systemically or locally in affected tissues. Thus it is hypothesized that the combined administration of topical and systemic glutathione will reduce the severity of cutaneous radiation injury and accelerate healing. A stable liposomal encapsulation of glutathione that can be orally and topically administered has recently become available and has been evaluated for efficacy in mitigating cutaneous radiation injuries using F344 rats exposed thorigh only. Visually, glutathione reduced cutaneous injury and increased healing compared to
control (non-glutathione treated) animals. An optical spectroscopic method was developed and used to analyze the functional characteristics of the exposed leg-tissue from day 1 to day 50 post-exposure. The system consists of a ultra-violet/visible/near-Infrared (UV-vis-NIR) spectrometer coupled fiber-optically with a reflectance/backscattering probe. A progressive time-change in the absorbance spectra of the irradiated skin was observed. A principal component analysis of the data was successful in monitoring progression of the skin injury with time, differentiating between levels of exposure, as well as differentiating between glutathione treated and non-treated animals.

Support by LDRD Program at the Pacific Northwest National Laboratory, operated by Battelle for the U.S. DOE under Contract DE-AC05-76RL01830.

120 RETINYL PALMITATE INCREASES THE MULTIPLICITY OF SKIN TUMORS IN CRL:SKH-1 (HR/HIR) HAIRLESS MICE EXPOSED TO SIMULATED SOLAR LIGHT.

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Vitamin A (retinol) plays an essential role in various biological processes; however, vitamin A is sensitive to heat and light. In order to increase its stability, esters of vitamin A, such as retinyl palmitate (RP), are employed in formulations for cosmetic purposes. Vitamin A is sensitive to heat and light. In order to increase its stability, esters of vitamin A, such as retinyl palmitate (RP), are employed in formulations for cosmetic purposes.

RETINYL PALMITATE INCREASES THE MULTIPLICITY OF SKIN TUMORS IN CRL:SKH-1 (HR/HIR) HAIRLESS MICE EXPOSED TO SIMULATED SOLAR LIGHT.

121 PERCUTANEOUS ABSORPTION OF CHEMICAL VAPORS THROUGH EXCISED PIG SKIN: A BIOLOGICAL ENDPOINT MODEL FOR EVALUATING PROTECTIVE CLOTHING.

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To define the kinetics of in vitro percutaneous absorption of solvent vapors, saturated concentrations of dioxane, isopropanol, methyl ethyl ketone, and toluene in static (still air at 30°C) and dynamic conditions (flowing air at 20°C) were exposed to freshly excised, split-thickness pig skin mounted in temperature controlled evaporation/penetration cells. Solvent concentrations in air (headspace over skin) were measured by gas chromatography (GC). Receptor fluid exiting the penetration cells was collected in 4 ml glass vials housed in brass jackets cooled to -20°C to reflect solvent loss, especially for toluene. Solvents were extracted from the receptor fluid with solid phase micro-extraction fibers (SPME) and quantified by GC. Static vapor phase permeability constants were determined over a 4 hour exposure period and mean values were 0.36 cm/hr for toluene, 0.41 cm/hr for isopropanol, 2.4 cm/hr for dioxane and 3.6 cm/hr for methyl ethyl ketone. Dynamic vapor phase permeability constants determined under analogous conditions were found to be approximately an order of magnitude lower and ranged from 0.045 to 0.083 cm/hr for the four solvents. Higher Kps for the static measurements may be due to a higher hydration state of the stratum corneum and may be more appropriate for solvent vapor trapped between protective clothing and the skin. Using the static vapor phase Kp values and acute reference exposure levels (California REL values, μg/m3), it is possible to calculate the necessary permeability values for protective clothing for a given air concentration of solvent. Such a biological endpoint model may be useful in striking a balance between protection and heat burden in the design of protective clothing.

122 ESTIMATED DERMAL PENETRATION OF PERMETHRIN IN HUMANS BASED ON IN VITRO AND IN VIVO DATA.

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The objective of this study was to develop an estimate of the percent dermal absorption of permethrin in man. This was accomplished by assuming the ratio of the in vivo dermal absorption to the in vitro dermal absorption by human skin was the same as the ratio of in vivo dermal absorption of rats to in vivo dermal absorption by humans. Three different concentrations of 14C-permethrin, ranging from 2.25 μg/cm2 to 200 μg/cm2, were used in vitro with human and rat skin preparations, and in vivo on bare rat skin. Twenty-four hours after application of 14C-permethrin to the human skin preparations, the radiolabel recovered from dermis (bound skin residues) and receptor fluid was summed to determine percent dermal absorption. At doses of approximately 2.25, 20 and 200 μg/cm2 of permethrin, values of 1.6% 3% and 6%, respectively, were obtained for percutaneous absorption (N = 9 /dose). The in vitro rat skin preparations incubated under the same conditions were found to absorb 20 +/- 9% 18 +/- 5% and 24 +/- 8%, respectively (N=6 /dose). In vivo rat dermal absorption was conducted in mice to determine whether the topical application of creams containing 0.1% or 0.5% increase squamous cell tumor multiplicity in the multiplicity of squamous cell neoplasms when compared with control groups. In mice that received topical applications of control cream or creams containing 0.1% or 0.5% RP significantly enhanced the incidence and multiplicity of squamous cell neoplasms when compared with no cream groups. In mice that received topical applications of control cream or creams containing 0.1% or 0.5% RP significantly enhanced the incidence and multiplicity of squamous cell neoplasms when compared with no cream groups.

123 DERMAL IRITATION OF ALIPHATIC HYDROCARbons (C9-C14) USING THE IN VITRO EPIDERM FULL THICKNESS (EFT-300) SKIN MODEL.

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Introduction: EpiDerm full thickness skin (EFT-300) cultures contain normal human-derived epidermal keratinocytes (NHKE) and dermal fibroblasts (NHFB) in a highly differentiated skin-like tissue. The objective of the present work was to systematically evaluate the EFT-300 skin equivalent for studying the dermal irritation of the aliphatic C9-C14 hydrocarbons, nonane, decane, undecane, dodecane, tridecane and tetradecane. Methods: The EFT-300 tissues were treated with 2.5ul preparations incubated under the same conditions were found to absorb 20 +/- 9% 18 +/- 5% and 24 +/- 8%, respectively (N=6 /dose). Dose and test duration did not have a statistically significant effect on percutaneous absorption of permethrin in the rat skin. The ratio of dermal absorption by in vivo rat skin to absorption by in vitro skin ranged from the 24-hr in vivo rat values to absorption by in vitro skin ranged from 7.3 to 28. This suggested that in vivo human skin absorption values for permethrin ranging from the 24-hr in vivo rat values to absorption by in vitro skin ranged from 7.3 to 28. This suggested that in vivo human skin absorption values for permethrin ranging from the 24-hr in vivo rat values to absorption by in vitro skin ranged from 7.3 to 28. This suggested that in vivo human skin absorption values for permethrin ranging from the 24-hr in vivo rat values to absorption by in vitro skin ranged from 7.3 to 28. This suggested that in vivo human skin absorption values for permethrin ranging from the 24-hr in vivo rat values to absorption by in vitro skin ranged from 7.3 to 28.
ethanol solution was applied to freshly excised human skin in an in vitro test system predictive of skin absorption in man. Twenty-four hours after application, the radiolabel recovered from carcass, urine and feces was determined for percutaneous absorption (N = 9 per dose). For reference purposes, excised human skin absorption of 14C-piperonyl butoxide (PBO) at 100 μg/cm2 was determined to be 7 ± 5% (N=9). For additional reference purposes, excised rat skin absorptions of permethrin at 2.25, 20 and 200 μg/cm2 were found to be 20 ± 9%, 18 ± 9%, and 24 ± 8%, respectively (N=6 per dose), approximately 10 times higher than human skin absorptions. Excised rat skin absorption of PBO was also higher (35 ± 9%) than the value obtained for human skin by a factor of about 5. The human skin results for PBO are lower than a previously reported value from our laboratory (13%), but higher than published values for human in vivo (1.3-3.7%), giving some confidence that the values for permethrin are not underestimated for man.

125 SKIN PENETRATION OF PERMETHRIN AFTER TOPICAL APPLICATION TO LIVE RATS.


The objective of this study was to determine the percutaneous absorption of radio-labeled permethrin in live rats to permit a calculation of the ratio of in vivo to in vitro values, determined in a separate study. Carbon-14 labeled permethrin in ethanol solution was applied to the clipped skin of live rats at doses of 2.25, 20 and 200 μg/cm2. As a reference compound, carbon-14 labeled piperonyl butoxide (PBO) was applied to live rat skin at a dose of 100 μg/cm2. All applications were decontaminated at 24 hours post-application, and rats were terminated either at 24 hours (3 dose levels of permethrin, 6 rats per dose level, 18 rats total) or 5 days (3 dose levels of permethrin and a single dose level of PBO, 6 rats per dose level, 24 rats total). Twenty-four hours and five days after application, and five days after PBO application, the radiolabeled and free carbon was determined in the carcass, urine and feces and was summed to determine percent absorption. Recoveries across all compounds and doses were 85-90% and percutaneous absorption values were corrected for recoveries. For the 24 hour time point, at doses of 2.25, 20 and 200 μg/cm2 of permethrin, values of 22 ± 8%, 22 ± 9%, and 28 ± 7%, respectively were obtained for percutaneous absorption (N = 6 per dose). For reference purposes, the five day percutaneous absorption of 14C-piperonyl butoxide (PBO) at 100 μg/cm2 was determined to be 42 ± 3% (N=6). Dose and test duration did not have a statistically significant effect on percutaneous absorption of permethrin in the rat in vivo.

126 TRANS-EPITHELIAL ELECTRICAL RESISTANCE (TEER) MEASUREMENT ON IN VITRO CUTANEOUS AND OCULAR 3-D MODELS: A SENSITIVE PARAMETER NEEDING STANDARDIZED CONDITIONS.


The barrier function of epithelial tissues is well described with passive permeability assays using hydrophilic or hydrophobic markers. Two major routes are mainly involved in small molecules flux: trans-cellular route mainly dependent on chemicals lipophilicity and paracellular route linked to intercellular spaces and under tight junctions (TJs) control. Together with the proteo-lipidic complex of the cornified layers, TJs are important effectors of the barrier function of tissues. When TJs are not mature or damaged in 3-D tissues, hydrophilic compounds are able to diffuse from the upper layers to the basal one. Consequently, modifications of barrier function linked structures can influence trans-epithelial electrical resistance (TEER) state of the tissue. Purpose: Modifications and quality of the barrier function could be detected by using TEER measurements. In the present work, we studied tissue integrity modifications after chemical treatments. We evaluated commercially available in vitro 3-D engineered models: two reconstructed human epidermis models (EpiSkin large model, RHE small) and a human corneal epithelial model (RHCE) supplied by skinEthnic Laboratories. TEER measurements were conducted before and after surfactants treatment.

Material and Methods: TEER was measured during a time period in order to define acceptable and standardized steps where results could be regarded as stable prior to measurements. Results: Comparisons between two electrolyte media were done in order to optimize measurements. After topical treatment with SLS and Triton X-100, results showed a sharp TEER decrease since the lowest concentrations tested reaching low resistance values at the highest surfactant concentrations. Complementary cellular viability assays showed clear effects at highest doses. Conclusion: TEER method could be a useful parameter for quality control assessments and comparisons between 3-D models. In addition it could be used as a suitable easy to use tool, to describe barrier function, complementary to other studies.

127 CYP450 CHARACTERIZATION OF THE RECONSTRUCTED HUMAN EPIDERMIS EPISKIN™ INVOLVED IN XENOBIOTIC METABOLISM.

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The main role of human skin is its barrier function to environment and exogenous chemicals. However, it is not merely an inert organ but it is responsible for a wide range of metabolic activities and detoxication reactions. Previous studies have shown that the reconstructed human epidermis EpiSkin™, an in vitro skin model, expresses mRNAs of several isoforms of phase I metabolizing-enzymes, such as cytochromes P450 (CYP). However, the presence of mRNA material does not necessarily mean that it is translated into proteins or that the corresponding enzymes are active. A western blot methodology applied to EpiSkin™ microsomes, allowed us to determine CYP1A1, CYP1B1, CYP2C18, CYP2D6, CYP2E1, CYP3A1, CYP3A4, CYP3A5 and CYP3A7, which are the main CYP involved in the xenobiotic metabolism expressed in skin and in this particular model. In addition, in order to confirm the epithelial CYP functionality, the catalytic activities of CYP1A1/1B1, 2B6/2C19/2E1 and 3A isoforms were measured by using fluorogenic substrates. Results showed that CYP1A1, CYP1B1, CYP2D6, CYP2E1, CYP3A4, CYP3A5 and CYP3A7 enzymes were present in EpiSkin™. CYP1A1/1B1 and CYP3A isoforms were the main isoforms determined in the epithelium. CYP2B6/2C19/2E1 activities were also detected but at a too weak level to be quantified. No real correlation was observed between the CYP mRNA expression, translation into protein and activity levels. This demonstrates that catalytic activity studies are necessary steps to prove the real metabolic capabilities of any tissue.

In conclusion, these findings show that EpiSkin™ is a pharmacologically active skin model for studying in vitro xenobiotic phase I metabolism and toxicity and selecting inducers or inhibitors for these enzymes.

128 QUANTIFICATION OF PHASES 1 AND 2 METABOLIZING ENZYMES IN EPISKIN™ AND NORMAL HUMAN EPIDERMIS.

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EpiSkin™ is a human skin model obtained from the in vitro differentiation of epidermal keratinocytes. To assess whether enzymes involved in detoxification of chemicals are expressed in EpiSkin™, we quantified, using the RT-PCR technique, their mRNA expression level in the model and biopsies of normal human epidermis and compared to each other. The data show that the expression profiles of phases 1 and 2 metabolizing enzymes in Episkin™ and epidermis are very similar, with low expression levels of CYP450s and very high expression levels of phase 2 enzymes. As example, glutathione-s-transferase P1 (GSTP1) is found at 5 to 13 millions copies/μg total RNA, catalase-O-methyl transferase (COMT) and reidox sulfotransferase (SULT2B1) at 0.5 to 1 million and 0.6 to 2 millions copies/μg total RNA, respectively. N-acetyl transferase (NAT5) is found at a lower level (~50000 to 200000 copies/μg total RNA) and monoamine oxidase (MAO) at approximately 10000 copies/μg total RNA. In contrast with total skin where alcohol dehydrogenase (ADH1B) is expressed at very high levels, this enzyme is almost absent in EpiSkin™ and epidermis. Other phase 1 enzymes are also expressed at low levels. The highest expression levels are found for CYP1B1, CYP2E1 and CYP4B1 at approximately 100000 copies/μg total RNA. Similar to human skin, CYP450s (CYP3A2, 4F12, 4F8, 7B1, 2B6 and 39A1) metabolizing endogenous compounds are also present. The highly similar expression profiles of metabolizing enzymes between the reconstructed skin and a normal human epidermis, strongly suggest that EpiSkin™ is a good model for studying detoxification in human skin.
129 AN INITIAL EVALUATION OF THE CELL SYSTEMS EST-1000 RECONSTRUCTED HUMAN SKIN MODEL FOR DISTINGUISHING R34 AND R35 CORROSIVES IN VITRO.


Skin corrosion refers to the production of irreversible damage to the skin following application of a test substance for up to 4 hours (Globally Harmonized System of Classification and Labelling of Chemicals (GHS)). Assessment of the potential of chemicals to induce skin corrosion is important in establishing procedures for their safe handling, packaging and transportation. Validation studies have shown that tests employing reconstructed human skin models are able to reliably distinguish between known skin corrosives and non-corrrosives. The reconstructed human epidermis model EST-1000 can be used in the assessment of skin corrosivity potential as indicated in the OECD Guideline for the testing of chemicals No 451. The purpose of this study was to assess whether the EST-1000 model can be used to correctly identify R 35 (UN packing group I) and R 34 (UN packing group II & III) chemicals by performing corrosion testing in accordance with INVITTOX Protocol No 118. Two R 35 corrosive chemicals (1,2 diaminopropane and acrylic acid), two R 34 corrosive chemicals (2-tert-butylphenol and octanoic acid) and two non-corrrosive chemicals (phenethyl bromide and 4- (methylthio)-benzaldehyde) were applied to EST-1000 tissues for periods of 3 minutes, 1 hour or 4 hours and tissue viability was determined by measurement of mitochondrial dehydrogenase activity using the MTT assay. The results of the study demonstrated that using the EST-1000 model in accordance with the INVITTOX protocol, it was possible to correctly identify the corrosive and non corrosive chemicals. Both R 35 corrosive chemicals were correctly identified and one of the R 34 corrosive chemicals was also correctly identified. The other R 34 chemical (2-tert-butylphenol) was overpredicted as a R 35 corrosive chemical. It is concluded that the EST-1000 model is a suitable candidate for distinguishing R 35 (UN packing group I) and R 34 (UN packing group II & III) chemicals.

130 CHARACTERIZATION OF OXIDATIVE DEAMINASE ACTIVITY IN THE RECONSTRUCTED HUMAN EPIDERMIS EPISKIN™


The skin is not just a passive physical barrier but is also a biological structure involved in a wide range of metabolic activities. The reconstructed human epidermis EpiSkin™ is a tool developed for studying, in vitro, skin toxicity of chemicals and their metabolism as alternative methods to animal experimentation. This implies that the model is metabolically active. Adenosine deaminase (ADA) and adenosine deaminase (AMPDA) are enzymes involved in the irreversible oxidative deamination of adenosine and adenosine derivatives to inosine and inosine derivatives, respectively. These enzymes, which are present in normal human epidermis, have a critical role in the adenosine homeostasis and may be in the proliferation and maturation of certain types of mammalian cells. In this work, we reported the characterization of oxidative deaminase activity in EpiSkin™ using adenosine derivatives as enzyme substrates (2’-O-iodosine, 2’-O-isopropylideneinosine...). Substrates degradation and the corresponding inosine derivatives appearance were detected by UHPLC and demonstrate that ADA and AMPDA are present and efficient in EpiSkin™.

131 IDENTIFICATION OF MOLECULAR THERAPEUTIC TARGETS IN PORCINE SKIN EXPOSED TO BROMINE USING TOXICOCOMPUTING.

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Bromine is a toxic industrial chemical that is irritating and corrosive, resulting in significant skin damage. Little is known about therapeutic treatments of bromine burns to reduce burn severity and healing time. Treatment selection and development requires the understanding of the underlying molecular mechanisms. This study utilized toxicocomputing analysis to evaluate transcriptional changes and identify potential molecular therapeutic targets in porcine skin exposed to liquid bromine. Ventral abdominal samples on each of four weanling swine were exposed to 600 μL undiluted bromine for 45 sec or 8 min. At 24 hr and 7 days post-exposure, skin samples were excised, and total RNA was isolated, processed and hybridized to Affymetrix GeneChip® Porcine Genome Arrays (containing 20,201 genes). Analysis revealed 30% of the transcripts changed at 24 hours were the same regardless of whether the bromine treatment was 45 seconds or 8 minutes, and 62% of the transcripts changed at seven days were the same regardless of the exposure. Both the 45 sec and 8 min exposures shared about 13% to 14% of transcripts at 7 days. Ingenuity Pathways Analysis (IPA) revealed 6 of the top 10 biological functions were commonly shared, while 11 genes were common shared among 24 signaling pathways. In addition, the functions of dermatological diseases and conditions, inflammatory disease, and immune response were significantly changed in which IPA identified drugs in Phase II or Phase III clinical trials, or FDA-approved for use against specific gene targets. The genes encoding CASP3, CD40, IL-6, and VEGFA were commonly shared among these three functional categories. These results indicated that the transcriptional changes in bromine-burned skin are primarily affected temporally rather than exposure duration, and the transcriptional responses can be differentiated using toxicocomputing analysis. This work was supported by the U.S. Army MRMC under Contract W81XWH-05-D-0001, Task Order 0010.

132 INTRACUTANEOUS ADMINISTRATION AS A METHOD FOR EVALUATION OF PHOTOTOXICITY.


To elicit phototoxicity, the material of interest must be in the target tissue at the appropriate concentration and be exposed to the appropriate activating wavelengths of light. In certain cases, clinical phototoxicity can be difficult to replicate in animal models, presumably because of differences in exposure patterns, light sources, metabolism, administration or unidentified variables. Direct administration to the skin by intracutaneous administration ensures that the putative phototoxin is present in the target tissue (skin) during irradiation, bypassing tissue accumulation, metabolism, or reasons that may not be known without extensive investigation. In this study, the PT of two known clinical phototoxins, doxycycline (DOX) and chlorpromazine (CTZ), were evaluated in albino hairless mice. The fluorescent source emitted principally ultraviolet A (UVA). A single UVA dose of 20 - 23 J/cm2 was used for all exposures. When DOX at the maximum tolerated dose was administered for five consecutive days by intraperitoneal injection followed by single UVA exposure, no cutaneous phototoxicity was elicited. To assess direct administration of DOX or CPZ to the skin, each phototoxin was administered by the IC route and the mice irradiated as soon as possible after administration to ensure their presence in the skin during exposure. Under these conditions, skin reactions consistent with phototoxicity (erythema, edema) were elicited in the exposure site. The same dose (total of both sites approximated the maximum tolerated dose) administered to each mouse without exposure served as control for any non-specific skin reactions. These results demonstrate that the intracutaneous delivery route is useful in assessing potential phototoxicity of clinically relevant materials that cannot be easily evaluated by other dosing routes.

133 THE USE OF HUMAN DATA WHEN CONDUCTING DERMAL SENSITIZATION QUANTITATIVE RISK ASSESSMENTS FOR FRAGRANCE INGREDIENTS; HOW WELL DOES THE LLNA PREDICT HUMAN NOELS?


Historical human data either from human repeated insult patch tests or human maximization tests are available for fragrance ingredients used in consumer products. When conducting a Quantitative Risk Assessment for the induction of dermal sensitization to a fragrance material, these data add an important aspect to the overall weight of evidence approach used to determine potency. A human sensitization test is not used to determine hazard; it is a test to confirm the lack of dermal sensitization at an exposure level previously identified as a NOEL in an animal model or derived as a likely NOEL from quantitative structure-activity relationships. The EC3 value calculated in the LLNA was demonstrated to closely correlate with the NOEL from human sensitization tests designed to confirm lack of induction. A detailed analysis of the dermal sensitization data for approximately 50 fragrance ingredients that have exhibited dermal sensitization has been conducted. The data show that for a majority of the materials reviewed, there is a very good correlation between the LLNA EC3 value and the NOEL in confirmatory human dermal sensitization tests. For a minority of materials the correlation is less predictive. When the correlation is not good, sometimes the data reveal that the LLNA EC3 value is much lower than the maximum tested NOEL in humans. For other materials the
data show that the EC3 value overestimates the NOEL in confirmatory human tests. These data illustrate the importance of conducting a confirmatory human sensitization test. The reason for this lack of correlation is not currently fully understood. It may be due to the amount of material that is absorbed and/or differences in the metabolic capabilities of mouse and human skin. As such, dermal metabolism and penetration studies are needed to help further our understanding of the discrepancy.

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

Keywords: skin burns, sulfur mustard, peptide, inflammation

134 HUMAN CADAVER SKIN: A VALID SURROGATE MODEL FOR THE DEVELOPMENT OF TOPICAL AND TRANSDERMAL DRUGS.

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Extensive use has been made of the human cadaver skin model in optimizing vehicles for topically applied products during the preclinical phase of the drug development process. However, its potential use in other phases of drug development has been limited as there is currently no formal regulatory acceptance of it as a valid surrogate model. Since validation of the model will require demonstration of good in-vitro/in-vivo correlation, an in-vitro comparison of five generic (test) glucocorticoids with the corresponding innovator (reference) products was conducted in the model and the data compared to the in-vivo data which were the basis for FDA approval. Split-thickness human skin was divided into multiple sections, mounted in Franz Cells, and products applied at a dose of 6 μL/cm2. Absorption was measured by sampling the receptor solution periodically over a 48-hour period and assuming for drug content by LC-MS. Both rate of absorption and total absorption were calculated. Four of the five generic products were found to be equivalent to the corresponding innovator product in the cadaver skin model, in agreement with the clinical data which was determined by vasoconstrictor assay (VCA). Test to reference ratios varied from 0.96-1.14 in vitro and 0.92-1.11 in vivo. However, one generic product was not equivalent to innovator (T/R = 0.63) and the reason for the discrepancy was found to be due to insensitivity of the VCA assay, not to a failure of the model. New data continue to validate the human cadaver skin model as a surrogate model for toxicity testing.

135 STRATATEST™ TISSUE. A NOVEL IN VITRO ALTERNATIVE FOR CYTOTOXICITY TESTING.

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There is a substantial and growing need for accurate, reproducible in vitro test systems capable of replacing animal models in the evaluation and classification of chemical agents. The available human epidermal cell lines poorly duplicate the in vivo response of human skin to chemical agents. Tissue engineering strategies provide for models that more closely recapitulate the native environment. Stratatech Corporation has developed and standardized Stratatest™ tissue, a novel in vitro human skin model, for consumer product and drug discovery testing. This model is generated using NIKS® keratinocytes, a consistent source of non-tumorigenic, pathogen-free human keratinocyte progenitors. Stratatest™ tissue, which contains both epidermal and dermal components, is a fully-stratified, multi-layered tissue. Stratatest™ tissue exhibits the biological characteristics of human skin. Histological analysis revealed appropriate tissue architecture formation. Indirect immunofluorescent detection confirmed the expression pattern of proteins essential for the integrity, structure, and function of stratified squamous epithelial tissue. By measuring skin electrical impedance, we determined that Stratatest™ tissue possesses robust barrier function, a critically important parameter of skin function. The utility of this model for toxicity testing was evaluated by measuring the response of Stratatest™ tissues to the known irritant SDS. Tissues exposed to a range of concentrations were assayed for cytotoxic effects. Cell viability was determined by using MTT reduction and the extent of IL-1α secretion was detected by ELISA. Both cell viability and IL-1α secretion revealed a clear dose-dependent response. Evaluation of four independent Stratatest™ tissue batches indicated a high degree of reproducibility. These results not only demonstrate the similarity of Stratatest™ tissue to intact skin, both in structure and function, but support use of this reproducible and reliable model for toxicological screening applications.

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


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

Keywords: skin burns, sulfur mustard, peptide, inflammation

137 A STUDY OF PHOTOTOXICITY EVALUATION BY ORAL ADMINISTRATION TO BALB/c MICE.


[Purpose] The phototoxicity of known phototoxic substances was evaluated in Balb/c mice by oral administration, in accordance with Wagai’s method (1989) since there is a dearth of information about phototoxicity studies via oral administration. [Method] Nalidixic acid (NA) and 8-methoxypsoralen (8-MOP) were used as positive phototoxic substances. Male Balb/c mice (BALB/cAnNCrlCrlj, Charles River Japan Inc.), aged 6 weeks were used in the study. On the day before administration, the backs of the animals were shaved, and they were fasted from 4 hours before administration. The dosing preparations (400, 200, 100, and 50 mg/kg NA, and 40, 20, and 10, and 5 mg/kg 8-MOP) were administered at 10 mL/kg. Thirty minutes after administration, the animals were irradiated with long-wave ultraviolet rays of approximately 20 J/cm2 (approximately 2.8 mW/cm2, approximately 120 minutes), using an Ultraviolet Irradiation Apparatus (Dermaray, M-DMR-50, Eisai Co., Ltd.). The thickness of both ears was measured with a dial thickness gauge (Ivaco G-2, Ozaki Mfg. Co., Ltd.), and the ears and back skin were observed before administration and at 0.5, 24, and 48 hours after irradiation. Skin reaction (erythema and edema) at the ear and back skin were scored in accordance with the Draize method criteria. [Result] In the 100 mg/kg NA group and above, erythema scored as 1 to 3 was observed at irradiated sites in all animals from 0.5 hours after irradiation, and edema scored as 1 was observed at 24 or 48 hours after irradiation. In the 10 mg/kg 8-MOP group and above, erythema scored as 1 was observed at irradiated sites in all animals from 0.5 hours after irradiation, and edema scored as 1 or 2 was observed at 24 or 48 hours after irradiation. Ear thickness incrementally increased at 600 and 200 mg/kg NA, and at 40, 20, and 10 mg/kg 8-MOP after irradiation. In this study, positive reactions were clearly observed, and ear thickness tended to reflect skin reaction.

138 TOPICAL APPLICATION OF ANTI-INFLAMMATORY COMPOUNDS IN AN EX VIVO HUMAN SKIN INFLAMMATION MODEL.


Atopic dermatitis (AD), a common chronic skin disease characterized by abnormal cytokine production and frequent skin infections, affects 10-20% of children and 2-3% of adults. AD has been reported to be associated with the activation of T cells that preferentially secrete Th2-like cytokines including IL-4, IL-5, IL-10, and IL-13; this can be mimicked by exposure of the skin to lipopolysaccharide (LPS). This study aimed to demonstrate the therapeutic effect of various anti-inflammatory drugs used in the treatment of AD can be measured in explant human skin cultures stimulated with LPS. Full thickness skin biopsies (3mm diameter) from ethically obtained tissue were inserted into 2mm holes cut into Tiswax filters (pore size 0.4μm). The filter was placed in a well of a 24-well culture plate.
with 1ml of culture medium (RPMI) containing 1% heat-inactivated human serum and 1% penicillin/streptomycin, with the epidermis facing upwards at the liquid-air interface and the dermis suspended in the culture medium. In order to stimulate inflammation, 10 μM LPS was added directly to the culture medium, whereas dexamethasone cream LAW (Riemser, 0.05%) or triamcinolone acetonide cream (Ratiopharm, 0.1%) were applied topically to the epidermis. Culture plates were incubated at 37°C in an atmosphere of 5% CO₂, in air, for 24h. LPS induced an increase in the concentrations of IL-6, IL-8 and IL-10 in the culture supernatants of 298%, 177% and 248% respectively. Topical application of dexamethasone and triamcinolone acetonide caused a reduction in the LPS stimulated release of IL-6 (32% and 82% respectively), IL-8 (114% and 121% respectively) and IL-10 (70% and 147% respectively) to almost control levels (100% reduction) detected in unstimulated skin biopsies. Triamcinolone acetonide was more effective at reducing the levels of inflammatory cytokines than dexamethasone. In summary, Biastra’s full-thickness human skin culture system appears to be a suitable model for assessing the effectiveness of potential new topical treatments for AD, using IL-6, IL-8 and IL-10 as markers of inflammation.

139 EFFECT OF BREVENAL ANTAGONISM IS CELL LINE DEPENDENT.

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The dinoflagellate responsible for Florida red tides, Karenia brevis, produces at least a dozen toxins called brevetoxins and curiously also produces an antagonist, brevemal. Brevemal is a non-toxic short-chain trans-syn polyether molecule that competes with brevetoxin for its active site on voltage-gated sodium channels. Previous studies in our laboratory have indicated that 0.1 μg/ml and 1.0 μg/ml brevetoxin in combination with brevemal 2 at 10-4M to 10-7M is capable of preventing inhibition of CHO-K1 BH4 cell proliferation by brevetoxin. For the current study Jurkat E6-1 Cells were treated with brevetoxin 2 at 10-5,25M (IC50 established in a previous experiment) in combination with 0.1 μg/ml, 0.5 μg/ml and 1.0 μg/ml brevetoxin and A549 bronchial epithelial cells were treated with Brevetoxin 2 at 10-12M to 10-5M in combination with 0.1 μg/ml, 0.5 μg/ml, and 1.0 μg/ml brevetoxin for 48 hours. Cell counts taken at 48 hours indicate that brevetoxin does not antagonize brevetoxin’s effect on cell proliferation in the T cell lymphoma line Jurkat E6-1. However, A549 bronchial epithelial cells showed a significant increase in proliferation for all doses of brevetoxen tested. The antagonistic effects of brevetoxen are dependent on the type of cell tested and it is possible that this difference may be exploited when determining the pharmacological efficacy of brevetoxen. Research supported by Marine Biotechnology in North Carolina.

140 DEVELOPMENT OF A SPECIFIC ASSAY FOR THE DETERMINATION OF AROMATASE ENZYME ACTIVITY.

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Concern over endocrine disruption is gaining momentum as reflected by the EPA’s Endocrine Screening Program as well as the possible inclusion of cut-off criteria for compounds with endocrine disrupting properties in the revised 91/414 guidelines for pesticides. One of the tests requested by the EPA is an in vitro assessment of aromatase activity; however there are drawbacks to this approach as only inhibition of the enzyme can be detected and there is no metabolic component to the assay. To address these shortcomings, we have developed an assay which can detect both increases and decreases in aromatase activity and can be used to assess tissue samples following in vivo exposure. The aromatase reaction proceeds by co-incubation of aromatase and aromatase inhibitor Anastrazole & Formestane. Under the same conditions of test, Aminoglutethimide inhibited aromatase activity to a lesser extent and Chrysin appeared to have no inhibitory activity. Marked increases in ovarian aromatase activity have been recorded when comparing ovarian samples from non-pregnant rats with samples from rats in late pregnancy. Our aromatase enzyme data are supported by corresponding gene and protein expression data and are consistent with literature reports. In conclusion, we have developed a specific assay to detect both aromatase enzyme inhibition and activation. This assay can be applied to both in vitro and in vivo assessments as well as to tissues other than the ovaries.

141 ESTROGEN RECEPTOR ALPHA OVEREXpressING MICE MAY BE A USEFUL TOOL FOR STUDYING THE EFFECTS OF ESTROGENIC COMPOUNDS ON THE REPRODUCTIVE SYSTEM.

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Estrogen is required during growth and development of the nervous, skeletal, immune, and reproductive systems. Estrogen receptor alpha (ESR1) acts as a physiological mediator of estrogenic action within these systems. To observe the effects of estrogenic compounds on the ESR1 pathway, we have generated and validated a transgenic mouse model that overexpresses ESR1 in several tissues, including the ovaries. Previous studies have shown that ovaries from ESR1 overexpressors (ESR1 OE) are morphologically normal. However, previous studies did not assess whether ovaries from ESR1 were functionally normal. Since estrogen is important for ovarian follicle growth and estradiol synthesis, this study tested the hypothesis that ovarian follicles from ESR1 OE grow faster and synthesize altered levels of estradiol compared to control mice. Antral follicles were isolated from ESR1 OE and control mice on postnatal day (PND) 30 and cultured in supplemented 6-minimum essential media for 168 hours. Individual follicle diameters were measured every 24 hours. After culture, media was collected to perform competitive enzyme-linked immunosorbent assays for estradiol levels. The data indicate follicle growth was similar in ESR1 OE and controls (controls at 168h = 461.25 ± 30.65 μm; ESR1 OE at 168h = 465.94 ± 30.39 μm; n = 4). In addition, estradiol levels were similar in ESR1 OE and controls (controls at 168h = 2103.75 pg/ml ± 411.49; ESR1 OE at 168h = 1905.45 pg/ml ± 391.5; n = 4). Estradiol levels were also normalized to follicle size and no significant difference was found between ESR1 OE and controls. Collectively, these data indicate that ESR1 overexpression has no effect on mouse follicle growth or estradiol production in vitro. Therefore, ESR1 OE mice could prove to be an important tool for determining whether and how other estrogenic and xenobiotic compounds act through ESR1 and affect the reproductive system.

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142 ACTIVATION OF THE AHR HYDROCARBON RECEPTOR (AHR) DURING PREGNANCY IMPAIRS MAMMARY EPITHELIAL CELL DIFFERENTIATION THROUGH DIRECT AND INDIRECT ACTIONS.

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Recently our laboratory made a novel discovery that exposure to the AHR agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) during pregnancy causes severe defects in mammary differentiation, with decreased ductal branching, poor formation of alveolar structures, suppressed expression of milk proteins, and failure to nutritionally support offspring. The development of the mammary gland during pregnancy is a complex process, driven by numerous molecules produced systemically and locally. AHR activation by TCDD may impair mammary development through a direct effect on mammary epithelial cells (MECs), or by altering paracrine or systemic factors that drive pregnancy-associated differentiation. Using AHR-deficient (AHRKO) mice, we show that pregnant females have normal development of the mammary gland in the presence or absence of TCDD. Therefore, the effects of TCDD on mammary development are AHR mediated, and the presence of AHR is not crucial for the development of the mammary gland. To experimentally determine whether exposure to TCDD alters mammary gland development during pregnancy by a direct or indirect effect on MECs, we transplanted MECs from pre-puberal AHRKO mice into wild-type (WT) mice and vice-versa. We analyzed glandular differentiation based on morphology and acini formation as well as of parturition. The glands were phenotyped for AHR protein expression to validate transplanted MECs from WT donors to AHRKO host gland. TCDD appears to impair mammary parenchymal tissue development in both a direct and indirect manner. This novel finding helps to explain the mechanisms by which AHR-mediated impairment of mammary development occurs during pregnancy.
ARSENIC INHIBITION OF T47D CELL GROWTH IS ASSOCIATED WITH PROMOTER HYPMETHYLATION OF RASSF1A AND CCND2 GENES.

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Recent evidence has shown that arsenic acts as an environmental endocrine disrupter and has anti-proliferative effects in breast cancer cells. We previously reported that sodium arsenite (As) inhibited DNA synthesis stimulated by 100 pM estrogen (E2) in human breast cancer derived T47D cells, whose growth is E2-dependent. Under a longer treatment (6 days), As (0.1–5 μM) also inhibited E2 stimulated T47D cell growth. Tumor suppressor gene RAS association domain family 1A (RASSF1A) controls mitosis/centrosome at early cancer stages. Cyclin D2 (CCND2) is an important gene involved in cell cycle regulation. Both genes are silenced in most human breast cells due to promoter hypermethylation. Using methylation-specific PCR (MSP), we found that promoters of these two genes are heavily methylated in actively growing T47D cells, but not in non-tumorigenic human breast MCF 10F cells. Since arsenic has been reported to interfere with DNA methyltransferase and cell cycle regulation, we investigated if As changed the methylation status of these two tumor suppressor genes. Compared to cells treated with E2 (100 pM) alone, cells treated with E2 (100 pM) and As (0.1 μM) together exhibited lower methylation levels of the promoter regions of both RASSF1A and CCND2.

AMPLIFICATION OF GLUCOCORTICOID-INDUCED CYTOXICITY OF NB2 LYMPHOMA CELLS BY RESVERATROL: EVIDENCE FOR A NOVEL MODE OF ACTION FOR POTENTIAL ENDOCRINE DISRUPTORS.

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NB2 rat lymphoma cells exhibit glucocorticoid receptor mediated apoptosis upon exposure to such compounds as dexamethasone, cortisol, or corticosterone. Accordingly, we have employed these cells as a “G-screen” assay system for the identification of xenobiotics with glucocorticoid or anti-glucocorticoid activity. Previous screening of 49 diverse chemicals implicated in endocrine disruption (e.g., heavy metals, organochlorines, flavonoids, phthalates, alkylphenols, diphenyl derivatives) exhibited no such activity over a 100,000-fold concentration range (0.1 nM to 10 μM) (Witorsch et al., The Toxicologist Supplement to Toxicological Sciences: 96: 399, 2006). In this study we report that addition of resveratrol (10 μM), a plant-derived stilbene with little or no cytolytic activity, amplifies the dose-dependent cytolytic effect of either dexamethasone (1.56 to 25 nM) or corticosterone (6.25 to 100 nM) two to four-fold. The enhanced cytolysis produced by addition of resveratrol to dexamethasone (6.25 or 25 nM) or corticosterone (100 nM) is inhibited by the further addition of the glucocorticoid receptor antagonist, RU 486 (500 nM). Whereas amplification of dexamethasone-induced cytolysis is observed with 10 μM resveratrol, no such effect was observed with lower concentrations (1 nM to 1 μM) of the stilbene. Studies are currently underway to identify other xenobiotics that might modify glucocorticoid-induced apoptosis of Nb2 cells. Since endocrine disruption is usually associated with hormonally active xenobiotics, this serendipitous observation with resveratrol suggests an alternative and novel mode of action for potential endocrine disruptors, modulation of endogenous hormone interaction with the target cell. (Supported by a grant from the Thomas F. and Kate Miller Jeffress Trust).

EVALUATION OF PITUITARY AND ADRENAL HORMONE RELEASE FOLLOWING EXPOSURE TO ATRAZINE AND ITS METABOLITE DEISOPROPYLATRAZINE (DIA), USING TISSUE PERIFUSION.


Atrazine (ATR) is one of the most widely used herbicides in the United States, with current total annual use of approximately 76 million pounds of active ingredient. Previous work in our laboratory has shown that ATR and its metabolite desisopropylatrazine (DIA) induce a dose-dependent increase in plasma ACTH, and serum corticosterone (CORT) and progesterone (P4), at 15 and 30 min after a single oral administration in vivo. Although the neuroendocrine mechanism for this increase in hypothalamic-pituitary-adrenal axis activation is currently unknown, ATR and DIA have been reported to directly alter steroidogenesis in vitro. To test whether ATR and DIA have a direct effect on the pituitary or adrenal glands, we examined hormone release from pituitary and adrenal tissue exposed to 50 μM ATR or DIA, using a perfusion procedure. Tissues from adult male Wistar rats were exposed to two, twenty-minute challenges over a three hour period. A positive control challenge was included for pituitary (10nM CRF) and adrenal (1nM ACTH) tissue, as well as final challenge with KC1 (60mM, pituitary), or ACTH (10nM, adrenal) to test for viability of the tissue at the completion of the perfusion. ATR and DIA had no apparent effect on release of ACTH or CORT. Interestingly, under these conditions, the parent compound (ATR) had no effect in either tissue, while treatment of adrenal tissue with its metabolite DIA produced a greater than twofold increase from basal P4 release (3-7 pg/mg/min) during the 90 min following the onset of the first challenge. The DIA-induced increase in P4 release from the adrenal demonstrates a direct effect of the chemical on steroidogenesis in this tissue. These results suggest that increased adrenal hormone release observed in the male Wistar rat following in vivo exposure to these chloroanilines is, in part, the result of a direct effect on the gland. This abstract does not necessarily reflect EPA policy.

EFFECTS OF 2, 3, 7, 8-TETRACHLORODIBENZOP-DIOXIN ON INSULIN SECRETION STIMULATED BY GLUCOSE IN MICE.

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Epidemiological and laboratory studies suggested that exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) affects glucose homeostasis and increased a risk of the onset of type-2 diabetes mellitus. In order to evaluate effects of TCDD on insulin secretion from Langerhans islets, we designed in vivo, ex vivo and in vitro experiments. In vivo Experiment: Male C57BL/6J and AhR-null mice were injected TCDD (10 μg/kg b.w.) i.p. fasted for 12 hours and administered glucose 24 hours post-administration. TCDD exposure significantly decreased plasma insulin concentration at 60 and 120 min after glucose challenge in C57BL/6J mice but not in AhR-null mice. In contrast, the plasma glucose concentration was not changed by TCDD exposure in the both strains. Ex vivo Experiment: We isolated Langerhans islets 24 h after TCDD administration, and determined glucose-stimulated insulin secretion from the islets. The insulin release was found to be significantly decreased by TCDD exposure at 60 min after glucose treatment. In vitro Experiment: Langerhans islets harvested from intact C57BL/6J mice were exposed to 0.1-100 nM TCDD for 24 h, followed by the determination of glucose-stimulated insulin secretion from the islets. Insulin secretion from the islets did not change by any doses of TCDD. In conclusion, insulin release in response to glucose stimulation was impaired by TCDD exposure and this effect was mediated by AhR signaling pathway. However, it is thought that TCDD did not directly affect the Langerhans islets. (This work was supported in part by Global COE Program ‘Medical System Innovation on Multidisciplinary Integration’ from MEXT, Japan.)

DISRUPTION OF STEROIDOGENESIS BY DI-(2-ETHYLHEXYL) PHthalate and its Metabolites in MA-10 Cells.

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To improve the flexibility of plastics and aid in processing, small molecules called plasticizers are often added to the polymer blend. These molecules readily leach out of plastics and therefore have become ubiquitous in the environment. It has been estimated that humans are exposed to plasticizers on the order of 10 mg/day. This is a concern as correlations between testicular dysgenesis syndrome and exposure to plasticizers have been found. The cause of reproductive tract abnormalities is believed to be reduced testosterone production by Leydig cells. In this study we use the MA-10 mouse Leydig tumor cell line which produces prostategen as a marker for testosterone. To test the effects of the common plasticizer di-(2-ethylhexyl) phthalate (DEHP) and four of its known metabolites; mono(2-ethylhexyl) phthalate (MEHP), 2-ethylhexanol, 2-ethylhexanal and 2-ethylhexanoic acid. Cell viability (MTT assay, Sigma-Aldrich, Inc.) and prostategen production (ELISA, Fitzgerald Industries Int.) were evaluated after 24hrs of exposure to varying concentrations. The dose dependent effects of DEHP, MEHP and 2-ethylhexaanal showed significantly reduced cell viability at high concentrations (≥ 1x10⁻⁴ M). The alcohol and acid had little effect on cell viability. DEHP, 2-ethylhexanal and 2-ethylhexanoic acid reduced prostategen production at concentrations as low as 1x10⁻⁴ M. 2-ethylhexanal showed the greatest suppression of prostategen (>60%), at concentrations that had little effect on cell viability. Understanding the response of Leydig cells to the degradation products of common plasticizers will ultimately allow the development of new plasticizers that avoid these bioactive metabolites.
While aryl hydrocarbon receptor (AhR) agonists including dioxins and polycyclic aromatic hydrocarbons (PAHs) are well known to disrupt thyroid function, we could study ethyl alcohol (EtOH) pharmacokinetics in sham-operated (SO) rats, because the absence of thyroid stimulating hormone (TSH) disrupts thyroid function and we could study EtOH pharmacokinetics in the TEN model in a low thyroid hormone state. We found that while body weight gains were lower (P<0.05) in EtOH rats, pulsatile BECs did not differ in FDX and HD rats. We conclude that metabolism of EtOH in the TEN model is not influenced by thyroid function. Thus, in the absence of data to the contrary, we have proposed that pulsatile BECs produced by cyclic EtOH metabolism and excretion of EtOH. French et al. previously proposed that these EtOH pulses were due to altered thyroid function at high blood EtOH concentrations and consequent effects on basal metabolic rate (Am. J. Physiol. 2000; 279:G118-G125). We tested this hypothesis in hypophysectomized (HX) or sham-operated (SO) rats, because the absence of thyroid stimulating hormone (TSH) disrupts thyroid function and we could study EtOH pharmacokinetics in the TEN model in a low thyroid hormone state. We found that while body weight gains were lower (P<0.05) in EtOH rats, pulsatile BECs did not differ in HX rats and HD rats. We conclude that metabolism of EtOH in the TEN model is not influenced by thyroid function. Thus, in the absence of data to the contrary, we have proposed that pulsatile BECs produced by cyclic EtOH metabolism caused by time-dependent EtOH pharmacokinetics which initiates inhibition of hepatic insulin signaling that leads to reduced SREBP1 and inhibition of class 1 ADH transcription (JBC 2006; 281:1126-34) Supported in part by NIAAA008645.

149 POTENTIAL ROLE OF THE ADRENA L AXIS ON THE REPRODUCTIVE EFFECTS OF ATRAZINE.

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We and others reported that atrazine (ATR) disrupts the regulation of the ovulatory luteinizing hormone (LH) surge and the hormonal control of other reproductive functions in the rat. In addition, administration of ATR or the intermediate metabolite desisopropylatrazine (DIA) stimulate adrenocorticotropic (ACTH), cortisol (CORT), and progesterone (P4). In contrast, the diaminochlortizaine (DACT) metabolite minimally affects the hypothalamic-pituitary-adrenal (HPA) axis. As adrenal activation may adversely affect LH secretion, these findings suggest that an atrazine-induced activation of the HPA axis may be an important part of the mode of action (MOA) through which the chlortizazines alter reproductive function. Therefore, we hypothesized that only those metabolites that activate the HPA axis will affect LH release. Long-Evans rats with 4-day estrous cycles were reared for 5 minutes or dosed by oral gavage at 0900h over one estrous cycle with 0.1% methyl cellulose vehicle, 75 mg/kg ATR, 10 or 60.2 mg/kg DIA, and 8.4 or 50.6 mg/kg DACT (14/10 light cycle, on 0500h). Dosing began on the day of vaginal estrus. On the day before estrous, doses of ATR were decapitated at 1600, 1800, and 2000h. Serum LH, prolactin (PRL), and P4 were measured by radioimmunoassay. Restraint, ATR, and both doses of DIA decreased the amplitude of the proestrous LH surge. In contrast, only the highest dose of DACT affected the surge. PRL was unchanged by any treatment. Unlike ATR, both doses of DIA decreased P4. Higher plasma levels achieved by direct dosing with DIA may have contributed to this. These results confirm that treatments which activate the HPA axis also alter LH release. These data suggest that activation of this axis contributes to the ATR- and DIA-induced disruption of LH secretion. Other MOAs may also be explored due to the ability of DACT to alter LH without significant adrenal activation. Additional studies are planned using specific inhibitors of the HPA axis to determine the role of this axis in the reproductive toxicity of ATR. This abstract does not necessarily reflect EPA policy.

150 TRANSCRIPTIONAL ACTIVATION OF ANDROGEN-REGULATED GENES BY ARYL HYDROCARBON RECEPTOR AGONIST IN ANDROGEN-RESPONSIVE CELL LINES.

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While aryl hydrocarbon receptor (AhR) agonists including dioxins and polycyclic aromatic hydrocarbons have attracted attention in terms of endocrine disrupting effects, little is known about effects of AhR agonists on androgen receptor (AR) sig-
concentrations and was more potent than methimazole for inhibition of T4 release.

The action of this chemical on thyroid hormone function will be evaluated further in vivo in an X. laevis-based amphibian metamorphosis assay. This suite of assays provides a framework for determination of the potential for chemicals to affect thyroid hormone status. This abstract does not necessarily reflect EPA policy.

153 ANTI-PROLIFERATIVE AND ANTI-STEROIDOGENIC EFFECTS OF POMEGRANATE COMPOUNDS IN HUMAN LNCaP PROSTATE AND H295R ADRENOCORTICAL CANCER CELLS.

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Prostate cancer is the second leading cause of cancer-related deaths among men in many Western countries. It is a hormone-dependent cancer and its proliferation is stimulated by endogenous steroid hormones. Aromatase (CYP19) and 5α-reductase (SRD5A) are the key enzymes that synthesize these hormones. Aromatase converts androgens to estrogens and 5α-reductase converts testosterone to DHT.

Natural compounds present in the pomegranate delay prostate cancer progression. They may act as inhibitors of steroidogenesis. Pomegranate compounds (punicic-, quinic-, gallic, trans-vaccenic, and cis-vaccenic acid, pelargonidin, cyanidin, malvidin- and delphinidin chloride, epicatechin gallate, epicatechin, kaempferol, and epigallocatechin) were tested in vitro in a hormone-dependent prostate cancer cell line (LNCaP cells) and steroidogenesis (human adrenocortical H295R cells) model. Cells were treated with 1x10^-5 and 2x10^-5 cells/well in 24-well culture plates exposed to various concentrations of pomegranate compounds for 5 days with a fresh re-exposure after 48 h. For cytotoxic effects, H295R were exposed once to pomegranate compounds for 24 h. Cytotoxicity and antiproliferative effects were measured with MTT reduction assay. Catalytic activity of CYP19 was determined in H295R cells by tritiated water-release assay. Enzymatic activity of 5α-reductase was determined in LNCaP cells by LC-MS by measuring conversion of testosterone to DHT. Punicic acid had an antiproliferative effect in LNCaP cells reducing cell viability for 33 and 87% at 30 and 100 μM respectively. Punicic acid had an antiproliferative effect in LNCaP cells reducing cell viability for 33 and 87% at 30 and 100 μM respectively. Punicic acid reduced aromatase activity by 77% compared to control. CYP19 was not expressed in LNCaP cells.

Preclinical results show that pomegranate compounds have an antiproliferative effect in LNCaP cells and punicic acid acts as an aromatase inhibitor in H295R cells.

154 CRF ANTAGONIST EFFECTS ON THE EXPRESSION OF GHRELIN, ITS RECEPTOR (GHSR) AND A POSSIBLE IMPACT OF THE GROWTH HORMONE AXIS.

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Ghrelin stimulates growth hormone (GH) secretion in a variety of tissues including the mammary gland where the hormone serves to maintain a proliferative, rather than a differentiated phenotype. Following repeat dosing of a CRF antagonist for 2 weeks, mammary gland hyperplasia was observed accompanied by a significant increase in mammary expression of mRNA for both ghrelin (GHRIL) and its cognate receptor, growth hormone secretagogue receptor (GHSR) in gland tissue. In proliferating (hyperplastic) mammary tissue from pseudo-pregnant castrated male rats, abundant expression of GHRIL is observed, well above that observed in control animals. We evaluated whether CRF antagonists could alter proliferation of human breast adenocarcinoma cells (MCF-7), and whether GHRIL and GHSR expression was changed. Increased proliferation of MCF-7 cells was observed following exposure to certain CRF antagonists. Using TaqMan™ real time PCR, we observed that GHRIL was upregulated (1.7 x control) with 17 hours of treatment, while GHSR expression was unchanged. By 20 to 24 hours, GHRIL was downregulated -1.4 x control) while the GHRIL mRNA was upregulated (1.3 x control). These data are consistent with our hypothesis that GHRIL expression increases in the mammary gland during proliferation stimulated by either pseudopregnancy or specific CRF antagonist treatment. The data further support a mechanism whereby CRF expression is upregulated until GHRIL binds to the receptor, which quickly triggers GHRIL downregulation to reduce the overall stimulatory GH effects.

155 HYDROXYLATED PCBs INHIBIT AROMATASE ACTIVITY IN HUMAN PLACENTAL MICROSOMES.

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PCBs have been widely used in industrial and commercial applications and largely prohibited since the 1980’s. PCDDs are still abundant pollutants in humans and wildlife and can be bio-transformed into two classes of metabolites, hydroxylated (OH) and methylsulfone (MeSO2) PCBs. Previous studies have shown that these metabolites act as endocrine disruptors by affecting steroidogenesis. In the present study, human placental microsomes were used to assess the effect of four common OH-PCBs on aromatase activity. Aromatase is a key enzyme in the steroidogenesis pathway and mediating conversion of androgens into estrogens. We tested four metabolites of the environmental common PCB 180. All four OH-PCBs showed similar inhibition of placental aromatase activity. More specifically, 4′-OH-CB172, 3′-OH-CB180, 3′OH-PCB182 and 5′-OH-CB183 had an estimated IC50 of 10.2 μM, 13.3 μM, 41.6 μM and 31.5 μM, respectively. Moreover, the LOAEL of these OH-PCBs was similar and in the low μM range. The human placenta is largely responsible for exposure of the foetus to estrogens and androgens and has a high expression of aromatase. Moreover, this tissue is essential in the blood flow exchange between mother and foetus and therefore an important route of exposure of the un-born to PCBs and their metabolites. OH-PCBs are known to be more efficiently transferred to the foetus than the parent compounds. Measured levels of individual OH-PCBs in cord plasma have found to be 0.5 nmol. Based on the results of our present study, no effect on placental aromatase by these PCB metabolites is to be expected. However, many more OH- and MeSO2-PCBs have been found in humans and wildlife and structure activity relationships (SAR) for aromatase inhibition are yet unknown. In this future exposure study, the SAR for placental aromatase inhibition should be determined to identify plausible risk. Further studies are now in progress in our laboratory to assess the specificity in relation to the position of the OH or MeSO2 groups.

156 ANTI DIABETIC AND ANTIOXIDANT ACTIONS OF TAURINE AND STRUCTURALLY RELATED SULFUR COMPOUNDS IN STREPTOZOicotIN-INDUCED DIABETES IN RATS.

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This work has assessed the ability of taurine (TAU) and the structurally-related compounds aminomethanesulfonic acid (AMSA), homotaurine (HMTAU) and pantoyltaurine (PTAU) to attenuate hyperglycemia, hyperlipidemia and oxidative stress associated with diabetes in a small animal model. For this purpose, groups of 6 male Sprague-Dawley rats were divided into three groups: control group, diabetic group, and diabetic group treated with a sulfur compound. Diabetes was induced using a single, 60 mg/kg, intraperitoneal (i.p.) dose of streptozotocin (STZ). A sulfur compound was administered as two 1.2 mM/kg i.p. doses, 75 min and 45 min before STZ. Control animals received saline. Blood samples were collected by decapsulation into tubes containing disodium EDTA, and processed for their plasma fraction. Plasma glucose (GLU), cholesterol (CHOL), triglycerides (TG), malondialdehyde (MDA), reduced glutathione (GSH) and nitric oxide (NO) were measured using standard methods. Diabetes elevated GLU (>400%), CHOL (135%), TG (184%), MDA (75%) and NO (~5.6-fold) and decreased GSH (79%). Regardless of their carbon-chain length and the presence or lack of N-substitution, all the test compounds demonstrated a significant attenuating action on all plasma alterations examined here, with the magnitude of the effects varying within a narrow range of values, especially in terms of their effect on indices of oxidative stress. The present results suggest the need for a future study that will evaluate the effects of the present sulfur compounds when administered to diabetic animals on a chronic basis.

157 DEVELOPMENT OF A HIGHLY SENSITIVE ANALYTICAL METHOD TO DETECT ESTROGEN METABOLITES IN BIOPROFILLET SAMPLES.


Endocrine disruption and development of hormone-dependent cancers in humans may be associated with dietary exposure to low concentrations of estrone (E1), estradiol (E2) and their estrogenic and carcinogenic metabolites, e.g. 4-hydroxyestradiol (4-OHE2). For experimental, epidemiological and dietary exposure studies, it is essential for these metabolites to be analyzed with detection limits in the pg/ml range, and lesser sensitivity on GC-MS or LC-MS. We developed an analytical method using HPLC-MS/MS, able to determine low levels (<2pg/ml) of hydroxylated estrogen metabolites. The method includes desulfuration of conjugated estrogens in the biological matrix, solid phase extraction and derivatization with either danyl chloride (DNS-CI) or pyridine-3-sulfonyl chloride (PS-Cl) to enhance ionization, detection and quantification by HPLC-MS/MS. The DNS-CI method worked efficiently for E1 and E2, while the PS-Cl method enhanced detection for...
2 and 4-hydroxylated metabolites of E1 and E2. Using human urine as initial biological matrix the following LODs were found: 10-30 pg/ml for E1, E2, 16αOHE1, 20αOHE1, 20αOHE2, 4αOHE2, corresponding to an instrument sensitivity of 1.6-3.1 pg on column for the DSN-Cl estrogens and a 1.4-7 pg on column sensitivity for the PS-Cl hydroxylated estrogens. Absolute recovery ranged from 50 to 70%. In view of the sensitivity of present method, estrogen metabolism can now be studied in a wide variety of biological matrices using small sample sizes, e.g. 1 ml. Applications can include serum, urine, culture medium from in vitro experiments and dairy products. Presently we focus on estrogen metabolism in in vitro systems and identification of estrogen metabolites in dairy products.

158 IODIDE DEFICIENCY, THYROID HORMONES, AND NEURODEVELOPMENT.

Iodide is an essential nutrient for thyroid hormone synthesis. Severe iodide insufficiency during early development is associated with cognitive deficits. Environmental contaminants can perturb the thyroid axis and this perturbation may be more acute under conditions of marginal iodine deficiency. The present study examined the relationships between graded levels of iodide deficiency in pregnant rats on hormonal status and behavioral indices of brain function in exposed offspring. This data is being generated to inform an exposure-dose-response model for developmental disruption of thyroid hormones. Female Long Evans rats (n=60, 60 days of age) were placed on diets with varying degrees of iodine deficiency for 7 wks prior to breeding. Iodine was added to a casein-based diet (25 μg/kg backgound levels) resulting in the following experimental groups: total 25 μg/kg (severely deficient), 75, 175, 275 (normal control diet) and 1025 μg/kg (excess). Pups were sacrificed on postnatal day (PN) 4, 14 and 21 and brain, liver, heart, thyroid glands, and brain were harvested. Dams were sacrificed at weaning for hormone analysis. Behavioral assessments were performed on male and female offspring (4-5 months of age) using a sensory gating paradigm based on auditory startle, spatial learning using a Morris water maze, and associative learning using a trace fear conditioning paradigm. Thyroxine was reduced (~45%) and TSH increased (2-3 fold) in dams and pups on PN21 in the 25 μg/kg iodine group. No changes in T3 were evident. Thyroid weights were significantly increased in pups on PN21 with nonsignificant increases also evident in dams. Preliminary analyses reveal no evidence of impairment in any of the behavioral tasks in either gender. These data demonstrate dose-dependent effects of iodine deficiency on thyroid hormones during development, however no impairments in behavioral indices of cognitive function were detected. (Does not necessarily reflect EPA policy)

159 IODIDE DEFICIENCY: METABOLIC AND HORMONAL STUDIES FOR PBPK MODELING.

Iodine is an essential nutrient and is critical for synthesis of thyroid hormones. Severe iodine deficiency is known to cause adverse health outcomes and remains a benchmark for understanding the effects of hypothyroidism. However, the implications of modest iodine deficiency on function of the thyroid axis remain unknown. The current study examined the relationship between graded levels of iodine deficiency in rats on thyroid hormones, thyroid iodine content, and urinary iodide excretion. Long Evans rats (n=25, female, 60 days of age) were placed on casein diets containing varying iodine concentrations for 8 weeks. Different iodine diets were created by adding 0, 50, 150, 250 and 1000 μg/kg to the base diet creating 5 iodine levels ranging from deficient (25 μg/kg) to normal (275 μg/kg) to excess (1025 μg/kg). Food and water intake were monitored daily. Urine was captured in metabolism cages over a 6 h period on 2 consecutive days each week. At 8 weeks animals were sacrificed and blood and thyroid glands collected for thyroid hormones and iodide content, respectively. Neither food nor water intake varied as a function of treatment group over the course of the study. Animals in the lowest and the two highest iodine groups were of similar weights at the end of the study, while the intermediate treatment groups were ~6% lighter in weight. Serum total thyroxine (T4) was dose-dependently reduced with significant declines (~35 and 60%) at the two lowest iodine groups. No change in serum T3 was detected. At the end of the study, dose-dependently increases in thyroid weight (5.3, 6.6, 7.4, 8.1 and 11.5 μg) and declines in thyroidal (6.06, 5.58, 5.08, 5.02, 2.53 ng/ml) and urinary (6.06, 165, 84, 28.5, 20.1 ng/ml) iodide content were observed as a function of iodine in the diet. These data document a viable model of iodine deficiency to inform the development of physiologically based pharmacokinetic models. (Does not necessarily reflect CDC or EPA policy)

160 REPRODUCTIVE TOXICITY AND PHARMACOKINETICS OF DI-N-BUTYL PHTHALATE (DBP) FOLLOWING DIETARY EXPOSURE OF PREGNANT RATS.
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Most rodent studies examining the developmental toxicity of DBP have relied on bolus gavage dosing. It has not been determined whether bolus dosing provides equivalent results to dietary dosing, which more accurately reflects human exposure. This study characterized the developmental toxicity of dietary DBP and compared these results to previous results obtained using gavage dosing. Pregnant CD rats were given 0, 100, or 500 mg DBP/kg/day in diet from gestational day (GD) 12 through 19. DBP exposure ended the morning of GD 19 and rats were killed 4 or 24 h thereafter. Similar to our previous bolus gavage studies, DBP dietary exposure resulted in significant dose-dependent reductions in testicular mRNA concentration of scavenger receptor class B, member 1; steroidogenic acute regulatory protein; aromatase P450, family 17, subfamily a, polypeptide 1; and in vivo P450 family 17, subfamily a, polypeptide 1. These effects were most pronounced 4 h after the end of exposure. Testicular testosterone was reduced 24 h post-exposure in both DBP dose groups and 4 h after termination of the 500 mg DBP/kg/day exposure. Maternal exposure to 500 mg DBP/kg/day induced a significant reduction in male offspring anogenital distance (AGD), and the presence of Leydig cell aggregates, increased cord diameters, and multinucleated gonocytes. Monobutyl phthalate, the developmentally toxic metabolite of DBP, and its glucuronide conjugate were found in maternal and fetal plasma, amniotic fluid, and maternal urine at levels previously reported following bolus gavage. Our results indicate that approximately equal doses from diet or gavage of DBP to pregnant rats result in qualitatively and quantitatively similar responses in male offspring. This study confirms that previous work using gavage is highly relevant for the risk assessment of DBP following human dietary exposure.

161 APPLICATION OF AN INTELLIGENT TESTING STRATEGY TO THE U.S. EPA ENDOCRINE DISRUPTOR SCREENING PROGRAM.
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The US EPA plans to initiate its Endocrine Disruptor Screening Program in early 2009. The EPA has designed its program in two tiers, consisting of in vivo and in vitro screens and tests; the results of Tier 1 screens would trigger further Tier 2 testing. However, the proposed screening battery has been criticized for the cost and time investments required, and its low specificity. We present the costs, in animal resources and result in source and method screening and characterization of the endocrine-disrupting potential of chemicals. The revised preliminary tier includes physical and chemical data, existing toxicological data, and in vitro and (Q)SAR methods that are either validated or nearly validated. The results of this alternative Tier 1 can be used in a weight-of-evidence approach to 1) identify priority chemicals and 2) design an intelligent, chemical-specific strategy for further screening or testing. Such a strategy would greatly reduce the use of animal testing for both Tier 1 and 2, while maintaining a high level of sensitivity, for identification and classification of endocrine disrupting chemicals.

162 SHORT-TERM EXPOSURE TO TRIFLURALIN DISRUPTS THYROID HORMONE HOMEOSTASIS IN RATS.
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Trifluralin is a selective, preemergence 2,6-dinitroaniline herbicide used to control many annual grasses and broadleaf weeds. Human exposure is likely to occur via consumption of vegetables and fruit and possibly fish from trifluralin run off in many annual grasses and broadleaf weeds. Human exposure is likely to occur via consumption of vegetables and fruit and possibly fish from trifluralin run off in water. It has previously been reported that trifluralin causes thyroid follicular cell tumors in rats from chronic feeding studies. Recently trifluralin was shown to reduce thyroxine (T4) by upregulating select hepatic UGT enzymes following a 2
week exposure. In order to generate baseline data for use in future mixtures studies we examined whether moderate to low doses of triclosan would cause any perturbation of thyroid homeostasis in a short-term (4 day) exposure model. Female Long-Evans rats (26 days old, n=66) were dosed via oral gavage with triclosan in corn oil vehicle (1mg/kg) at 0, 0.3, 1, 3, 10, 30, 50 or 300 mg/kg/day for 4 consecutive days. Serum and livers were collected 24 hours after the last dose. Body weight gain was not affected by treatment. Liver to body weight ratio was significantly increased with 300 mg/kg/day triclosan (24%). T4 was significantly reduced by 25% only at the 300 mg/kg/day dose. This work confirms that short-term exposure to triclosan disrupts thyroid hormone homeostasis in rats. (This abstract does not necessarily reflect EPA policy)

163 TRICLOSAN DISRUPTS THYROIDINE: MECHANISMS AND LIFE-STAGE SUSCEPTIBILITY.


Triclosan (5-chloro-2-(4-chlorophenoxy)phenol) decreases serum thyroxine (T4) in the weanling rat. Scientific uncertainties include: by what mode of action (MOA) does triclosan decrease T4, and does triclosan induce hypothyroxinemia in dams and neonates? To test the hypothesis that triclosan decreases T4 via activation of the pregnane X and constitutive androstane receptors, and subsequent up-regulation of hepatic catabolism of hormones, weaning female Long-Evans rats received triclosan po (0-1000 mg/kg/day) for 4 days. Pentoxysorin-0-dihydrase (PROD) and uridine diphosphate glucuronosyltransferase (UGT) enzyme activities were measured in liver microsomes. qRT-PCR was used to measure mRNA expression of cytochrome P450s 1a1, 2b2, and 3a, UGTs 1a1, 1a6, and 2b5, sulfotransferases 1c1 and 1b1, and hepatic transporters OATP1a1 and 1a4, MRP2, and MDR1b. PROD activity increased ~800 percent and T4 glucuronidation increased 2-fold at 1000 mg/kg/day. CYP2b2, CYP3a, UGT1a1, and SULT1c1 mRNA expression levels were induced 2-fold, 4-fold, 2.2-fold, and 2.6-fold at 300 mg/kg/day, respectively. No changes were observed in mRNA levels for any transporters. These data support the hypothesized MOA for that triclosan-induced hypothyroxinemia. To determine if this MOA applies to dams and neonates, pregnant Long-Evans rats received triclosan po (0-300 mg/kg/day) in corn oil from gestation day (GD) 6 to postnatal day (PND) 21. Serum and liver were obtained from pups on PND 4, 14, and 21, and dams on PND 22. Maternal weight gain (GD19-PND1) decreased ~10% with 300 mg/kg/d treatment. Serum T4 was reduced in dams by ~30 percent at 300 mg/kg/day, with no T4 effects observed for PND 4, PND 14, or PND22 pups. This work suggests susceptibility differences between weanlings directly exposed and neonates exposed via dams. This abstract does not necessarily reflect the policy of the US EPA.

164 COMPARATIVE DEVELOPMENTAL THYROID STUDIES WITH 6-PROPYL-2-THIOURACIL (PTU) USING TWO RAT STRAINS (WISTAR VERSUS SPRAGUE-DAWLEY) – RESULTS OF TREATMENT-RELATED EFFECTS IN THYROID HORMONES AND THYROID MORPHOLOGY.

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Two comparative developmental thyroid studies with PTU (Study Design according to EPA Guidance Document [October 2005]) were conducted using different strains of rats (Wistar versus Sprague-Dawley) to determine relative sensitivity. Also, confirmation was needed to determine if Wistar controls had potential residual effects resembling “thyroid gland dysplasia” (term used for condition of follicular cell hypertrophy, decreased T4, & increased TSH in control Wistar rats as reported for RCC breeding colony in mid-1990’s to 2000) (“Thyroid Gland Dysplasia in WistarHan Rats” Researcher–The RCC Magazine. No.21, April 2005). Results are presented following treatment of time-mated female rats [Crl:WI(Han)] and [Crl:CDSII] with PTU at identical low and high oral gavage dose levels (namely, 0.1 and 2.5 mg/kg bw/day) daily on GD 6 through PND 10, as compared to respective sham-treated control groups. Results indicate that PTU-treated rats of both strains, demonstrate clear dose response relationships for Thyroid Hormones (decreased T4 (lesser effect for T3) & increased TSH for dams [GD 20] (PND 21) and fetuses [GD 20] and pups (PND 4 & 21)]. At these timepoints, correlated changes were observed for increased Relative Thyroid Weight in treated dams (GD 20) (PND 21), and for Thyroid Morphology (increased incidence/severity of follicular cell hyperplasty / hyperplasia) in treated Dams / Fetuses / Pups, as compared to controls. Importantly, results indicate no apparent significant differences between the Wistar and S-D strains when tested in this study design. The results indicate both strains may be used to test for potential effects on thyroid hormone and light microscropy in fetuses/pups to ascertain NOAELs/LOAELs following exposure to dams during pregnancy and lactation. We confirm a lack of “dysplasia” in Wistar controls.

165 EVALUATION OF SUBCHRONIC TOXICITY AND ESTROGENIC ACTIVITY OF BLACK COHOSH IN FEMALE WEANLING WISTAR HAN RATS EXPOSED BY GAVAGE.

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Black cohosh is a popular herbal supplement taken by women for the treatment of menstrual cramps and menopausal symptoms. The recommended daily dose is 40 mg/day (~0.5-0.6 mg/kg/day). An often reported mechanism of action alludes to estrogen-mediated effects; however, results found in the literature are mixed. No significant estrogenic effects were observed in an untransreted assay conducted by NTP. To address these findings, we examined whether moderate to low doses of trifluralin would cause any perturbations of estrogenic activity in Wistar and Sprague-Dawley strains of rats (Wistar versus Sprague-Dawley) to determine relative sensitivity. Also, confirmation was needed to determine if Wistar controls had potential residual effects resembling “thyroid gland dysplasia” as reported for RCC breeding colony in mid-1990’s to 2000 ("Thyroid Gland Dysplasia in WistarHan Rats” Researcher – The RCC Magazine. No.21, April 2005). Results are presented following treatment of time-mated female rats [Crl:WI(Han)] and [Crl:CDSII] with PTU at identical low and high oral gavage dose levels (namely, 0.1 and 2.5 mg/kg bw/day) daily on GD 6 through PND 10, as compared to respective sham-treated control groups. Results indicate that PTU-treated rats of both strains, demonstrate clear dose response relationships for Thyroid Hormones (decreased T4 (lesser effect for T3) & increased TSH for dams [GD 20] (PND 21) and fetuses [GD 20] and pups (PND 4 & 21)]. At these timepoints, correlated changes were observed for increased Relative Thyroid Weight in treated dams (GD 20) (PND 21), and for Thyroid Morphology (increased incidence/severity of follicular cell hyperplasty / hyperplasia) in treated Dams / Fetuses / Pups, as compared to controls. Importantly, results indicate no apparent significant differences between the Wistar and S-D strains when tested in this study design. The results indicate both strains may be used to test for potential effects on thyroid hormone and light microscropy in fetuses/pups to ascertain NOAELs/LOAELs following exposure to dams during pregnancy and lactation. We confirm a lack of “dysplasia” in Wistar controls.

166 IN UTERO EXPOSURE TO CHLOROQUINE REDUCES TESTOSTERONE AND STEROIDGENIC GENE EXPRESSION IN FETAL RAT TESTES.

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Chloroquine (CQ) is a commonly prescribed drug in the treatment and prevention of malaria, as well as several connective tissue disorders at doses in the range of 5 - 25 mg/kg/d. CQ is considered safe for use in pregnant women based on lack of evidence of increased miscarriage or overt birth defects in offspring of exposed mothers. Yet despite widespread use, few reproductive toxicity studies are available for this drug, particularly at human relevant doses. CQ disrupts steroid homeostasis in the adult rat. Since CQ is efficiently transferred to the fetus, the potential to interfere with androgenesis at a time when testosterone is critical for male sexual development exists. To investigate the effect on sexual development, pregnant Sprague-Dawley rats were given an oral gavage of water or CQ (10, 50, or 100 mg/kg/d) on gestation days (GD) 16-18. Maternal blood and male fetuses were collected on GD 19 and examined for early markers of endocrine disruption. Maternal serum estradiol levels were reduced at 10 mg/kg/d and increased at 100 mg/kg/d when compared to controls, while progesterone increased in a dose-dependent manner at doses ≥50 mg/kg/d, suggesting compensation by the maternal hypothalamus-pituitary-gonadal axis. Anogenital distance was not affected by CQ on GD 19, but reduced coiling of the testicular artery at the high dose indicates delayed testes development. Fetal testes testosterone was decreased in all treatment groups in a dose-dependent manner. Microarray analysis of testses revealed significant down-regulation of several genes associated with fatty acid, cholesterol and steroid regulation at 100 mg/kg/d, including Npgc, Srebp1 and Tm7sf2. Reproducible, but statistically insignificant, dose-related decreases were also seen in the expression of steroidalogenic genes Star, Gpr17a1, P450sc and 3Hofd7t which
are modulated by other known anti-androgens. This study provides strong evidence that CQ is likely to disrupt testosterone-dependent development in the rat fetus at human relevant doses.

167 KETAMINE-INDUCED NEURODEGENERATION IN THE NEWBORN RHESUS MONKEY.

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Ketamine is a widely used pediatric anesthetic, but the types of toxic insult that lead to ketamine-induced neuronal death are not adequately understood. One of the main goals of this study was to determine whether there is a duration of ketamine-induced anesthesia below which no significant ketamine-induced neurodegeneration can be detected. Newborn rhesus monkeys (postnatal day 5 or 6) were administered ketamine intravenously for 3, 9 or 24 hours to maintain a steady anesthetic plane, followed by a 6-hour withdrawal period. The 24-hour duration was selected as a relatively long duration, while the 3-hour duration more closely approximates typical pediatric general anesthesia. Animals were subsequently perfused and brain tissue processed for analyses using silver and Fluoro-Jade C stains and caspase-3 immunostain. The results indicated that no significant neurotoxic effects occurred if the anesthesia duration was 3 hours. However, 9 and 24-hour ketamine infusions significantly increased the number of silver-impregnated granules and the number of caspase-3 and Fluoro-Jade C-positive neuronal profiles in layers II and III of the frontal cortex compared with controls. The extent of neurodegeneration seen with 24-hour exposure is indeed greater than that seen after a 9-hour exposure. Although a few caspase-3 and Fluoro-Jade C-positive neuronal profiles were observed in some additional brain areas including the hippocampus, thalamus, striatum and amygdala, no significant differences were detected between ketamine-treated and control monkeys in these areas. These data suggest that the newborn monkey is sensitive to anesthetic-induced neurodegeneration and that exposure of 9 hours or longer can induce neurodegeneration during this early stage of development. Supported by FDA/NCTR E-7189, CDER/FDA and NICHD.

168 DIFFERENTIAL SPATIOTEMPORAL REGULATION OF BASIC HELIX-LOOP-HELIX (BHLH) AND HOMEOMODE (HD) TRANSCRIPTION FACTORS DURING GESTATIONAL LEAD EXPOSURE (GLE) SELECTIVELY INCREASES THE NUMBER OF LATE-BORN RETINAL NEURONS.

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GLE increases and prolongs the proliferation of retinal progenitor cells (RPCs) resulting in an increased number of late-born retinal neurons (rods and bipolar cells), but not Müller glial cells [SOT 2008]. Our present goals was to determine the spatiotemporal mRNA and protein expression pattern of transcription factors (TFs) that produced this novel retinal phenotype. C57BL/6 female mice were exposed to water or 55 ppm lead throughout gestation (E) and until postnatal day 10 (PN10): period equivalent to human gestation. Affymetrix arrays and real-time PCR (RT-qPCR) determined retinal gene expression at E18.5 and PN2-10. Western blots and immunocytochemical confocal (IHC) studies used PN2-10 retinas. Peak (BPb) at PN10 was 20 μg/dL. Microarray and RT-qPCR analysis revealed that Mash1 and Chx10 were upregulated in GLE retinas from PN6-10 and PN2-6, respectively. However, He1, NeuroD1, Otx2, Notch1, STAT3, CNTF, r-p130, SOCS3 and Sp1 mRNA expression were not different from controls. Immunoblot analysis revealed that GLE upregulated Mash1 expression from PN6-10; upregulated Hes1 expression from PN2-6 and downregulated its expression at PN6; and did not change STAT3 mRNA expression. In PN5 GLE retinas, Hes1 mRNA revealed that Mash1, Hes1 and Chx10 were upregulated in postmitotic and differentiating neurons. The GLE-induced upregulation of the Hes1 (BHLH TF)-Notch1 pathway is consistent with increased and prolonged RPC proliferation. The GLE-induced upregulation of Mash1 (proneural BHLH TF) and Chx10 (bipolar cell promoting HD TF) in post-mitotic RPCs in concert with the downregulation of Notch1-Hes1 and no change in STAT3 mRNA is consistent with the selectively increased rods and bipolar cells. Thus, low-level GLE targeted specific BHLH and HD TFs during a critical stage of late retinal development, which produced a unique retinal phenotype. Supported by NIH Grants E0914282, EY07751 and EY07024.

169 ASSESSMENT OF SYNAPSE FORMATION IN RAT PRIMARY NEURAL CELL CULTURE USING HIGH CONTENT MICROSCOPY.


Cell-based assays can model neurodevelopmental processes including neurite growth and synaptogenesis, and may be useful for screening and evaluation of large numbers of chemicals for developmental neurotoxicity. This work describes the use of high content screening (HCS) to detect chemical effects on synaptogenesis in vitro. HCS was performed with a Calomics ArrayScan VTiTM, an automated epifluorescence microscope and image analysis system, using cells in a microtiter plate format. Cerebellar granule cells (CGCs) from PND7 LE rats were seeded at 1.3 x 10^5 cells/cm^2 on laminin coated 96-well plates and grown for 2, 3, 5, 7, 9 or 14 days in vitro (DIV). Cells were then fixed and fluorescently labeled with anti-bodies against βIII-tubulin and synapsin I to visualize neuronal cells bodies and qualitatively assess the development of pre-synaptic terminals. Synapsin staining was diffuse up to DIV3. Synapsin puncta appeared at DIV5 and were observed up to DIV14. DIV7 CGCs were then used to optimize a protocol for neurite and synapsin I quantification using an ArrayScan VTiTM. The number of synapsin puncta and total area of synapsin stained associated with neuronal cell bodies and neurites were used as endpoints to measure synaptogenesis. CGCs were then treated with 3 or 10 μM of the PKC inhibitor Bis1, the MEK inhibitor U0126, the Src kinase inhibitor SU6656 or vehicle control (0.1% DMSO) at 2 h post-seeding and evaluated on DIV7 with the ArrayScan protocol (n=4-6 wells/group). 3 μM SU6656 increased the density of synaptic puncta associated with neurites on the cell bodies on DIV7. 3 μM Bis1 and 10 μM U0126 decreased the number and total area of synapsin puncta associated with cell bodies and neurites. 10 μM Bis1 produced large reductions in synapsin staining and decreases in total neurite length (16% vs control), indicative of overt cellular toxicity. These data demonstrate this method can be used as a screening tool to detect chemical effects on synaptogenesis in primary cultures. This abstract does not necessarily reflect USEPA policy.

170 LEAD EXPOSURE AND EXTERNALIZING BEHAVIOR IN KINDERGARTEN CHILDREN.

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Objectives: Studies on the effect of lead exposure on children's externalizing behavior (e.g., aggression, hyperactivity) are mixed. The purpose of this study was to provide further data on whether lead exposure is associated with increased externalizing behavior in kindergarten children, and assess whether the effect is independent of psychosocial factors in a non-clinical community sample of Chinese children. Method: Participants consisted of 650 5-6 years old kindergarten children in Jintan City, China. The Chinese version of the Child Behavior Checklist (CBCL) was used to measure behavioral outcomes. Blood lead level (BLL) was measured by a reference laboratory of the Centers for Disease Control and Prevention (CDC) in the Shanghai Lead Prevention Center. A self-administered parental questionnaire was used to collect information on potential confounds, including parental socioeconomic status, education, occupation, and marriage status. Results: Geometric mean (±SD) BLLs ranged from 8.7±11.4 μg/dL (range 0.0-99.9 μg/dL). Geometric means of CBCL T-scores (T-Mean ± 1SD) were: 50.4 (±6.0) total score, 55.5 (±6.2) social problems score, 51.7 (±5.9) attention problems score, 63.1 (±8.4) emotional problems score, 59.0 (±7.3) hyperactivity score, 54.2 (±6.9) peer problems score, 50.3 (±6.6) internalizing score, 50.0 (±6.6) externalizing score and 50.5 (±6.9) total problems score. The group difference remained after controlling for potential demographic and psychosocial factors. Conclusions: This population-based study suggests that increased lead levels are associated with increased externalizing behavior and it has potential public health implications for prevention of lead exposure in children.

171 CHANGE OF GENE EXPRESSION PROFILES IN ZEBRAFISH EMBRYOS AS AN ENDPOINT IN NEUROTOXICITY SCREENING.

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Chemicals that adversely affect the developing nervous system may have long-term consequences on human health. Little information exists on a large number of environmental chemicals to guide risk assessments for developmental neurotoxicity (DNT). As traditional DNT testing using rodents are costly, time consuming and labor intensive, alternative species such as zebrafish are being adapted for high-throughput toxicity screening. Hence, it is necessary to identify and characterize zebrafish biomarkers for assessing the DNT potential of chemicals. First, we described the developmental expression profile of 9 nervous system genes expressed in
untreated zebrafish embryos and larvae from 1 dpf to 6 days post-fertilization (dpf) determined using RT-qPCR. The data indicated that synapsin I and myelin basic protein (mbp) showed the largest increase in transcript levels. HuC, gap43, neurogenin 1 (ngn1), t1, tubulin, and gfp showed gene expression profile with an early increase followed by down-regulation. The transcripts of nestin and sonic hedgehog decreased during zebrafish development. We next investigated the changes in gene expression profile in zebrafish embryos exposed to two known developmental neurotoxicants (ethanol or valproate), from 0 to 5 dpf. Both toxicants affected gene expression at 3 and 6 dpf with some differences between the two chemicals. There were, however, some similarities with both valproate or ethanol exposure producing changes in mbp, nestin, and ngn1 expression when assessed at 3 dpf, and in mbp, gfp and ngn1 expression when assessed 24 hours after exposure had ceased (i.e., 6 dpf). Collectively, these data indicate that transcript levels of genes are responsive to developmental neurotoxicant exposure. It is possible to utilize the change of gene expression profiling as an endpoint to evaluate the developmental neurotoxicity.  

(This abstract does not necessarily reflect USEPA policy)

EXPOSURE TO PYRETHROID INSECTICIDES ALTERS EXPRESSION OF GENES IMPORTANT FOR DOPAMINERGIC NEURAL DEVELOPMENT

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Pyrethroids are widely used residential and agricultural insecticides, and recent studies indicate that the developing embryo may be especially susceptible to pyrethroid exposure. Data from the Richardson laboratory show that developmental exposure of mice to deltamethrin (a type II pyrethroid) results in increased dopamine transporter (DAT) expression and increased expression of Nur1 and Pitx3, transcription factors involved in dopaminergic system development. These mice also displayed increased locomotor activity. We have chosen to use the zebrafish to explore the hypothesis that developmental pyrethroid exposure alters dopaminergic neuronal development. To investigate the toxicity of pyrethroids in this model system, zebrafish embryos (3hpf) were exposed to pyrethroids and observed individually every 24h until 144 hpf. High doses of pyrethroids caused mortality (LCS0 of 55g/L for deltamethrin), pericardial edema, body axis malformations, and spams. Lower doses caused hyperactivity, similar to the effects seen in mice. To evaluate changes in gene expression, quantitative RT-PCR was used to determine expression of DAT, Pitx3, Nur1, and tyrosine hydroxylase (a marker of dopaminergic neurons). Deltamethrin (15g/L) caused an increase in expression of DAT (3.4 fold), TH (3.2 fold) and the transcription factors Nur1(3.3 fold) and Pitx3 (3.6 fold) without causing overt toxicity. Furthermore, these changes persisted in 2 week old larvae that had been exposed to deltamethrin during early embryogenesis. These data validate the zebrafish as a model for studying pyrethroid neurotoxicity, and demonstrate that short-term exposure to deltamethrin during early development results in alteration of gene expression that persists for at least 2 weeks. Supported in part by NIEHS grants P30ES005022 and RO1ES015991.

DEVELOPMENTAL NEUROTOXICITY OF ACRYLAMIDE IN WISTAR RATS

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There are innumerable hazardous contaminants in our environment. Some of these agents are known to be neurotoxic in animals and humans. Focusses and neostines are known to be a high-risk groups. The present study was carried out in order to see the enhanced developmental neurotoxic effects in pregnant female rats using acrylamide. Pregnant Wistar rats (9-11 weeks old), weighing 190-205 gms were dosed with acrylamide at 0.8, 12 and 16/18 mg/kg b.w. form day 6 of gestation to day 21 of lactation. No maternal death or distinctive toxic signs were noted during gestation however, two dams from high dose group were found dead during lactation. The maternal body weight and feed consumption were significantly decreased in mid and high dose groups during gestation and lactation. The mean body weight and feed consumption of pups were significantly decreased at all the treatment groups. Survival index of the pups was decreased at high dose group on postnatal day 4, 21 and 28. During neurobehavioral observation of pups, circling behaviour (PND 11), abnormal gait (PND 21) and impaired mobility (PND 21) were observed in male and female pups from high dose group. The pups from the high dose groups were falls immediately during wire manoeuvre observation. The mean ambulatory activity on postnatal day 15 and 17 was decreased in the male and female pups from the mid and high dose groups. On PND 23 abnormal air righting reflex of the male and female pups were observed at mid and high dose groups and mean foot splay (mm) of male and female pups were significantly decreased at high dose group. It can be concluded that acrylamide did not show selective developmental neurobehavioral toxicity. It was evident that the test substance was found to possess maternal toxicity but failed to reveal potential developmental toxicity.

NEUROTOXICITY OF TBT IN F1 RATS AS EVALUATED BY AN OPEN FIELD TEST AND PREPULSE INHIBITION TEST


Neurotoxicity is one of the major effects of trichlorobutyltin (TBT). The effects on the next generation of F1 rats exposed to TBT via the placenta and their dams’ milk may be stronger than those on adults. Pregnant Winter rats were exposed to TBT at 0 and 125 ppm in their food. Half of the female F1 rats in both groups were exposed to TBT at 125 ppm in their food from 9 to 15 weeks of age. Female F1 rats were divided into: the control-control (CC), TBT-control (TC), control-TBT (CT), and TBT-TBT (TT) groups (n = 10/group). After the administration, an open field test and prepulse inhibition (PPI) test were performed at 15 weeks of age. The mean values in the CT, TC and TT groups were significantly lower than that of the CC group. The mean value of face washing in the TT group was significantly lower than that in the TC group. There were no significant differences in indexes of the PPI test. Exposure to TBT via the placenta and their dams’ milk inhibited the TBT rach, which contains neurotoxicant. Inhibition of the rach activity induced by exposure to TBT via the placenta and their dams’ milk and/or via food was suggested. The effects were most evident in the TT group.

QUANTITATIVE ASSESSMENT OF [18F]-ANNEXIN V UPTAKE BY MICROPET IMAGING AS A BIOMARKER OF KETAMINE-INDUCED NEURONAL DEATH


Ketamine is a dissociative anesthetic that is primarily used for the induction and maintenance of general anesthesia. Recent reports indicate that ketamine anesthesia for 6-12 h triggers neuronal apoptosis in postnatal day (PND) 7 rats. In all apoptotic neurons, phosphatidylserine (PS) is rapidly redistributed from the inner to outer surface of the plasma membrane and can be selectively recognized and bound by annexin V. Because the high-resolution positron emission tomography scanner (microPET) provides 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 annexin V. In this study, the effect of ketamine on the uptake and retention of [18F]-annexin V in the rat brain was investigated using microPET imaging. On PND 7, rat pups in the experimental group were exposed to 6 injections of ketamine (20 mg/kg at 2 h intervals) and control rat pups received 6 injections of saline. On PND 35, [18F]-annexin V (1 mc) was injected into the tail vein of treated and control rats and static microPET images were obtained over 2 h following the injection. Radiolabeled tracer accumulation in the region of interest (ROI) in the frontal cortex and hippocampus were converted into Standard Uptake Values (SUVs). After the injection, radiotracer was quickly distributed into the brains of both ketamine- and saline-treated rats. Compared with the control group, the uptake of [18F]-annexin V was significantly increased in the ROI of ketamine-treated rats. Additionally, the duration for wash-out of the tracer was prolonged in the ketamine-treated animals. This preliminary study demonstrates that microPET imaging is capable of distinguishing differences in retention of [18F]-annexin in different brain regions and suggests that this approach may provide a minimally invasive biomarker of neuronal apoptosis. Supported by NICHHD, NTP/NIEHS and FDA.
176 EARLY EXPOSURE TO OXYTOCIN ALTERS ZEBRA FINCH VOCAL DEVELOPMENT.

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Autism spectrum disorder is associated with deficits in both social and language skills, and evidence suggests a role for oxytocin signaling in the etiology of autism. Peripheral administration of oxytocin (widely used during parturition) has been shown to distribute to the central nervous system, particularly in newborns (McEwen 2004). Oxytocin signaling has been established to be important in the modulation of social behaviors. The extent to which oxytocin signaling is involved in vocal development has not been established, possibly due to a lack of appropriate animal models. Since songbirds learn a form of vocal communication, they represent a promising model for evaluating effects of oxytocin-related peptides on vocal development. As an avian species, zebra finches utilize mesotocin rather than oxytocin signaling (these nonapeptides differ by a single amino acid). To establish the suitability of zebra finches as an animal model to study developmental effects of oxytocin-related peptides, we have begun to characterize the receptor likely involved. Immunohistochemical experiments using an antibody raised against the mesotocin receptor specifically cloned in our laboratory suggests that distinct mesotocin receptor expression occurs within the vocal motor brain regions and that mesotocin receptor expression varies as a function of age during vocal development. To determine if early exposure to oxytocin could alter normal vocal development, birds were treated with either oxytocin (500 mcg/kg, 1000 mcg/kg, or 3000 mcg/kg) or vehicle for five days (days 5-9). The birds were then raised by the same adult tutor. Songs were recorded at adulthood and ten song files were randomly selected for analysis. There was no statistical difference on note stereotypy by oxytocin treated animals due to dosage and thus the animals were pooled into one group. The songs produced by the oxytocin treated animals were less stereotyped than those of the vehicle treated animals (p < 0.015, two sample t-test). These preliminary results suggest that early post-natal oxytocin exposure may alter zebra finch vocal development.

177 GESTATIONAL EXPOSURE TO TOLUENE DID NOT ALTER THE BEHAVIOR OR VISUAL FUNCTION OF OFFSPRING RATS.


Children of women who were exposed to organic solvents during pregnancy have shown altered physical development, behavior (Pearson et al. 1994; Arnold et al. 1994) and visual function (Till et al. 2001; 2003; 2005). We evaluated the offspring of pregnant Long-Evans rats who were exposed to toluene vapor at concentrations of 0, 10, 100 or 1000 ppm from days 8-19 of gestation. There were no effects of toluene on maternal body weight during pregnancy or preweaning growth of offspring at any concentration, but postweaning offspring weights were significantly lower through 18 weeks in the 1000 ppm group. One set of offspring was killed at 3 and 8 weeks of age using a functional observational battery (FOB) and motor activity (n-9/10/dose, 1 male/litter). There were no treatment effects on horizontal or vertically directed activity. All of the FOB endpoints, only slight effects on reactivity and the click response were detected; while significant, these changes were neither dose-responsive nor consistent. The rats were then trained to perform a visual signal detection task to assess learning and sustained attention. No effects of toluene exposure were observed in a lever press task, visual discrimination task or the signal detection task. Beginning at approximately 75 days of age, electroretinograms (ERG) and pattern-electric evoked potentials (VEP) were recorded from a second cohort of offspring (n=10/dose, 1 male offspring/litter). No statistically significant effects of toluene exposure were observed in any ERG or VEP parameter, or in contrast thresholds extrapolated from VEP contrast-amplitude functions. These experiments have not confirmed that gestational exposure to toluene alters the visual function or behavior of offspring. This abstract does not reflect EPA policy.

178 PROTECTION FROM KETAMINE-INDUCED DNA DAMAGE IN RAT FOREBRAIN CULTURE BY L-CARNITINE.


Ketamine, a noncompetitive NMDA receptor antagonist, is used as a general anesthetic, and may cause neurodegeneration if administration occurs during critical periods of development. L-carnitine plays an integral role in reducing brain injury associated with mitochondrial neurodegenerative disorders. The purpose of this study was to investigate whether co-administration of L-carnitine could protect against or attenuate ketamine-induced cell death using newborn rat forebrain cultures. Neural cells collected from the newborn rat forebrain were incubated for 24 h with 1, 10 or 20 μM ketamine, normal culture medium (control), or ketamine (10 μM) plus 100, 500 or 1000 μM L-carnitine. A modification of guanine (8-oxoguanine) presumably induced by the reaction of endogenous and exogenous reactive oxygen species with nucleic acids was used as a biomarker for oxidative DNA damage. 8-oxo-dG ELISA and antibody to human 8-oxoguanine DNA glycosylase (8OGG1) which catalyzes the removal of the 8-oxoguanine through cleavage of the DNA phosphodiester bond, were used to explore the potential regulatory DNA repair system. The 8-oxo-dG ELISA and immunostaining data indicate that ketamine (10 or 20 μM) produced elevated levels of 8-oxoguanine, and a reduction in mitochondrial metabolism of 8OGG1. Preliminary data also show increased DNA migration as indicated by an alkaline single cell gel electrophoresis (Comet assay). No significant effect was observed in the release of lactate dehydrogenase. Ketamine-induced DNA damage in forebrain culture was effectively blocked by L-carnitine (500 or 1000 μM), suggesting its potential protective effects via prevention of apoptosis and chromosomal damage, and changing cellular transcriptional responses. Supported by NCTR/NTP IAG # 244-07-0007 (E-2155).

179 VALPROATE-INDUCED ABNORMAL DEVELOPMENT NEUROTOXICITY IS AFFECTED BY MATERNAL CONDITIONS, INCLUDING SHIPPPING STRESS.

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Prenatal stress is known to affect the development of the brain, and to exaggerate the developmental toxicity of chemicals. Many studies on developmental neurotoxicity (DNT) use pregnant rodents mated at the supplier, which consequently suffer from stress of shipping and environmental changes. We have demonstrated differences in developmental neurotoxicity induced by valproate (VPA) between pregnant rats mated at our own animal facility (in-house group) and rats purchased pregnant (supplier group). Rats were treated with VPA (800 mg/kg) orally on gestation day (G) 9 or 11 (VPA9 or VPA11), and the fetal brain was examined at E16 using immunohistochemistry for serotonin, tyrosine hydroxylase (TH), and TuJ1 (a neuronal marker). Cortical dysgenesis (abnormal cortical plate) was induced in both VPA9 and VPA11 groups. Abnormal traveling of the neural fasciculus at the isthmus, resulting in disturbance of the migration of TH-positive and serotonin neurons was observed in the hindbrain of only the VPA11 group. Comparing the incidence of these abnormalities between the in-house and supplier groups, the incidence of the cortical dysgenesis was increased slightly, and that of the abnormal ponts was clearly increased in the supplier group. Thus, observation of E16 brain clearly demonstrated that a difference in mating location (own animal facility or supplier) affects the reproducibility of VPA-induced DNT. This research is supported by a grant for Long-range Research Initiative from the Japan Chemical Industry Association.

180 DISTRIBUTION OF C-FOS-LIKE IMMUNOREACTIVITIES IN THE NEONATAL RAT BRAIN SHORTLY AFTER MATERNAL DEPRIVATION IN THE BRDU-INDUCED HYPERACTIVITY MODEL.

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c-Fos has been used as a marker of neuronal activity, and immunohistochemistry of this protein is one of the most useful methods to investigate active brain areas in experimental animals. We applied c-Fos immunohistochemistry to develop a method to detect abnormal brain function during the neonatal period in a chemical-induced developmental disorder model. To investigate neonatal brain function, we induced maternal deprivation, which was enough to stimulate them and induced c-Fos immunoreactivities in the brain areas related to the olfactory system, and stress response. To produce a chemical-induced hyperactivity rat model, we treated pregnant rats with 50 mg/kg of 5-bromo-2-deoxyuridine (BrDU) from gestation day 9 to 15. Neonatal rats at 11 days of age were perfused with a fixative following one-hour maternal deprivation (neonatal rats were placed in a new empty cage for one hour). The number of c-Fos immunoreactive cells was counted in the olfactory brain areas and compared between the control and BrDU groups. The number of c-Fos immunoreactive cells in the BrDU group was decreased in the piriform cortex, the locus coeruleus and dorsal part of the septum, which plays an important role in neonatal learning and memory. We further analyzed Pearson product-moment cor-
TIME COURSE OF CHOLINESTERASE INHIBITION FOLLOWING DEVELOPMENTAL CHLORPYRIFOS EXPOSURE USING DIFFERENT ADMINISTRATION PARADIGMS.

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Chlorpyrifos (CPS) is widely used in agricultural settings and residue analysis has suggested that children in agricultural communities are at risk of exposure. This has resulted in a large amount of literature investigating the potential for CPS-induced developmental neurotoxic effects. Three of the most common routes of administration of CPS are orally in oil, subcutaneously in oil, and subcutaneously in DMSO. For comparison between these methods, rat pups were exposed daily from days 10-16 to CPS (5 mg/kg) either orally dissolved in corn oil (PO-Oil), subcutaneously dissolved in oil (SC-Oil), or subcutaneously dissolved in DMSO (SC-DMSO) at a rate of 1.0 ml/kg. A representative vehicle/route group was present for each treatment. A time course of acetylcholinesterase inhibition on the last day of exposure was conducted to determine the peak time of inhibition following each route. Samples were taken at 2, 4, 6, 8, 10, and 12 hours. In the forebrain, the time to peak inhibition was 4 hours following all routes and the level of inhibition was greatest in the SC-DMSO (~70%) followed by PO-Oil (~50%) and then SC-Oil (~42%). In the cerebellum, the time to peak inhibition was 6 hours following PO-Oil (~45%) and SC-Oil (~37%) but was 2 hours with SC-DMSO (~68%). Recovery in the forebrain was observed with all routes but in the cerebellum, recovery occurred in the oil routes but was not evident in the SC-DMSO route. These data suggest that using DMSO as a vehicle for CPS not only induces greater AChE inhibition in the oil routes but was not evident in the SC-DMSO route. These data suggest that using DMSO as a vehicle for CPS not only induces greater AChE inhibition in the oil routes but was not evident in the SC-DMSO route. These data suggest that using DMSO as a vehicle for CPS not only induces greater AChE inhibition in the oil routes but was not evident in the SC-DMSO route.

HIGH-THROUGHPUT BEHAVIORAL QUANTIFICATION OF DI(2-ETHYLHEXYL) PHOSPHATE (DEHP) EXPOSED RATS.

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Differences in performance between male and female rats are used to assess substances thought to affect sexual development of the brain. Most current methods for measuring sexually-differentiated behavior in rats have been limited to human observation requiring both subjective interpretation and limited quantification. Human observation is labor-intensive, restricting these techniques from being used as high-throughput screens for agents such as endocrine-active substances. This poster presents apparatus and methods for quantifying sexually-differentiated behavior as well as the effects of 125 or 250 mg/kg of di (2-ethylhexyl) phosphate (DEHP) given perinatally. Specifically, male and female pups were exposed, via the dam, from gestational day 14 to postnatal day (PND) 3 and then measured for activity and play behavior. Ninety-six rats were processed in a between-group design with negative- (corn oil) and positive- (50 mg/kg vinclozolin (Vz)) control groups. Sex differences in activity did not appear until PND 54 in control and 125 mg/kg DEHP groups. The sex differences were not statistically significant for the 250 mg/kg DEHP and Vz groups. Quantitative assessment of play behavior showed negative-control male rats engaging in more rough-in-tumble play than females at all ages. DEHP and Vz did not impact male play behavior, but did increase female but results in regional differences in the pattern of AChE inhibition and recovery.

DEVELOPMENTAL EXPOSURE TO DELTAMETHRIN RESULTS IN REGIONAL ALTERATIONS IN SODIUM CHANNEL SUBUNIT AND BRAIN-DERIVED NEUROTROPHIC FACTOR mRNA EXPRESSION.

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Developing animals are known to be more sensitive to the neurotoxic effects of the pyrethroid insecticide deltamethrin. Deltamethrin is thought to exert toxicity by delaying the closure of voltage gated sodium channels and leading to a persistent depolarization. In vitro studies have found that exposure to toxins that increase sodium flux into cultured neurons causes down-regulation of sodium channel subunits, which in turn affects the firing properties of the cells and synaptic activity. However, it is not clear if such an effect occurs in vivo. Here, we sought to determine whether developmental exposure to deltamethrin affects sodium channel subunit expression. Pregnant mice were exposed throughout gestation and lactation to either 0 or 3 mg/kg deltamethrin every three days. The offspring were tested as adults for behavioral deficits and male offspring sacrificed at 10-11 months of age for determination of isoform-specific sodium channel (Nav1 and 2.1) and BDNF mRNA expression by QPCR. Following developmental exposure to deltamethrin, Nav1.3 expression was decreased by about 27% and Navβ3 by about 26% in the frontal cortex, with no significant effects on other α or β isoforms. In the striatum we observed an approximate 24% decrease in Nav1.1, Navβ2 and Navβ1 along with a 15% reduction in Navβ3. Because sodium channel expression could potentially lead to alterations in synaptic activity, we determined the mRNA expression of brain-derived neurotrophic factor (BDNF), which is known to be regulated by neuronal activity. We saw a slight but non-significant reduction of BDNF mRNA in the frontal cortex. However, BDNF mRNA was reduced by about 66% in the striatum. Taken together, these data suggest that developmental deltamethrin exposure results in long-term alterations of isoform-specific sodium channel subunit expression which may result in region-specific alterations in neuronal activity and contribute to the behavioral deficits observed in mice developmentally exposed to deltamethrin.

CA1 SYNAPTIC EXCITABILITY MEASURED IN VITRO IN HIPPOCAMPAL BRAIN SLICES IS CHANGED IN ADULT RAT OFFSPRING AFTER MATERNAL EXPOSURE TO MEHg.

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In vitro exposure to methylmercury (MeHg) induces developmental deficits in offspring, such as loss of memory function [1]. A key brain region involved in learning and memory is the hippocampus. It is known that acute in vitro exposure of hippocampal brain slices to MeHg may affect the amplitude of electrically induced field potentials [2]. We investigated ex vivo the excitability of the in vitro hippocampus of juvenile (35 days) or adult rats (68 days) born from mothers which...
186 ASSESSMENT OF THE NEUROTOXICITY OF ACRYLAMIDE AND TRIMETHYLTIN CHLORIDE IN THE HARLAN HAN WISTAR RAT.

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Acrylamide and Trimethyltin chloride are known to induce peripheral and central nervous system damage. This study was designed to identify the neurotoxic properties of Acrylamide and Trimethyltin Chloride following repeated dose administration for up to twenty-eight consecutive days in the Han Wistar rat. Open-field arena assessments, motor activity assessments and grip strength tests were performed weekly, together with body weights and dietary intake. Clinical observations and visual inspection of water bottles was also undertaken. At study termination, animals were subjected to in situ perfusion fixation. The brain was weighed and the mesencephalon was dissected and examined. Reduced activity, lethargy, hunched posture, body tremors and respiratory patterns were detected following Acrylamide administration. Reduced transfer of d-amphetamine and increases in urination and defecation were detected. Mortality noted was noted to females. Reduced forelimb grip strength and motor activity were detected, together with reduced body weight gains, dietary intake and food efficiencies. Changes in brain weights were detected and histopathological examination revealed changes in the spinal cord, ventral and roof ganglia, and sciatic and tibial nerves. Altered motor activity and reduced activities were noted together with increased respiratory activity. Increases in motor activity and reduced grip strength were noted. Histopathological examinations revealed changes in the spinal cord, dorsal root ganglia, dorsal and ventral roots and skeletal muscle. This study confirmed that the procedures employed are effective in identifying the neurotoxic effects of Acrylamide and Trimethyltin Chloride in the Han Wistar rat.

187 DEVELOPMENTAL NEUROTOXICITY: PRACTICAL EXPERIENCE FROM THE CONDUCT OF A VALIDATION STUDY.

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A validation study was carried out to establish a “best practice” in our laboratory environment. Since a single positive control agent could not demonstrate the sensitivity and specificity of the battery of methods and functional tests, Methylenoxan methanol acetate (MAM) was selected, which de Groot et al. (2005) used in a model study for the morphologic approach of DNT testing. MAM was given to dams during days 15 to 15 of pregnancy at dose levels of 1.25, 5.0 and 7.5 mg/kg body weight/day. The pups were examined at postnatal days 11, 12 and 13 post gestation. Brain weights were reduced at 7.5 mg/kg, independent from the fixation method. Prolonged fixation of the brains in formalin had an influence on brain weight. For instance, at 65 days, the fresh brain weight or the weight immediately after the perfusion fixation was reduced to 16% less than the weight in the first postnatal week. Therefore, the nature of brain fixation is critical for historical control data. For macroscopic morphometry the brains were photographed from the dorsal aspect with a millimeter scale, enabling the assessment of the length of forebrain and cerebellum. There was a dose-dependent reduction in the length of forebrain and a tendency for an increased length of cerebellum. At the microscopic level, the decrease in brain size was not observed, demonstrating the importance of accurate homology of brain sections and performance of linear morphometry for the detection of subtle differences. As some guidelines (US EPA) require examination of the peripheral nervous system in “plastic” sections, epoxy resin sections were produced, containing in continuity in a single section the dorsal and ventral nerve roots and the dorsal root ganglia. The peripheral sciatic nerve and its branches could be examined effectively in transverse epoxy resin sections, but for longitudinal sections paraffin embedding, preshake large nerve portions, was superior. Overall this study confirmed the feasibility of the methodology applied in our laboratory.

188 BIOLOGICALLY-BASED MODELING TO ASSESS EARLY EMBRYONIC EXPOSURE TO XENOBIOTICS SUITABLE FOR NEUROBEHAVIORAL TERATOLOGY STUDIES.


Quantitative means of assessing early embryonic exposure to xenobiotics (XB) via maternal exposure early in the 1st trimester (GD15-50) remain relatively scant in the literature. This developmental stage is marked by unique physiology (i.e. cellular growth rates, nutrient mechanisms, vasculature, neuroendocrine modulation, metabolism and plasma/cell structures) not found in other life stages, suggesting a need to incorporate and translate these vital temporal details into a stage-specific, biologically-based kinetic model (BBPK) for estimating embryonic dose-metrics early in organogenesis. We believe that neurobehavioral teratogens or NB1-exclusive “window of susceptibility” for XB coincide with EEE. To address both exposure and dosimetry at this life-stage we describe/develop an early gestational BBPK model that includes embryonic stage-relevant: (1) physiological growth functions for prordial organs, (2) diffusion-limited maternal transfer kinetics, (3) tissue-specific partition functions, and (4) t-cell apoptosis and receptor fusion. We use this model to probe exposure-dose scenarios of specific xenobiotic and endogenous molecules, including: (1) physicochemical/ADME properties and embryonic plasma binding (PB) of NB1s. Similar models may be important tools in the exploration of other agents, environmental and therapeutic, in the NB1-associated risk of PDD etiology in presumably genetically susceptible populations to identify research needs, both mechanistic and epidemiological, that will partially disentangle the complex underpinnings of neurobehavioral teratogenicity.
190 MOLECULAR AND CELLULAR CHARACTERIZATION OF METHYLMERCURY AND SELENIUM SYNAPTOTOXICITY IN THE DEVELOPING HIPPOCAMPUS OF RATS AND MICE.

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Methymercury (MeHg) exposure from occupational, environmental, and contaminated foods is a significant threat to public health. MeHg readily passes the blood-brain barrier causing severe psychological and neurological problems. The developing nervous system is particularly vulnerable to the toxin, and although MeHg poisoning has been studied for decades, the molecular components and pathways involved in the cellular pathology are largely unknown. Several studies have demonstrated that selenium can be also neurotoxic, yet protect against the MeHg-induced pathology. The molecular mechanisms involved in the selenium induced neurotoxicity and protection are ill-defined. An aim of this study is to evaluate the neurotoxic effects of MeHg and selenium on developing neurons, and to identify cytoskeletal and synaptic proteins that may contribute to the neuropathology. Hippocampal primary cell cultures from rats and mice at 14 days were exposed to either MeHg or sodium selenide (NaSe) (0–50 μM), or in combination, for 24 hours and the number of dead cells determined using propidium iodide as a marker for cell death. Immunocytochemical quantification of dendritic and synaptic proteins, including MAP2 and synaptophysin, was evaluated. A concentration-dependent increase in mortality was observed with single toxin exposures in either species, and results in dendritic fragmentation, loss of cellular integrity, and dramatic changes in a number of cytoskeletal and synaptic proteins. In contrast, co-exposure with the toxins resulted in maintenance of dendritic and synaptic integrity, that includes diffuse synaptic protein staining. Toxin-induced changes in the dendritic spines (size, volume, contact to presynaptic structures), as well as synaptic protein expression and localization are also described. This study is supported by NS5641A/0507.

191 LIFETIME STUDIES OF DEVELOPMENTALLY PB-EXPOSED MICE AND ALZHEIMER'S DISEASE BIOMARKERS IN THE BRAIN AND BLOOD: ALTERATIONS IN AMYLOID PRECURSOR PROTEIN, SPECIFICITY PROTEIN 1 (SP1) AND AMYLOID-BETA.

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Alzheimer’s disease (AD) is a progressive neurodegenerative disease that will affect 50 million Americans by 2025. Disease pathology is hallmarked by senile plaques composed of Amyloid-beta (Aβ) peptide aggregates and neurofibrillary tangles composed of hyperphosphorylated tau. Although AD is a disease of old age, it is not known when it begins or the underlying etiology. Thus, it is vital to pinpoint not known when it begins or the underlying etiology. Thus, it is vital to pinpoint when in the lifespan the levels of Aβ peptide as well as its precursor Amyloid Precursor Protein (APP) depart from normal expression in the brain. Our lab has previously shown that the neurotoxicant, lead (Pb), causes a latent increase cortical brain APP expression by transcriptional up-regulation, thereby initiating an increase in Aβ. The objective of this study is to quantify APP and Sp1 (the regulator of APP expression) protein levels by Western blot analysis and APP by ELISA assay, in control and lead-exposed animals throughout the lifespan, in both the plasma and the brain. This will allow us to see if alterations in the brain are reflected in the blood and thus present these proteins as potential biomarkers to screen human populations.

192 GENETIC VARIATION IN BEHAVIORAL AND BRAIN TRANSCRIPTIONAL EFFECTS OF MEHg EXPOSURE.

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Methylmercury (MeHg) is a potent neurotoxin that affects the developing nervous system. It accumulates in the brain, most notably the cerebellum and hippocampus, causing irreversible damage. The neurotoxic effects and the mechanisms by which MeHg acts remain to be elucidated, especially in cases of long-term exposure. A study was conducted to evaluate genetic variation in MeHg effects on brain and behavior. Mice of both sexes from the BXD mouse progenitor lines (C57Bl/6 and DBA/2) and several BXD lines were exposed to MeHg perinatally. Pregnant mice were injected subcutaneously with 3mg MeHg/kg body weight or saline during gestation. Neonate offspring were injected during the first week of birth on postnatal days 3 and 5. Mice were assessed in open field, light-dark box and the Rota-rod apparatus at 4-5 weeks of age. Strain specific effects of MeHg on behavior were observed. Cerebella from separate mice, treated with the same MeHg exposure regimen, were dissected at 4-5 weeks of age and used for gene expression analysis using the Illumina Mouse Sentrix 6 array. Significant two-way (treatment x strain) and three-way (treatment x sex x strain) effects were detected. Analysis of significantly over-represented pathways and functional Gene Ontology categories reveals genetic variation in MeHg effects on neural development, Wnt signaling (Wnt, DVL, and Axin), the MAP kinase pathway, and developmental GABAergic systems (GABA-C rh). The lipid metabolism category was also over-represented, particularly due to sterol metabolizing genes. MeHg alters the expression of Glutathione-S-transferase, but these effects were not found to be strain specific in this subset of the BXD population. The implication for this project is that in addition to effects of MeHg on oxidative stress related genes, there are specific neurodevelopmental effects that are the result of an interaction of a genetic predisposition and an environmental exposure.

193 LOW-DOSE METHYLMERCURY (MEHG) EXPOSURE ACTIVATES CASEPASE-3 IN POSTNATAL RAT HIPPOCAMPUS.

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MeHg, an environmentally persistent toxicant, induces a spectrum of harmful effects on neurodevelopment. Our recent studies (Fallul-Morel et al. 2007) have shown that a single sc injection of 5μg/gm MeHg in 7 day old (P7) rats acutely activates caspase-3, an apoptotic protease, leading to cell cycle arrest and cell death in hippocampus. Furthermore, reduced cell number and organ size were detected at P21 in addition to behavioral effects at P35. Caspase-3 activation, a marker for apoptosis, may involve either the extrinsic pathway that depends on caspase-8 or the intrinsic pathway (including caspase-9), which we found to underlie hippocampal neurotoxicity (Sokolowski et al. 2008). Based on the pathway definition, we used low doses of MeHg in our model of hippocampus toxicity, to define the effects of environmentally relevant exposures. Of all the molecular markers we studied, including [SH]-rhamidine incorporation, BstU1, total DNA content and absolute cell number, activated caspase-3 immunohasay was the most sensitive. Thus, caspase-3, a significant endpoint protein, was detected using the most sensitive assay, immuno-histochemistry, in the low-dose exposures. After 24hr treatment with MeHg (10, 0.6, 0.2, 0.1μg/g) or vehicle in the P7 rat, whole brains were prepared for frozen sectioning and caspase-3 staining. We found two-fold more activated caspase-3-positive cells in the hippocampus than in the control. The next higher dose, 0.6μg/g MeHg, elicited a five-fold increase in caspase-3-positive cells while the highest dose, 10μg/g MeHg, increased positive cells eight times greater than control. By using the most sensitive marker for apoptosis, caspase-3 staining, we now detect significant MeHg neurotoxicity at a 25-fold lower dose than previously defined. Future studies will characterize acute effects on hippocampal neurogenesis and later consequences on organ cell composition and cognitive functions. NIEHS: ES07148, ES50522, ES11256; USEPA R823931; FRM SPE2006.
Manganese (Mn) is an essential metal, necessary for the normal functioning of a variety of physiological processes. In humans, Mn neurotoxicity is characterized by a variety of psychiatric, cognitive and motor disturbances, the latter resembling parkinsonian symptoms. Among brain cells, astrocytes, which provide a major support for neurons, accumulate this metal at higher extent. Using a co-culturing system, we investigated the ability of Mn to alter the ability of astrocytes to promote neuronal outgrowth in primary hippocampal neurons. When astrocytes were pre-incubated with different concentrations of Mn (50, 100, 200, 500 μM) followed by treatment wash-out, before being co-cultured with neurons for 48 hours, their ability to promote neurite outgrowth was impaired in a concentration-dependent manner. Only the highest Mn concentration was toxic to astrocytes. At the concentration of 200 μM, Mn significantly decreased axon length (to 59.2±13.5% of control), neurite length (to 64.0±2.2% of control) and the number of neurites per cell (to 76.4±1.1% of control). These effects were prevented by pre-incubating astrocytes with the antioxidants melatonin (200 μM) and N-t-butyl-alpha-phenyl-nitron (100 μM). Increasing astrocytic glutathione (GSH) levels with GSH ethyl ester (2.5 mM), or decreasing it with the GSH synthase inhibitor buthionine sulfoxime (25 μM) significantly decreased Mn toxicity, and respectively, the ability of Mn to inhibit neurite outgrowth promoted by astrocytes. Mn also caused a decrease of fibronectin, an extracellular matrix protein, which plays a relevant role in neuronal differentiation promoted by astrocytes. These findings suggest that Mn impairs the ability of astrocytes to promote neuronal differentiation by a mechanism that may involve oxidative stress and impaired fibronectin expression. (Supp. in part by grant W91XWH-05-1-0239 from the US DOD)

Perinatal lead exposure alters postnatal cholinergic and amineergic system in rat brain: Reversal effect of calcium co-administration.

Lead (Pb) is known to exert its neurotoxic effects by competing with calcium for calcium receptors coupled with second messenger functions. Our earlier physiological and pharmacological studies indicated the role of transmitter systems in Pb induced behavioral perturbations. In the present study, we have examined the alterations in synaptosomal acetylcholine (ACh), epinephrine, dopamine, acetylcholinesterase (AChE), and mitochondrial monoamine oxidase (MAO) by spectrophotometric assays in the cerebellum and hippocampus of perinatally Pb-exposed rats. Rats (Wistar) were gestationally exposed to 0.2% Pb (Pb acetate in drinking water of mother) from day 6 and the pups were exposed lactationally till PND35. The changes observed in synaptosomal ACh, dopamine, and epinephrine levels in both cerebellum and hippocampus. These alterations were greater at PND35. The changes in the cholinergic system (ACh and AChE) were more pronounced in hippocampus (ACh,37%; AChE,44%). The cerebellum exhibited similar changes in the amineergic system (MAO,41%; epinephrine,31%; dopamine,34%). The Pb-induced changes in cholinergic and amineergic systems correspond to the cognitive and behavioral involvement of these neurotransmitters and brain regions. Calcium co-administration to Pb-exposed rats significantly reversed the Pb-induced alterations in these transmitters and enzymes. The reversal effect was greater at PND35. These data suggest protective effect of calcium in Pb-exposed animals. This study was supported by UGC grants No.F-33-364/2007, and UGC-MRP-2503/2008.

Advantages of monkey neurobehavioral assessment using the functional observational battery (FOB) – comparative study with dogs and rats.


Background and Purpose The FOB is widely used to identify potential neurotoxicity of various chemicals, mainly in rats; however, there have been few FOB studies in dogs and non-human primates. The purpose of this study was to compare neurobehavioral changes between rats, dogs and monkeys. [Methods] Six male cynomolgus monkeys, four male beagle dogs and six male SD rats were used. The FOB methods consisted of home-cage observation, handling-observation, open-field observation (except for monkeys) and manipulative measurements of activity/excitability, neuromuscular, sensorimotor and autonomic nervous functions. Neurobehavioral functions were determined by the FOB methods after dosing with chlorpromazine, haloperidol, diazepam or caffeine. [Results] Chlorpromazine induced signs of central nervous system (CNS) depression, including sedation, decreased sensory reactivity and hypothermia in all species, and a cataleptic state in monkeys and rats. Haloperidol induced similar signs of CNS depression, but did not induce sedation in rats or hypothermia in dogs or rats. Diazepam appeared to have an anxiolytic effect at a low dosage in monkeys and dogs, but did not induce excessive sedation. However, in rats, diazepam induced only sedation. Caffeine induced hyperactivity in monkeys and dogs, but produced a biphasic response of hyperactivity (low-dosage) and hypactivity (high-dosage) in rats. The lowest doses effective in producing observable neurobehavioral changes were seen in monkeys, followed by dogs, and then rats, and the observed effects in monkeys most closely resembled the known clinical effects of these drugs in humans at clinical dosages.

Synapse formation during nervous system development and degeneration in the pathogenesis of human neurological diseases is a highly regulated process. Subtle changes in the environment of the complex neuronal network may cause either breakdown or creation of synaptic connections. Discovery screening for neurological diseases and compound neurotoxicity evaluation would benefit from robust, automated, quantitative in vitro assays that monitor neuronal function. We hypothesized that (1) toxic insults to the nervous system will cause neuronal synapses to deteriorate in the early phase of neurotoxicity, eventually leading to neurodegeneration and neuronal cell death if the damage is severe; and (2) an in vitro functional assay for synapse formation and neuronal morphology could be used to monitor and identify such neurotoxic events. We thus developed an automated, functional, high-content imaging assay to track and quantify the dynamic changes in neurite morphology and synapses. This assay identifies primary neuronal cells by a neuron-specific marker and detects synapses on the spines of neurons with pre- and post-synaptic markers. The multiplexed targets are simultaneously detected with four fluorescent colors, and the fluorescent images of the labeled neurons and synapses are acquired by an automated imaging instrument. The phenotypic features of neuronal morphology and the synapse are automatically identified and quantified by specialized image-analysis software. Such features are potential indicators for neuronal development, differentiation and neurotoxicity, and we could quantify changes in these features under different experimental conditions. By monitoring changes in these features, we could also quantitatively evaluate compounds involved in developmental neurotoxicity. In summary, this assay facilitates automation and streamlining of a laborious process in drug discovery screening and compound neurotoxicity assessments; it enables quantitative comparisons between compounds in neuronal morphology and function, particularly in neurite and synapse associated events.

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Pulmonary exposure to particulate matter (PM) is a known cause of systemic cardiovascular dysfunction; however, the mechanism(s) of damage are not fully understood. While this laboratory has characterized microvascular dysfunction after exposure to PM, the specific hemodynamic adjustments that follow PM exposure are unknown. The purpose of this study was to determine the effects of pulmonary exposure to diesel exhaust particles (DEP) on peripheral microvascular function and hemodynamics. Rats were intratracheally instilled with SRM 1650b (NIST, 0.100 µg/rat). The spinotrapezius muscle was prepared for intravital microscopy 24 hr after exposure. Arteriolar reactivity was assessed by iontophoretic application of endothelium-dependent (acetylcholine, ACh, 0.025 M) and endothelium-independent (nitric oxide synthase inhibitor, NG-monomethyl-L-arginine (L-NMMA, 10-4 M)). In denuded arteries from air rats (max: 2.5 ± 4% denuded) but was increased in arteries from air rats (max: 2.5 ± 4% denuded) but was increased in arteries from air rats (max: 2.5 ± 4% denuded) but was increased in arteries from air rats (max: 2.5 ± 4% denuded) but was increased in arteries from air rats (max: 2.5 ± 4% denuded). The spinotrapezius muscle was prepared for intravital microscopy 24 hr after exposure. Arteriolar reactivity was assessed by iontophoretic application of endothelium-dependent (acetylcholine, ACh, 0.025 M) and endothelium-independent (nitric oxide synthase inhibitor, NG-monomethyl-L-arginine (L-NMMA, 10-4 M)). In denuded arteries from air rats (max: 2.5 ± 4% denuded) but was increased in arteries from air rats (max: 2.5 ± 4% denuded) but was increased in arteries from air rats (max: 2.5 ± 4% denuded) but was increased in arteries from air rats (max: 2.5 ± 4% denuded) but was increased in arteries from air rats (max: 2.5 ± 4% denuded).
to influence blood pressure (BP) and vascular reactivity. The mechanisms by which DEP affect vascular reactivity and BP are mostly unknown. In addition, it is not known if inhaled DEP target and remain in the lung or if these particles can pass through the lung epithelia into the systemic circulation. If DEP enter into the systemic circulation during in vivo exposure, endothelial cells lining the vasculature will come into direct contact with DEP. We investigated the effects of DEP on an immortalized mouse lung epithelial node endothelial cell line (SVEC4-10). PM2.5 from a Cummins diesel engine operating under load were collected on a Teflon filter. SVEC4-10 cells were exposed to DEP in vitro. Cell viability, glutathione (GSH) depletion and mRNA expression levels for 6 genes (Edn1, Ece1, Ednra, Ednrb, eNos, iNos) were measured by qPCR with DEP concentrations up to 200μg/ml culture media. DEP induced no more than a 20% loss of cell viability (24hr exposure). However, DEP for 3 hrs decreased Edn1 mRNA expression. Edn1 is the precursor gene of endothelin-1 (ET-1), a potent vasomotor. Additionally there was a >2-fold increase in eNos mRNA and a substantial induction of iNos. These responses were found in DEP exposures at concentrations as low as 10 μg/ml. Although these effects are not characteristic of endothelial responses expected in vivo (vasoconstriction), these results do suggest that DEP can influence the expression of vasoactive mediators. This work was supported by NIEHS grants 1P50ES015915, P30ES07033 and T32ES07032.

However, each BD exposure indicated a lesser toxic response than its corresponding PDE exposure. In addition, our results suggest that aging significantly enhances the toxicological responses of fresh BD and PDE emissions in the particle phase, and decreases in the gas phase (when particles are present). This suggests that the gaseous components are partitioning onto the particles during the aging process. Taken together, these results further support the importance of fuel type and atmospheric aging when examining the toxicity of vehicle emissions.

EXPOSURE TO COMBINED VEHICLE EMISSIONS ALTERS VASCULAR REACTIVITY IN APOE-/- MICE.

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Traffic-related air pollution is consistently associated with cardiovascular morbidity and mortality. Recent human and animal studies suggest that exposure to vehicular exhaust (VE) in air pollution affects vascular function, specifically enhancing arterial vasoreactivity. The goal of this study was to examine the effects of short-term exposure to VE on vascular reactivity and on mediators of vascular tone. Two groups of ApoE-/- mice were exposed to 300μg PM/3m3 of VE (a combination of diesel and gasoline engine emissions) by whole body inhalation for 6 h, then sacrificed either immediately or 18 h post-exposure. Control ApoE-/- mice were exposed similarly to filtered air (FA). The aorta was extracted from each animal and 3mm segments were mounted on a tension myograph system to measure contractile response. Data revealed no significant differences in the contractile response to phenylephrine (PE) in either exposure group. However, when comparing acute FA, VE exposed mice elicited an increase contractile response to the thromboxane mimetic, U46619, and a decrease in relaxation after administration of acetylcholine (ACh). These findings suggest that short-term exposure to VE is associated with acute endothelial response and vasocostriction and is consistent with similar findings from coronary and mesenteric vessels. Identification of the target pathways controlling vascular tone that are susceptible to emissions requires further study.
that DEP make capillaries leaky by disrupting endothelial adherens junctions and inducing secretion of VEGF-A. The production of potentially damaging ROS is also induced by DEP. Supported by NIEHS P30ES05022.

208 ACTIVATION OF GSK-3β PLAYS A CRITICAL ROLE IN DIABETES-RELATED CHANGES IN CARDIAC ENERGY METABOLISM, LIPID ACCUMULATION, INFLAMMATION, AND REMODELING: METALLOTHIONEIN PREVENTION VIA AKT2 PRESERVATION.

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Glycogen synthase kinase-3β (GSK-3β) was found to play a pivotal role in a few cardiac disorders. We have shown that cardiac-specific metallothionein (MT)-overexpressing transgenic (MT-TG) mice were highly resistant to diabetic cardiomyopathy. Therefore, we investigated whether MT’s cardiac protection against diabetes (Supported, in part, by research grants from ADA, JDRF) is mediated by inactivation of GSK-3β. Diabetes was induced by streptozotocin with single dose injection in both MT-TG and wild-type (WT) mice. Cardiac changes including energy utilization, lipid accumulation, inflammatory response and fibrotic remodeling were examined at 2 weeks, 2 and 5 months after diabetes with Western blotting, RT-PCR, and immunohistochemical assays. Results showed that GSK-3β was significantly activated in the hearts of WT diabetic mice, but not MT-TG diabetic mice. Consequently, cardiac glycogen synthase (GS) phosphorylation, hexokinase (HK) II and PPARα expression, which mediate glucose and lipid metabolism, were also significantly changed along with cardiac lipid accumulation, inflammation (TNF-α, PAI-1 and ICAM-1), and fibrosis only in the WT diabetic mice. To further confirm the pivotal role of GSK-3β in the above pathogenic changes, pharmacological inhibition of GSK-3β activity in the WT diabetic mice was found to completely prevent these effects. To define how MT inactivates GSK-3β, Akt2 expression levels were found to be significantly reduced in the hearts of WT diabetic mice, but not MT-TG diabetic mice. Knockdown of Akt2 expression by siRNA resulted in a significant activation of GSK-3β, along with inactivation of GS, decrease of HK II and increase of PPARα expression. These results suggest that activation of GSK-3β plays a critical role in changing cardiac energy utilization, increasing cardiac lipid accumulation, inflammation, and remodeling. Inactivation of GSK-3β by MT is mediated by preventing diabetes-attenuated Akt2 expression and plays a critical role in its cardiac protection against diabetes (Supported, in part, by research grants from ADA, JDRF).

209 ARYL HYDROCARBON RECEPTOR HETEROZYGOUS MICE EXHIBIT ENHANCED SENSITIVITY TO VASOCONSTRICTORS.

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Aryl hydrocarbon receptor (AHR) null mice are hypertensive at modest altitude and hypotensive at sea level. We tested the hypothesis that blood pressure would be normal in AHR heterozygous mice at modest altitude, but these mice would exhibit altered vascular responses to vasoconstrictors. We measured mean arterial blood pressure (MAP) in C57Bl/6 AHR wildtype (WT) and heterozygous (Het) mice by in vivo telemetry and compared the untreated mice treated with inhibitors of angiotensin II formation (captopril), endothelin-1 signaling (PD155080) or both. In addition, we assessed vasoconstrictor responses to phenylephrine (PE) ± endothelial nitric oxide synthase (eNOS) inhibitor, LNNa, in isolated aortic rings. AHR Het mice were normotensive, compared to WT mice (MAP: 105±1.3 mm Hg, WT; 106±2.1 mm Hg, Het; n=8/genotype). While captopril significantly reduced MAP in both genotypes, the decrease in AHR Het mice was significantly greater (A BP: -10.6±1.3 mm Hg, WT; -15±4.1±1.1 mm Hg; n=8/genotype; p<0.05). In contrast, PD155080 did not decrease MAP in WT mice, but did decrease it in AHR Het mice (A BP: -1.8±0.9 mm Hg, WT; -5.7±1.2 mm Hg, Het; n=8/genotype; p<0.05). The two drugs together were additive in reducing MAP in both genotypes. In addition, aorta from AHR Het mice constricted to a significantly greater degree to PE than from WT mice (% KCl constriction: 29±6, WT; 40±5, Het). Since PE constriction is offset by eNOS production of the vasodilator, nitric oxide (NO), blocking eNOS activity reveals the degree of NO production. PE-induced constriction was not significantly different between AHR WT and Het mice following pretreatment with LNNa±% KCl constriction: 11±4, WT; 12±9, Het). Taken together these data suggest that AHR Het mice exhibit an enhanced response to vasoconstrictors, including angiotensin II, endothelin-1, and PE, and this enhancement may be mediated, in part, by a reduction in NO production. Supported by HL078914.

210 DOXORUBICIN INDUCED PLATELET CYTOTOXICITY AND APOPTOSIS, POTENTIAL CONTRIBUTING FACTOR TO DOXORUBICIN ASSOCIATED THROMBOCYTOPENIA.

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Doxorubicin (DOX) is a widely used anti-cancer drug for solid tumors and hematological malignancy, yet its active use is impeded by serious adverse effects including thrombocytopenia. While bone marrow toxicity of DOX has been suggested to be the sole mechanism underlying the reduced platelet counts, direct effects of DOX on platelets have never been examined. Here we investigated the toxicity of DOX on platelets and its underlying mechanism in an effort to elucidate the contribution of platelet cytotoxicity in DOX-induced thrombocytopenia. In freshly isolated human platelets, DOX induced platelet cytotoxicity in a time and concentration dependent manner. DOX-mediated ROS generation, reduced glutathione and subsequent protein thiol depletion were shown to mediate the DOX-induced platelet cytotoxicity. Conspicuously, DOX-treated platelets displayed apoptotic features like caspase-3 activation, reduced mitochondrial transmembrane potential and phosphatidylserine exposure as shown by flow cytometric analysis. More importantly, DOX-mediated platelet cytotoxicity was significantly enhanced by shear stress, important complicating factor in cancer patients and these results could be further confirmed in vivo animal model where intravenous infusion of DOX damaged platelet integrity significantly. In summary, we demonstrated that DOX can directly induce platelet cytotoxicity through ROS generation, GSH decrease and protein thiol depletion. Protein thiol depletion was the key link in DOX-mediated ROS generation with platelet cytotoxicity and apoptosis. With this study, we believe that important evidence was given into the role of DOX-induced platelet cytotoxicity in the development of thrombocytopenia in DOX treated patients.

211 ARSENIC REQUIRES SPHINGOSINE-1-PHOSPHATE TYPE 1 RECEPTORS TO STIMULATE VASCULAR REMODELING.

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Environmental arsenic exposure from drinking water is a major public health concern associated with increased risk and incidence of cardiovascular diseases and cancers. Even with low environmental exposures, arsenic affects a number of vascular beds causing liver diseases (sinusoidal capillarization and portal hypertension), ischemic heart disease, peripheral vascular disease (arteriosclerosis obliterans), and tumor angiogenesis. We previously established that activating Rac1-GTPase and NADPH oxidases is essential for arsenic-stimulated pathogenic vascular cell signaling. However, the mechanism for initiating this arsenic signaling is unknown. Here we show that arsenic stimulated the sphingosine-1-phosphate type 1 receptor (SIP1,) to initiate Rac1-mediated signaling for angiogenic gene expression and tube formation in human microvascular endothelial cells (HMVEC). In addition, we demonstrated that SIP1 is highly expressed on liver sinusoidal endothelial cells (LSEC) and is required for arsenic-stimulated LSEC deendothelialization. Arsenic-stimulated signaling in HMVEC and LSEC was inhibited by Pertussis toxin or a selective inhibitor of Rac1. Arsenic- and SIP-stimulated LSEC deendothelialization and HMVEC tube formation were blocked by VPC 23019, a SIP antagonist. SIP, knockdown with siRNA inhibited arsenic-stimulated HMVEC angiogenic gene induction and tube formation. In contrast, SIP, knockdown did not affect arsenic induction of HMox1 or IL8. Since excess or inappropriate signaling through SIP1, contributes to oxidant-mediated vascular disease and increased tumor angiogenesis, these findings may provide mechanistic insight into an initial step in the vascular pathogenesis of arsenic. Supported by NIEHS R01ES013781.

212 MITOCHONDRIAL REACTIVE OXYGEN SPECIES CAUSE ZIDOVIDINE-INDUCED CARDIAC MITOCHONDRIAL TOXICITY IN VIVO.

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Transgenic mice (TG) were used to explore mechanisms of mitochondrial oxidative stress from zidovudine (AZT) used to treat HIV/AIDS. TGs either depleted or over-expressing mitochondrial superoxide dismutase (SOD2-2X or SOD2-2KO, respectively) or expressed mitochondrially-targeted catalase (mCAT). TGs and wild type (WT) littermates were treated (oral AZT: 0.22 mg/day; 35d). As a biological read out, cardiac mitochondrial oxidative events and dysfunction were defined in ‘2 by 2’ protocols. Left ventricle (LV) mass and LV end diastolic dimension
were determined echocardiographically. Mitochondrial H2O2, aconitase activity, histology and ultrastructure were analyzed. AZT-treated WTs exhibited oxidative stress and cardiac dysfunction. Mitochondrial H2O2 increased and aconitase was inactivated in SOD2−/− KO, and cardiac dysfunction worsened with AZT. Conversely, native cardiac function was preserved in SOD2−/− hearts and in mCAT hearts. In SOD2-OX and mCAT TGs, mitochondrial H2O2, aconitase, LV mass and cavity volume resembled that of vehicle-treated WTs. Both SOD2-OX and mCAT prevent or attenuate oxidative stress and AZT-induced cardiac dysfunction. AZT worsens SOD2−/− KO cardiac dysfunction and increases mitochondrial H2O2. Since dysfunctional changes are attenuated by decreasing H2O2 and worsened by increasing H2O2, oxidative stress from H2O2 is crucial pathogenetically in AZT-induced mitochondrial toxicity.

**213 THE PHARMACOKINETIC/PHARMACODYNAMIC RELATIONSHIP: IS THE KINETIC PROFILE AFFECTED BY CARDIOVASCULAR EFFECTS?**

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To achieve toxic effects in a toxicology study or observe cardiovascular changes in a safety pharmacology study, selection of doses that will also allow for determining a maximum safe starting dose in man is critical. The selected high doses for non-clinical studies often differ from those used in nonclinical kinetics. Cardiac profiles characteristic of non-linear kinetics (e.g., plateau before decay) are indicative of saturation. An alternative theory is the physiologic response, particularly the cardiovascular (CV) response, may result in a kinetic profile similar to those attributed to nonlinear kinetics. We investigated the effects of decreasing BP and HR on the kinetics of diltiazem (D) in a rat hemodynamic model and cisapride (C) in a guinea pig CV model. These studies were conducted remotely using the Empia/Clexus to dose/collect blood samples while measuring HR and BP simultaneously using telemetry. A blood collection catheter was placed in the carotid artery (femoral vein in rats) and a dosing catheter in the jugular vein. Rats were iv dosed with 3 doses of D (2.5, 5, and 10 mg/kg) with washout between doses. Guinea pigs were given a single iv dose of C (3 mg/kg). D at 10 mg/kg decreased HR and BP significantly in all animals. Nonlinear (atypical) PK profiles were found in these animals and associated with the significant decreases in HR and BP. C decreased HR in 4 guinea pigs however, transient decreases in BP were recorded in only 2 guinea pigs. PK profiles for those 2 guinea pigs resulted in atypical profiles during early time points when changes in the CV endpoints were maximal. However, the animal that demonstrated less CV effects showed typical decay in C concentrations over time. There was a strong association between atypical PK profiles and duration/extent of decreases in HR and BP after dosing with D and C. The simultaneous collection of cardiovascular and kinetic information demonstrated that alterations in BP and HR may also explain the changes in the kinetic profile.

**214 DEVELOPMENT OF METHODS TO IMAGE CALCIUM TRANSIENTS IN HUMAN CARDIOMYOCYTES.**


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The early identification of potential cardiovascular effects, as well as detection of drug-induced arrhythmias including torsades de pointes is critical to the evaluation of potential new chemical entities. Determination of effects on additional channels may provide further insight into mechanistic understanding of a compound's action on cardiac function. Once established for a compound and/or compound class, such an assay may provide a relatively fast and easy way to de-risk cardiovascular liability. The ability to test compounds on human cardiac function, and specifically effects on calcium channels/handling, has been limited by the availability of human cardiomyocytes. Human cardiomyocytes are now available commercially in sufficient number to study effects of compounds on cardiac channel function and calcium signaling, in the most relevant species. We sought to establish conditions to study cardiac function and calcium handling in primary human cardiomyocytes by imaging intracellular calcium using calcium sensitive fluorescent dyes and then to determine the sensitivity of the assay to known cardiotoxins that affect the cardiac signaling pathway. We first established conditions to image calcium levels in cardiomyocytes using Furaz-2AM dye and then evolved reproducible responses using high potassium extracellular solution. Our results indicate that the calcium influx induced by high potassium was blocked in a concentration-dependent manner by calcium channel antagonists nifedipine and verapamil, indicating that activation of voltage-gated calcium channels mediated the signal, at least in part. Finally, we tested doxorubicin, a well-known cardiotoxicant, and effects on calcium signaling. Doxorubicin also inhibited calcium responses concentration-dependently. These preliminary results suggest that human cardiomyocytes may be useful tool to evaluate compounds effects on calcium signaling, either directly or through downstream effects on cell health/viability. Additional compounds will be evaluated to establish broad utility of the assay.

**215 DOBUTAMINE CARDIAC “STRESS” TEST IN THE RAT: STRAIN COMPARISON AND POTENTIAL UTILITY IN CARDIOVASCULAR TOXICITY STUDIES.**


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Electrocardiogram (ECG) exercise testing is used in patients with risk of heart disease. The test stresses the patient while monitoring them for ECG abnormalities, possibly indicating underlying cardiovascular (CV) disease. Dobutamine, a β1-adrenergic agonist that increases heart rate (HR) and contractility, can be administered intravenously (i.v.) and used in place of exercise. We examined the utility of a dobutamine stress test in rats to assess differences in strains and use as a measure of cardiac toxicity. Wistar-Kyoto (WKY), spontaneously hypertensive (SH) and spontaneously hypertensive heart failure (SHHF) rats were surgically implanted with radiotelemeters and a chronic i.v. catheter in the left jugular vein. After recovery, conscious rats were monitored with ECG and placed in a whole-body plethysmograph (WBP) to monitor ventilatory parameters. The i.v. catheter was exteriorized from the WBP and attached to an infusion pump, delivering increasing doses of dobutamine (10, 20, 40 and 80 μg/kg/min) to the conscious animal. WKY rats experienced a dose-dependent increase in HR (average maximum of 511 bpm at highest dose) and cardiac arrhythmias (7% total on average), both of which were potentiated in SHHF rats (53% bpm and 23 arrhythmias). There were no significant differences between WKY and SH results or in any ventilatory parameters. A group of animals from each strain was also tested under urethane anesthesia (1.5g/kg ip). Each strain had reduced HR response to increasing doses of dobutamine and minimal arrhythmia. In conclusion, the dobutamine stress test shows that SHHF rats are prone to elevated HR and arrhythmic events when compared to the healthier strains. As a methodology, this test can be a valuable tool to assess the impacts of air toxics on the CV system of animals with pre-existing disease. (This abstract does not reflect EPA policy)

**216 INCIDENCE AND SPECIFICITY OF DRUG-INDUCED TRAFFICKING INHIBITION OF CARDIAC ION CHANNELS.**

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The hERG potassium channel is the most common target for drug-induced QT prolongation resulting in torsades de pointes (TdP). HERG or hERG current can be compromised in two ways: via drug-induced direct block of the channel and/or drug-induced trafficking inhibition resulting in fewer channels at the cell surface. To determine the incidence of trafficking inhibition, the Prestwick Chemical Library (1120 structurally diverse compounds) was screened in HERG-Lite®, the only high-throughput assay able to predict HγR trafficking inhibitors and blockers. A total of 12.4% (138/1120) of the compounds in the library showed significant trafficking inhibition in HERG-Lite®. Of these, 49 were pure trafficking inhibitors as no block was detected in automated patch clamp (APC; PatchXpress®, MDS-AT). The remaining 89 were dual risk compounds in that they showed both trafficking inhibition in HERG-Lite and direct block in APC. Of the 285 compounds identified by APC as hERG blockers, 31% were also trafficking inhibitors. The specificity of drug-induced trafficking inhibition was assessed by monitoring the cell surface expression of six other cardiac ion channels: Nav1.5, KOLQT1/minK, Kir2.1, Kir2.2, Kv1.5, and Kv4.3 in an assay modeled after HERG-Lite®. The well-known hERG trafficking inhibitors — gendamycin, arsenic trioxide, pentamidine, and cardiac glycosides—all showed distinct patterns of effects on the other cardiac ion channels, including increases in surface expression in some cases, suggesting considerable specificity in drug-induced trafficking inhibition.

**217 CELLULAR OXIDATIVE STRESS ALTERS ENOS FUNCTION BUT NOT EXPRESSION IN EA.HY926 CELLS.**

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Vascular endothelium is both a target for angiotensins, many of whom serve to increase cellular oxidative stress, and a source of protection against vascular damage. A key player in the maintenance of endothelial function and vascular homeostasis is
nitric oxide (NO) production mediated by endothelial nitric oxide synthase (eNOS). To examine the interaction between oxidative stress and endothelial function, the NO production capacity by human EA.hy926 cells, a cell model for vascular endothelium was studied. Nitric oxide (NO)-specific difluorofluorescein dyes, DAF-FM and DAF-FM-DA (5 Cy3 Calbiochem) were used to measure extracellular and intracellular NO production and total and active (phosphorylated) eNOS expression measured by western blotting under basal and oxidative stress induced conditions. Oxidative stress was verified by increased dichlorofluorescein fluorescence. EA.hy926 cells exhibited comparable total eNOS and somewhat greater phosphorylated eNOS expression (normalized to β-actin) relative to human umbilical vein endothelial cells; basal cell-mediated NO production, however, was negligible. Induction of cellular oxidative stress by glutathione depletion and/or hydrogen peroxide increased intracellular NO but not extracellular NO by approximately 2-fold. This induction of cellular oxidative stress had no effect on total or phosphorylated eNOS expression, or the ratio of phosphorylated to total eNOS. These data suggest that while EA.hy926 cells contain adequate and active eNOS expression, demonstrable NO production in these cultures requires induction of cellular oxidative stress. This oxidative stress-induced NO production may be specific for certain reactive oxygen species such as hydrogen peroxide, and is not mediated by changes in total or active eNOS expression.

218 HYPOXIA-INDUCIBLE FACTOR 1α IS CRITICALLY INVOLVED IN METALLOTHIONEIN PROTECTION AGAINST DIABETIC CARDIOMYOPATHY.

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Background: Previous studies have shown that metallothionein (MT) is effective in the prevention of diabetic cardiomyopathy, and hypoxia-inducible factor 1α (HIF-1α) is known to be a master transcription factor regulating a variety of genes involving in angiogenesis and glycolysis in the heart. Given that the expression of a variety of angiogenic factors, such as vascular endothelial growth factor (VEGF) and glycolysis enzymes, such as hexokinase II (HK-II), are regulated by transcription factor HIF-1, it is interesting to investigate the role of MT in the regulation of HIF-1 activation. Objective: The purpose of this study was to examine whether or not MT affects HIF-1α activity in the heart of diabetic mice and in the cardiac cells cultured in a medium that mimics diabetic conditions leading to the regulation of angiogenic factors and glycolytic enzymes. Methods: Diabetes was induced by streptozotocin (150 mg/kg) in a cardiac-specific MT or HIF-1α-over-expressing transgenic mouse model. The primary mouse neonatal cardiacmyocytes and embryonic rat cardiac fibroblast H9c2 cells were cultured. mRNA and protein levels of VEGF and HK-II and activity of HIF-1 were analyzed by RT-PCR, Western blotting and HIF-1α dependent luciferase activity assays. Results: Diabetes decreased cardiac VEGF and HK-II protein level in mice; both cardiac-specific MT and HIF-1α-over-expression could relieve this suppression. Further analysis showed that MT over-expression increased HIF-1α protein level in diabetic heart. Stably over-expressing MT in H9c2 cells increased HIF-1α mRNA level, indicating the transcriptional modulation of HIF-1 by MT. The addition of MT into the cultures of H9c2 cells transfected with HIF-1α-specific reporter gene relieved high glucose suppression of hypoxia-induced luciferase activity. Conclusion: MT rescues HIF-1α transcriptional activity leading to normalization of VEGF and HK-II in cardiomyocytes under diabetic conditions.

219 ENHANCED CONSTITUTIVE ENDOTHELIAL-DEPENDENT VASODILATION IN ARYL HYDROCARBON RECEPTOR NULL MICE.

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Aryl hydrocarbon receptor (AHR) mice were reported hypertensive at modest high altitude and hypotensive at sea level. After being acclimated to altitude for generations, the AHR null mice start losing their high blood pressure phenotype. Since the constitutive expression of NO (NO) production plays a crucial role in maintaining normal blood pressure, we tested the hypothesis that AHR null mice has enhanced constitutive endothelial-dependent vasodilatation mediated by elevated endothelial nitric oxide synthase (eNOS) expression. We assessed vascular responses to vasoconstrictor phenylephrine (PE) and endothelial nitric oxide synthase inhibitor, N-nitro-L-arginine (LNAME) in aortic rings isolated from C57Bl/6 wildtype and AHR null mice. We also measured aortic eNOS protein expression by western blot and 24 hr nitrate/nitrite (NOx) excretion by Griess assay in mouse urine. In addition, we measured eNOS mRNA expression in cultured human aortic endothelial cells treated with AHR siRNA. Compared to PE treatment, AHR null mice aortic rings showed a greater change in response to PE treatment after preincubation with LNAME (4% of maximal KCl constriction: 85±6, WT; 123±5, AHR null; p<0.05), which revealed an enhanced constitutive NO production in AHR null mice. AHR null mice aortic eNOS protein expression in AHR null mice was significantly increased by 3-fold compared to wildtype mice. The 24 hr urine NOx excretion was also significantly increased (267±66 pmol, WT; 96±237 pmol, AHR null; p<0.05). In human aortic endothelial cells culture, eNOS mRNA was induced 2-fold with AHR siRNA treatment (p<0.05). Together, these data suggest an enhanced constitutive endothelium-dependent vasodilation mediated by elevated eNOS expression. In order to estimate the role of this vasodilatory effect on blood pressure regulation, we will assess the blood pressure of wildtype and AHR null mice with LNAME treatment. Supported by HI078914.
222 ELECTROPHYSIOLOGICAL PROFILING OF CARDIAC PROGENITORS FROM ADULT HUMAN MYOCARDIUM.

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Background: Development in vitro of assay techniques based on the human stem cell-derived cardiomyocytes would provide an opportunity to assess the specific cardiac safety end-points early in the drug development process. Objective: Electrophysiological properties of cardiac progenitors from adult human myocardium were investigated with special focus on establishment of cell-based high throughput cardiac toxicity screens. Methods: Characterization of ionic currents in clonal cardiac progenitors (CPs) derived from adult human heart was performed using automated patch clamp system PatchXpress 7000A. The cells were maintained in M-199 medium containing 10% FBS and subcultured every 48 hours on collagen/laminin extracellular matrix. Results: Outward potassium current mainly consisting of slow and rapid delayed rectifier (IKs, IKr) and Ca2+-activated chloride-muzzle-sensitive (IKCa) components were recorded. Inward cardiac sodium current suppressed by felodipine was present in all cells after 4 weeks in culture. Nifedipine-sensitive L-type Ca2+ current (ICa,L) was detected in ~10% of CPs. The inward rectifier Kir3.1 (KIR3.1) current could also be recorded. These results demonstrate the presence of functional cardiac currents in cardiac progenitors derived from adult human myocardium. For the first time assay protocols and procedures were established to measure the main cardiac currents in CPs and characterize their electrophysiological properties in a high throughput electrophysiology platform. Conclusion: Cardiac progenitors from adult human myocardium appear to be a promising model for the early in vitro cardiac safety assessment. Further validation of the model with tool compounds toward regulatory acceptance should evaluate predictivity of the model for clinical effects.

223 NEAR INFRARED (NIR) IMAGING OF LYMPHATIC ACTIVITY IN CONTROL SUBJECTS AND IN PERSONS WITH LYMPHEDEMA.

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Although the lymphatics are thought to play a crucial role in cancer metastases, diabetes, asthma, and obesity, there is little understanding of lymph function even in health. Acquired lymphedema can be caused by the filarial parasite or by trauma due to radiation therapy, infiltrating cancer, surgery, or infection, and congenital lymphedema involves structural changes in the architecture of lymphatic transport. A method to image lymphatic function was developed using indocyanine green (ICG) as a contrast agent and detection of its fluorescence following excitation by near infrared (NIR) light. After obtaining IRB approval and informed consent, 24 normal volunteers and 20 persons with stage 1 or II unilateral lymphedema of one limb were enrolled in a clinical study to evaluate lymphatic function. Intradermal injections of ICG (0.025 mg/injection) were made in arms or legs of all subjects, with up to 0.4 mg injected per subject after all injections were made. Dynamic imaging in arms of control subjects revealed a mean velocity of 0.62 cm/sec, 0.82 cm/sec in symptomatic arms of control subjects, and 0.69 cm/sec in symptomatic arms of persons with lymphedema. In conclusion, we observed four distinct structural patterns associated with lymphatic disorders in persons with lymphedema: absence of lymph flow or dysfunction in lymphatic transport, abnormal lymphatic tortuosity, and reversal of lymph flow. Structural changes are likely responsible for functional effects. This distinct lymph channels, lymphatic hyperplasia, lymphatic tortuosity, and reversal of lymph flow. Supported in part by the National Institutes of Health (R01 HL064391).

224 GERANYLGERANYLACETONE (GGA) PROTECTS THE HEART FROM DOXORUBICIN CARDIOTOXICITY WHILE REDUCING TUMOR VOLUME OF BREAST CANCER.


Background: Doxorubicin is widely used for the treatment of both solid tumors and hematological malignancies; however, its use is limited by cardiotoxic adverse effects. Geranylgeranylace tone (GGA), an anti-angiogenic agent, was used in Japan since 1984, with no known adverse effects, has been shown to inhibit ovarian cancer progression in vivo in mice. In oxidative stress animal models, GGA also reduces cell death. We hypothesized that GGA would prevent doxorubicin cardiac toxicity, limited cardiac cell death and oxidative stress, yet improve doxorubicin’s chemotherapeutic effect. Nude mice implanted with human breast cancer cells (MDA-231) were studied after single or combination chronic treatments. Results: GGA reduced doxorubicin toxicity by significantly maintaining heart fractional shortening after chemotherapy and significantly decreasing the TUNEL positive cells in the heart. This was accompanied by a reduction in cardiac scar formation, partially mediated through the source nitric oxide synthase. In xenografts implanted nude mice, GGA+DOX treatment also significantly reduced tumor mass versus DOX treatment alone. Metastases were reduced in the combined GGA-DOX treatment as observed by histopathology and by fluorescence optical imaging of remaining in tumor cells. In vitro, GGA inhibited lysophosphatic Acid (IPA)-induced matrix metalloproteinase (MMP) invasion by MDA-231 cells suggesting the pathway inhibition. Conclusion: GGA is a prospective drug for use as a anti-cancer treatment scheme.

225 COMPARISON OF ECG TELEMETRY DATA IN FREELY-MOVING DOGS, USING NON-INVASIVE AND INVASIVE METHODS.

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Electrocardiography (ECG) investigations are routinely performed during preclinical toxicology studies in non-rodent species. The pertinence and reliability of these data may be compromised, however, by the need to restrain the animal during the recording of surface ECG traces and by their limited duration (a few minutes). Telemetry may be employed to improve both the quality and duration of ECG investigations, but the surgical methods used to implant the sensors are often incompatible with the objectives of a regulatory toxicology study. Surface ECG recording and data transmission through Bluetooth technology may be used to address these limitations, allowing long-term ECG signal acquisition from freely-moving and socialised animals. Our objective was to compare surface ECG data recorded with a Jacket External Telemetry device (JET, DSI, USA) with those obtained using gold-standard invasive telemetry. Six dogs were surgically equipped with a telemetry implant and data collected simultaneously from both devices for 24 hours before and after oral administration of 30 mg/kg of sparfloxacin. The duration of the RR, PR and QT intervals and of the QRS complex were measured with each device at specific time-points. There was an excellent correlation of the data generated by the two devices (r = 0.95) both before and after sparfloxacin treatment. It was concluded, therefore, that non-invasive surface ECG recording may be reliably achieved in the free-moving dog during the course of a regulatory toxicology study using the JET device. This technology may prove to be a powerful tool for the detection of ECG-modifying properties of new drugs in regulatory toxicology studies (e.g. QT prolongation).

226 METALLOTHIONEIN PROTECTS CARDIAC CELLS AGAINST ANGIOTENSIN II-INDUCED APOPTOSIS THROUGH SUPPRESSION OF P3 EXPRESSION AND ACTIVATION IN VITRO AND IN VIVO.


Our previous study has shown the antioxidant protein metallothionein (MT) significantly prevented Ang II-induced NADPH oxidase activation, nitrosative stress, and apoptosis in the heart, which led to a significant prevention of cardiac remodeling and dysfunction. Since we found that tumor suppressor protein p53 played an important role in Ang II-induced cardiac apoptotic cell death, the present study was designed to determine whether MT protection against Ang II-induced apoptosis was mediated by suppression of p53 expression and activation. Cardiac H9C2 cells were pretreated with and without 25-50 μM zinc (Zn) to induce MT synthesis and then exposed to 100 nM Ang II for 7 hr or 24 hr. Exposure of cells to Ang II for 7 hr resulted in a significant increase apoptotic cell death which was enhanced by DNA fragment ELISA assay, and caspase-3 and caspase-8 cleavage, along with significantly increased total and phosphorylated p53 expressions. Pretreatment with Zn induced MT synthesis, detected by Western blotting assay, and also significantly inhibited Ang II-induced total and phosphorylated p35 expression and apoptotic cell death. To eliminate the inhibitory effect of Zn directly on Ang II-induced p53 expression and apoptosis, pre-exposure of cardiac cells to low dose of cadmium that significantly induced MT synthesis also showed a protection against Ang II-induced p53 expression and apoptosis, suggesting the protective role of MT, instead of Zn, in the cardiac cell death. Nude mice implanted with human breast cancer cells (MDA-231) were studied after single or combination chronic treatments. Results: GGA reduced doxorubicin toxicity by significantly maintaining heart fractional shortening after chemotherapy and significantly decreasing the TUNEL positive cells in the heart. This was accompanied by a reduction in cardiac scar formation, partially mediated through the source nitric oxide synthase. In xenografts implanted nude mice, GGA+DOX treatment also significantly reduced tumor mass versus DOX treatment alone. Metastases were reduced in the combined GGA-DOX treatment as observed by histopathology and by fluorescence optical imaging of remaining in tumor cells. In vitro, GGA inhibited lysophosphatic Acid (IPA)-induced matrix metalloproteinase (MMP) invasion by MDA-231 cells suggesting the pathway inhibition. Conclusion: GGA is a prospective drug for use as a anti-cancer treatment scheme.
of Zn, against Ang II effect on p53 and cell death. To validate the in vitro finding into an in vivo model, Ang II was infused into transgenic mice with cardiac-specific overexpression of MT (MT-TG) and their wild-type (WT) mice. Ang II induced significant up-regulation of total and phosphorylated p53 expression along with induction of apoptotic cell death in the hearts of WT mice, but not in those of MT-TG mice. These results suggest that MT protect the heart against Ang II-induced apoptotic cell death via suppression of p53 expression and activation (Supported in part by ADA and AHA grants).

227 EGGC, A MAJOR GREEN TEA POLYPHENOL, PROTECTS ENDOTHELIAL CELLS AGAINST PCB-INDUCED CELL DAMAGE VIA INCREASED NFR2 SIGNALING.

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Polychlorinated biphenyls (PCBs) are widespread and persistent environmental contaminants that have been shown to induce oxidative stress and inflammation in endothelial cells. Epigallocatechin gallate (EGGC) is believed to improve health and protect against vascular diseases such as atherosclerosis. Recently, EGGC has been demonstrated to induce NF-E2-related factor (Nrf2), a transcription factor that regulates expression of many antioxidant and phase II detoxifying enzymes. Thus, we hypothesized that EGGC may enhance the defensive mechanism of vascular endothelial cells against exposure to environmental contaminants such as PCB126 via upregulation of Nrf2. To test this hypothesis, we studied inflammatory parameters, including adhesion molecules. Primary vascular endothelial cells were pretreated with EGGC, followed by exposure to PCB126. Both EGGC and PCB126 significantly increased Nrf2 protein expression in a dose-dependent manner. In contrast, Nrf2-regulated phase II detoxifying enzymes, such as NADPH:quinone oxidoreductase-1 (NQO1), were significantly increased by EGGC treatment but not after exposure to PCB126. Both PCB126 and EGGC activated extracellular signal-regulated protein kinase 1/2 (ERK1/2), and the pharmacologic inhibition of this kinase reduced Nrf2 protein expression. Caveolin-1 silencing abolished both the PCB- and EGGC-stimulated Nrf2 and NQO1 protein expression. In addition, treatment with EGGC decreased the level of platelet/endothelial cell adhesion molecule-1 in the cells independent of PCB126 exposure. These findings suggest that EGGC induces Nrf2 expression mainly via an ERK1/2 signaling pathway, with subsequent upregulation of its target protein NQO1. Furthermore, functional caveolae appear to be necessary for Nrf2-mediated regulation of EGGC-associated cell protection. This may explain in part the protective properties of EGGC against PCB-induced cellular toxicity. (Supported by grants from NIEHS, NIH (P20ES07380), and the UK AES)

228 INTRANASAL BENZO[A]PYRENE ALTERS DIEL RHYTHM OF BLOOD PRESSURE IN RATS.

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Polycyclic aromatic hydrocarbons (PAHs), including benzo[a]pyrene (BaP), are environmental contaminants formed during organic material combustion (e.g. burning fossil fuels and cigarette smoke). BaP toxicity may be mediated by activation of the aryl hydrocarbon receptor and/or formation of reactive metabolites, both of which lead to increased oxidative stress. Since air pollution and cigarette smoking are known to increase cardiovascular disease in humans, it is hypothesized that increased oxidative stress from BaP exposure will increase blood pressure in rats. Rats implanted with femoral radiotransmission pressure catheters were exposed daily to BaP (0.01 mg/kg, intranasal) or vehicle (tricaprylin) for 7 days. Since vascular oxidative stress can reduce nitric oxide, causing endothelial dysfunction and increased peripheral resistance, plasma nitrate/nitrite levels were also measured. The slope of the rise in blood pressure (dP/dt) with each pulse was extracted from the pressure waveforms as a measure of vascular stiffness. BaP exposure significantly increased diastolic and systolic pressure (p<0.0005 and p<0.015 for BaP factor in 3-way ANOVA, respectively) compared to control. BaP exposure also significantly decreased pulse pressure and increased heart rate. Paradoxically, no significant difference in dP/dt was detected between treatment groups, while plasma nitrate/nitrite levels in the BaP-exposed rats increased to approximately 125% of the control group. Systolic, but particularly diastolic pressure failed to decrease in BaP-exposed rats primarily during the afternoon (period of inactivity/sleep for rats). This failure of blood pressure to dip during sleep is associated with increased risk of serious cardiovascular events in humans. The increased diastolic pressure observed in this study is not consistent with the elevated nitric oxide production. Furthermore, increased nitric oxide levels should have caused a decrease in dP/dt. Therefore, factors other than endothelial function appear to be altered to produce the elevated peripheral resistance observed in this study, particularly during sleep, in BaP-exposed rats.

229 CHANGES OF DYNAMIC BEAT-TO-BEAT QT-TQ INTERVAL RELATIONSHIP IN CONSCIOUS DOG MODEL OF SHORT AND LONG QT SYNDROMES.

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Repolarization restitution refers to the relationship of QT duration to the preceding TQ duration, and has been hypothesized to predict the arrhythmia due to reentry. Changes of ventricular repolarization time can affect restitution and possibly lead to untoward arrhythmia. This study was designed to evaluate restitution parameters (the 5th percentile of QT interval (TQ5min), QT/TQ ratio, the percent-age of beats with QT/TQ ratio >1) in the conscious, sling-trained canine model of short QT syndrome in comparison with long QT syndrome. The RR, QT, and TQ intervals were examined in sling-trained, conscious dogs infused with escalating concentrations of pinacidil, nicardipine, atorvastatin, and quinine. The QT shortening drugs (pinacidil and nicardipine)  significantly decreased QT, QTc(B,E,F), and TQmin intervals in a dose-dependent manner. The percentage of beats with a QT/TQ ratio > 1 were increased significantly (p<0.01) after giving pinacidil and nicardipine. In contrast, QT lengthening drugs (atorvastatin and quinine) significantly prolonged QT, and QTc(B,E,F) intervals in a dose-dependent manner. The percentage of beats with a QT/TQ ratio > 1 were significantly reduced (p<0.05) and the lower TQ interval boundary (TQmin) was significantly increased (p<0.05) after infusion of QT lengthening compounds. In conclusion, ECG restitution changes can be use as biomarkers to predict arrhythmia vulnerability in a conscious sling-trained dog model of short and long QT syndromes.

230 A DISSECTING AORTIC ANEURYSM MODEL INDUCED BY N-(2-AMINOETHYL) ETHANOLAMINE IN RAT.

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Dissecting aortic aneurysm (AAA) is a tear in the wall of the aorta that causes blood to flow between the layers of the media of the aorta and forces the layers apart. Aortic dissection is a medical emergency and can quickly lead to death, even with optimal treatment. Our aim is to study the toxicity of the industrial chemical N-(2-aminoethyl) ethanolamine (AEEA) in the development of AAA. In an in vivo study, pregnant Sprague-Dawley rat dams were treated with AEEA by intraperitoneal [IP] injection on gestation days 14-21, a period of rapid aortic development. Seven groups were treated (1 mg/kg to 100 mg/kg AEEA). The control group received normal saline. The aortas from newborn pups were harvested and examined for dissecting aneurysm. Pregnant rats died with the two highest doses of AEEA (75 mg/kg, 100 mg/kg) after 5-6 IP injections. No lesions were found in pups (0/12) at 1 mg/kg and in pups in the control group (0/23). The DAA were detected in other groups: 8/27 pups at 10 mg/kg, 14/29 pups at 25 mg/kg, and 6/9 pups at 50 mg/kg. In a preliminary in vitro study, the primary cultured vascular smooth muscle cells (SMC) of aortas from fetuses of Sprague-Dawley rats were exposed to AEEA. The cytotoxicity of AEEA was determined over a 24-hour incubation period with a range of concentrations from 5 to 200 mM AEEA. Using the standard MTT measure of cellular viability. An approximately 50-60% decrease in viability was noted at 25 mM AEEA or above. Our data indicate that AEEA given IP results in the development of DAA.

231 A NINETY-DAY SUBCHRONIC INHALATION TOXICITY STUDY OF GOLD NANOPARTICLES IN SPRAGUE-DAWLEY RATS.

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Gold nanoparticles (NPs) have been used extensively for therapeutic purpose or food supplement. Especially inhalation toxicity of gold NPs could be urgent information to ensure workers and consumer’s health. Gold NPs (4-5 nm) were generated and distributed of NPs measured. Rats were divided into 4 groups with 10 rats in each group: control group (low (2.36 × 101 particle/cc), middle (2.36 × 104 particle/cc, 1.68 × 105 particle/cc), and high-dose group (1.85 × 106 particle/cc)). The animals were exposed to Gold NPs 6/day, 5 day/week, for 90-day duration. Mortality and clinical observations were conducted daily and body weight and food consumption were recorded weekly. Lung
function tests were performed weekly. At the end of the study, all rats were subjected to full necropsy and absolute and relative organ weights were recorded. Blood samples were collected for hematologic and clinical chemistry. Male rats did not show significant changes in body weight during the 90-day experiment, but female rats in the low-dose group showed significant body weight changes when compared with the control group from day 42 of exposure. There were no significant changes in food consumption, lung function test or hematologic values in male or female rats during the study. There were no specific symptoms observed in ophthalmological examination. Blood analysis for male or female rats. There were no dose-dependent changes in blood biochemical values or absolute and relative organ weights for male and female rats. Histopathological examination showed some inflammatory changes in the high dose group. Therefore, the current results indicate inhalation exposure of gold NPs at the concentrations described in this report have no significant influence on health.

### 233 COMPARISON OF THE PULMONARY TOXICITY OF MICRON AND NANOSIZED GOLD PARTICLES IN RATS


**Introduction:** Due to physical characteristics such as size and surface to volume ratio, the interaction of nanoparticles versus micron sized particles of the same material with biological systems might be markedly different. Pure gold was chosen as a model particle to study effects on systemic and lung inflammatory markers. In addition, gold particles, especially in the nano size range are produced for medical application but much is known about potential toxic effects. This study has demonstrated a more widespread distribution for 10 nm sized particles compared to 250 nm after i.v. injection in rats. The present study was focused on the toxicity of gold particles. Methods: A single dose of 50 μg of spherical gold particles in aqueous suspension of 10 nm and 250 nm (SPI supplies) was administered to male WU Wistar-derived rats by intratracheal instillation. A single dose of 50 μg and 250 μg DQ12 quartz was used as a positive control. Dynamic light scattering (NanoSight) was used to determine the particle size distribution in the suspensions prior to administration. Bronchoalveolar lavage fluid and blood was collected to assess the effect on cell differentials, oxidative stress and inflammation after 19 and 72 hrs. Results: The 10 nm gold particles formed aggregates and had a mean particle size distribution of 175 nm, while 250 nm gold particles had a size distribution of 220 nm. A significant increase in monocytes and IL-6 levels is observed in the lung 72 hrs after 250 nm gold application, but this was not evident for 10 nm gold particles. After 19 hrs, an oxidative stress response is seen in the lung using 250 nm, but not with 10 nm particles. No sign of systemic inflammation by gold particles is seen. Conclusion: A difference was found for the 10 nm gold particles compared to 250 nm after instillation. The 250 nm particles elicited a mild inflammatory response in the lung, whereas the 10 nm particles did not.

### 234 DISTRIBUTION OF [14C]C60 IN PREGNANT AND LACTATING RATS


The potential for workplace and environmental exposure to carbon nanoparticles such as Buckminsterfullerene (C60) has generated a need to understand basic properties of C60 in biological systems. This study was conducted to determine the distribution of [14C]C60 in the pregnant rat and fetus, and in the lactating rat and offspring. Adult female Fisher rats were dosed via tail vein injection on gestational day (GD) 15 with 0.28 mg/kg body weight [14C]C60 (~3 μCi per rat) prepared in 5% polynvinyllpyrrolidone in saline (PVP), and four dams were dosed with PVP alone. Urine (0-24hr) and tissues (24 hr) were collected from the dams. Eight lactating rats were dosed on postnatal day (pnd) 8 with 0.36 mg/kg [14C]C60 prepared in PVP and sacrificed at 24 hr or 48 hr after exposure. In the pregnant dams, radioactivity was distributed to the placenta (approximately 2% of the dose), to the fetus (1.0%), and to the female reproductive tract (3.0%). Radioactivity was distributed to the milk (<1%) and mammary tissue (<1%) in the lactating rats, and to the GI tract (<1%) and liver of the pup (<1%). For the pregnant dam, radioactivity was distributed to the urine (<2%), blood (0.9% per mL) and plasma (1.7% per mL), brain (<1%), lung (<1%), heart (<1%), liver (<43%), spleen (4%). In comparison to the pregnant dam, lactating rats had a similarradioactivity distribution to the blood and plasma at 24 hr after exposure (with a 50% decrease by 48 hr), a higher distribution to the lung, and a decreased distribution to the liver. Metabolism analysis of urine indicated that dams exposed to C60 had a decrease in metabolites derived from the Krebs cycle and an increase in metabolites derived from the urea cycle or glycolysis; with alterations in the levels of some sulfur-containing amino acids and purine/pyrimidine metabolites. This study indicated that C60 can cross the placenta and can be transmitted from mother to offspring via milk. This research was funded by RTI International.

### 235 ASSESSMENT OF THE DISTRIBUTION OF INTRAVENOUSLY-INJECTED SILVER NANOPARTICLES IN PREGNANT MICE AND DEVELOPING EMBRYOS


The translocation of nanoparticles across the placenta and accumulation in developing embryos in vivo has not been widely studied. The objective of this study was to evaluate the distribution of silver (Ag) nanoparticles in pregnant mice and translocation to developing embryos. Ag nanoparticles (fabricated in-house, 7-9 nm in diameter as measured by dynamic light scattering) in citrate buffer were injected i.v. into pregnant CD-1 mice on gestation days (GD) 7, 8, and 9 (total: ~3 μg Ag/mouse). Controls received citrate buffer only. Mice were euthanized on GD10 and brain, heart, kidneys, liver, lungs, lymph nodes, ovaries, spleen, and thymus were collected from each dam. From each litter, the myometrium and endometrium of the uterus, chorio-allantoic placenta, visceral yolk sac, and embryos were collected. Tissue Ag levels were quantitated using inductively coupled plasma mass spectrometry (ICP-MS). In treated dams, Ag (in ng Ag/mg tissue) was detected at statistically significant (p<0.001) concentrations (compared with controls) in the liver (2.94), spleen (1.35), lungs (0.676), and visceral yolk sacs (1.95). Average accumulation in the liver was 64% of total detected Ag; average accumulation in the spleen, lungs, and visceral yolk sacs was 4, 2, and 1.6% of total detected Ag, respectively. Thus, Ag nanoparticles injected into pregnant mice distributed to maternal liver, spleen, and lungs, with the highest accumulation in liver. Ag nanoparticles distributed to the visceral yolk sacs, but did not accumulate in uterine tissue, chorio-allantoic placenta, or developing embryos to statistically significant levels.

### 236 ASSESSMENT OF THE TOXICITY OF MULTI-SIZED SILVER AND GOLD NANOPARTICLES IN ZEBRAFISH EMBRYOS

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Nanoscale engineering represents a current dynamic area of interdisciplinary research incorporating nanoparticles into a diverse product matrix. Due to the disparate physicochemical properties and functions correlated with nanoparticles,
there is an acute need for assessing nanotoxicity. Nanoparticle size, surface functionalization, and chemical composition are but three critical nanotoxic metrics. Zebrasuf (Danio rerio) embryo exposures with non-functionalized, colloidal silver (cAg) and gold nanoparticles (cAu) in an array of sizes (5, 10, 50, and 100 nm) produced a range of toxic effects. Using a semi-quantitative scoring system, cAg, 10, 50, and 100 elicited nearly 100% embryonic mortality 120 h post-fertilization (hpf); cAu, 10, 50, and 100 exposures resulted in less than 3% mortality 120 hpf. While cAu induced minimal sublethal toxic effects, cAg exposures generated various embryonic and morphological malformations including stunted growth, opaque and non-depleted yolks, circulatory malformations, and jaw defects amongst others at 120 hpf. Both cAg and cAu were taken up by the embryos and results from vector control experiments suggest cAg sublethal toxicity and mortality were a result of the nanoparticles themselves or Ag ions formed during in vivo nanoparticle destabilization or as a residual synthetic contaminant. Although cAg toxicity was size-dependent at specific concentrations and time points, the most striking result is that parallel sizes of cAg and cAu induced significantly divergent toxic profiles, with the former being toxic and the latter inert at all sizes tested. Thus, we propose that nanoparticle chemistry is as, if not more, important than specific nanosizes at inducing toxicity in vivo. Ultimately, nanotoxicity assessments using the zebrafish embryo model should identify nanomaterial physicochemical characteristics that yield minimal or no toxicity for improved rational designs of nanomaterials.

237 EXAMINATION OF POTENTIAL DERMAL IRRITATION FOR DIFFERENT SIZES OF METAL NANOPARTICLES.

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Nanoparticles can have properties different than larger sizes of the same material. Knowledge about potential health effects is limited. Exposure of skin to nanoparticles is possible and could result in dermal irritation. In an effort to test nanoparticles in vivo while minimizing the number of rabbits used, nine nanoparticles were placed on the backs of 4 rabbits to determine if they cause skin irritation. The study followed the Environmental Protection Agency Health Effects Test Guidelines, Office of Laboratory, Peripherals and Toxic Substances 870.2500, Acute Dermal Irritation, as the basic procedure. We determined the ideal number of sites when placing Hilltop Chambers on the skin is 8 sites per rabbit. Three other sites are used as a control with no chamber at all. Four rabbits were used to expose 9 nanoparticles, three times for each nanoparticle. A randomized rotation was employed to blind those reading the sites. Each of the 4 rabbits had at least one negative control site with a Hilltop Chamber only and seven sites with nanoparticles (six for one rabbit with two negative controls). Each nanoparticle site on each rabbit was a different nanoparticle so that each nanoparticle was still placed on 3 different rabbits. Nanoparticles used were suspended in 0.5 ml of saline: Aluminum (50, 80 nm); Silver with proprietary organic coating (10 and 80 nm); Silver without coating (10, 25, 80 nm). Tungsten (60 nm); and Aluminum Oxide (30 nm). After the 72-hour observation, skin was collected for histopathology. The results demonstrated that none of the nanoparticles tested produced skin irritation.

238 LACK OF DERMAL PENETRATION FOLLOWING TOPICAL APPLICATION OF COATED AND UNCOATED NANO AND MICRONIZED TITANIUM DIOXIDE TO INTACT AND DERMABRADED SKIN IN MICE.

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Dermal transport and possible toxicity of nanoscale metal oxides used in cosmetic and personal care products are relatively unexplored and is the focus of this study. We previously reported that ~10% of intradermally injected PEG-coated nanoscale titanium dioxide (nano-TiO2) bioaccumulate in the liver and lymph nodes. The topical application of QDs to dermabraded skin resulted in accumulation of ~1% and ~0.1% of the applied dose in the liver and lymph nodes, respectively. We also demonstrated that following intradermal injection, the biodistribution of three different types of TiO2, uncoated nano- and micro-sized particles, and coated nanoparticles, depended upon the size and coating properties. In that study an increase in titanium (Ti) in liver accounted for ~10% of injected dose of all three TiO2, in lymph nodes the Ti accounted for ~1% of injected dose seen with coated nano and micro TiO2, and Ti accumulated in kidneys; exclusively with micro TiO2. (~10% of dose). In the present study, emissions containing 5% of either nanoscale (25 nm primary particle size) uncoated anatase TiO2, micron-scale anatase TiO2 and nanoscale (14 x 7.9 nm rods) polystyrene/alumina-coated TiO2, were topically applied to intact dorsal or inguinal and axillary regions or to dermabraded skin of hairless mice. Lymph nodes, liver, spleen, kidneys and blood were collected at 6 and 24 hours post application and Ti levels were quantified by ICP-MS. No significant elevations in Ti levels were observed in all the organs analyzed for the three different TiO2 formulations when applied to intact and dermabraded skin. The results suggest that both intact and compromised skin of hairless mice may be an effective barrier in preventing dermal penetration and subsequent organ bioaccumulation of topically applied nano- and micron-sized TiO2, of different surface coatings. These studies were supported by an IAG between the Toxicology National Program and FDA (IAG# 244-07-0007).

239 PULMONARY RESPONSES TO DIESEL FUEL CATALYST CERIUM OXIDE NANOPARTICLES.

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Cerium oxide has been used as a fuel-borne catalyst to lower the mass of diesel exhaust particles (DEP), but is emitted as nanoparticles in the diesel exhaust. While the health effects of DEP have been investigated for decades, the potential pulmonary toxicity of cerium oxide nanoparticles has not been characterized. The objectives of this study were to investigate the effects of cerium oxide on the pulmonary immune/inflammatory responses. Rats (Sprague Dawley) were exposed to cerium oxide (0.5 to 20 mg/kg) by intratracheal instillation. Alveolar macrophages (AM) were isolated via bronchoalveolar lavage (BAL) the next day. Cerium oxide exposure was found to induce significant neutrophil infiltration and elevate lactate dehydrogenase activity and albumin content in the BAL fluid, suggesting that these particles induced inflammation, cytotoxicity and epithelial damage. The results also showed direct interaction of cerium oxide with AM, resulting in AM phagocyte activity as monitored using confocal microscopy; increased reactive oxygen species production in response to zymosan challenge as indicated by enhanced chemiluminescence generation, and increased production of the pro-inflammatory cytokines IL-12 and TNF-α. However, cerium oxide significantly reduced nitric oxide production by AM in response to ex vivo LPS challenge, demonstrating that these particles may modify AM host defense capability. Analysis of mRNA levels of several genes using real time RT-PCR indicate that cerium oxide significantly increased suppressor of cytokine signaling and osteopontin (OPN) in BAL cells, while OPN was also increased in the lung tissue. When OPN was analyzed at 28 days post exposure, there was a further increase of mRNA in lung tissue. These results show that cerium oxide-mediated lung toxicity includes pulmonary inflammation and lung injury, and a persistent induction of OPN in lung tissue, which has been associated with lung fibrosis.
241 INVESTIGATION OF NANO PARTICLES-INDUCED TOXICITY USING CAENORHABDITIS ELEGANS FUNCTIONAL GENOMICS.


In the present study, toxicity of nanoparticles (i.e. silver nanoparticle, fullerene, carbon nanotube) was investigated in Caenorhabditis elegans using functional genomic tools. Whole genome microarrays were used to screen for global changes in C. elegans transcription profiles following nanoparticle exposure and with subsequent quantitative analysis conducted on selected genes. The integration of gene expression with organism and population level endpoints was investigated using C. elegans functional genomic tools, to test the physiological relevance of nanoparticle-induced gene expression. Silver nanoparticle exerted considerable toxicity in C. elegans, observed most clearly as a dramatic decrease in reproduction potential after 24 hours of silver nanoparticle exposure. Significant decreases in reproduction parameter concomitantly occurred in silver nanoparticle exposed C.elegans. Overall results of functional genomic using mutant analysis suggested that the sod-3 and dal-12 genes may be related to silver nanoparticle-induced reproductive failure in C. elegans and that oxidative stress may be an important mechanism in nanoparticle-induced toxicity. This study also suggested that the interpretation of microarray and subsequent quantitative expression data was greatly strengthened by linking them with organism and population level experiments and functional genomics using mutant strains appears to be an ideal tool for biomarker discovery in toxicological research as it can reveal the physiological meaning or function of observed altered gene expressions. Acknowledgment: This work was accomplished through the supports of the Ministry of Environment as “The Eco-technopia 21 project”.

242 INVESTIGATION OF THE ROLE OF AIRWAY INFLAMMATION BY NANOPARTICLES IN RESPIRATORY ALLERGY INDUCED BY TMA.

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Nanoparticles are increasingly used in a wide range of applications, and may have a higher tissue potential than similar particles of larger sizes. Nanoparticles can induce inflammatory reactions in the lung and may thus interfere with existing allergic inflammation. Our study aimed to assess whether inhalation exposure to nanoparticles aggravated a respiratory allergic response in Brown Norway (BN) rats. The rats were topically sensitized to trimellitic anhydride (TMA) and were challenged with TMA 14 days later. Next, animals were exposed by inhalation to carbon nanoparticles (about 30nm), aggregated carbon nanoparticles, fine carbon nanoparticles (about 300nm), multi-walled carbon nanotubes (MWCNT) or clean air 5 hours/day, 2 days/week during 3 weeks, and thereafter challenged with TMA again. The allergic rats exhibited an altered breathing pattern (apneoeas), and a slightly increased breathing frequency during challenge, allergic laryngitis and increased airway reactivity in the lungs. Exposure to nanoparticles and fine particles induced accumulation of black particles in the larynx, lungs and mediastinal lymph nodes. Exposure to nanotubules induced black granula, tubules and aggregate in the lungs only. Exposure to carbon nanoparticles or carbon particles did not aggravate the TMA-induced allergic reaction. Therefore, it was concluded that nanoparticles or nanoparticle aggregates did not alter the allergic response against TMA in BN rats, despite the presence of distinct particle accumulation in case of the nanoparticle aggregates.

243 PBPK MODELING OF MICRO AND NANO SIZED FLUORESCENT POLYSTYRENE SPHERES.

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To understand the kinetics of nanoparticles in comparison to that of chemically identical larger particles, we developed a physiologically-based pharmacokinetic (PBPK) model for micro and nano sized fluorescent polystyrene spheres using distribution data in rats. Four differently sized spheres (1000, 100, 40, 20nm) were dosed to rats as a single intravenous (IV) injection or pharyngeal aspiration into the airways, and the subsequent tissue distribution was assessed. Using these data, a full tissue compartment model was constructed consisting of blood, lymphatic system and twelve tissues, each described using first-order kinetics. The model was initially fit to the IV data on 1000nm spheres and applied to predict kinetics of the 3 nanosized spheres with same exposure route. The model successfully simulated the kinetics of 1000nm spheres. It also predicted tissue kinetics well from micro to nano in several major organs such as liver, lung, and heart. The model prediction suggested that compared to microspheres, the 40 and 20 nm spheres had a reduced distribution to the spleen and bone marrow and an increased distribution to the brain. However, it is unclear whether the slight increase in distribution to the brain for the small particles is real because the particle concentration in brain samples was near or below the limit of detection (<0.04% of total spheres). By re-optimizing certain parameters, we also fit the model to IV data on each of the 3 nanosized spheres respectively. The resulting model fit well for 100nm spheres, but was inadequate for 40 and 20nm spheres, for which further modifications of model structure may be needed. A comparison of model parameters optimized specifically for each sized particle indicated that the smaller particles tend to stay longer in blood circulation. Regardless of particle size, the estimated partition coefficients are always high for spleen and liver, but low for brain, suggesting a common kinetic mechanism of these particles in major organs.

244 ALTERED GENE EXPRESSION PROFILES IN MURINE BRAINS FOLLOWING EXPOSURE TO INHALED NICKEL NANOPARTICLES.

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The respiratory tract is the primary target for inhaled nanoparticles (NPs). However, there is evidence that once deposition occurs, these particles can escape clearance mechanisms and target secondary organs like the brain. The aim of this study was to examine the transcriptional response of three regions (olfactory bulb, midbrain, and cerebellum) in the mouse brain following exposures to inhaled nickel nanoparticles (Ni NPs). Utilizing a whole-body exposure system, C57 male mice inhaled either 100 μg/m3 of spark generated Ni NPs or filtered air for 5h/d, 5d/w, for up to 5m. The three regions were collected 1w, 5m, and 5m, post exposure (24h) for gene expression analyses (n=4/group). Since studies suggest that NPs damage tissue through oxidative stress and inflammatory mediated pathways, PCR profiling systems for both pathways were used to evaluate the change in expression of 168 genes in each region as compared to controls. A change of 3-fold or higher was considered important. After 1w of exposure, the expression of approximately 50 oxidative stress and inflammation related genes were altered in all regions. Following 5m of exposure, more changes in gene expression were observed in the olfactory bulb (85 and 72, respectively) followed by the cerebellum (35 and 45, respectively) and the midbrain (21 and 28, respectively). To confirm these results, individual real time RT-PCR was performed on selected genes: Mep-1, Trifl, Il-1β, Ho-1, and Cps-1. Brain deposition of inhaled Ni NPs was determined for all regions, using graphite furnace atomic absorption spectroscopy. A significant difference was only observed for the olfactory bulb (n=8, P<0.05). These results suggest that all three regions are affected by exposures of inhaled Ni NPs via oxidative stress-inflammatory mediated pathways; but, the olfactory bulb may be a more sensitive target to adverse effects. Additional studies are needed to link the changes in gene expression to mechanisms of toxicity.

245 POTENTIAL CARDIOVASCULAR EFFECTS OF FULLERENE C60 INHALATION EXPOSURE.

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Fullerenes are forms of carbon nanomaterials used in a wide variety of commercial applications and have the potential for significant human exposure. Based on ambient ultrafine particle research, it is predicted that small particles may have systemic toxicity related to cardiovascular outcomes. This study evaluates the cardiovascular effects of subchronic inhalation exposure of B6C3F1 mice to C60 particles (diameter 1 and 0.05 μm). The initial screening for cardiovascular toxicity was conducted applying a custom-designed TaqMan array for genes known to play an important role in the mechanisms of atherosclerosis such as inflammation, oxidative stress, and coagulation. We observed changes on expression of several genes related to stress response, including c-fos, and heat shock protein (hsp) 25, 70, and 90 in the aortas of the exposed mice independent of the size of the particles. These findings were confirmed by a real-time RT-PCR. Hsps are incremented in triggering many autoimmune/chronic inflammatory diseases. In this regard, it has been suggested a multifaceted role for hsps in atherosclerosis. For example, it has been reported that the expression of hsp90 is upregulated very early at lesion-prone sites in the aortas of young apoe-/- mice. Thus, fullerene exposure of wild type mice, not prone to develop atherosclerosis, resulted in a cardiovascular stress response which may provide a predisposition to atherosogenesis. Disclaimer: The findings and conclusions in this abstract are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.
246  GOLD NANOPARTICLE (AUNP) SURFACE CHARGE INFLUENCES TOXICITY.

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Nanoparticles can vary in size, chemical composition, surface chemistry and surface area. Small changes in these features impact different properties to a nanoparticle, including how they interact with and potentially influence biology. Early life stages are often sensitive to chemical insult, which make embryonic zebrafish as an ideal platform to investigate the biological activity and toxic potential of gold nanoparticles (AUNP). In initial studies, developmental responses to waterborne exposure to AuNPs were determined using a wide range of concentrations (0.016 to 250 μg/mL (ppm)). Cationic, N,N,N trimethylammoniummethanethiol (TMAT), and anionic, 2-mercaptosethanesulfonate (MES), AuNPs produced with a 1.5nm core size induced developmental toxicity in embryonic zebrafish. TMAT was more overtly developmentally toxic, while MES induced more sublethal effects at this developmental stage. The most susceptible early developmental stage to nanomaterial exposure was determined to be 24 hours post fertilization (hpf) and 48 hpf for TMAT and MES, respectively. Narrowing the window of exposure allowed for genomic evaluations to be conducted in order to determine gene expression responses to each AuNP exposure. Nimblegen zebrafish arrays with 37,157 genes were used with RNA isolated from embryonic zebrafish exposed to the lowest observable adverse outcome effect (LOAEL) for TMAT (50ppm) and MES (10 ppm) at 24 and 48 hpf. The combination of these studies illustrates the value of the embryonic zebrafish model to determine structure activity relationship, and that the surface charge of AuNP influences biological responses. Supported by NIEHS P3000210, EPA RD-833520, T32 ES07060, and Air Force Research Laboratory under agreement # FA6500-05-1-5041.

247  PULMONARY BIOASSAY STUDIES WITH SEPiolITE NANOCLAY SAMPLES IN RATS.

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Sepiolite is a magnesium silicate nanoclay mineral. A pulmonary bioassay toxicity study was conducted in male rats with sepellite, PBS, quartz particles and ultrafine titanium dioxide particles. Following intratracheal instillation exposures at doses of 1 and 5 mg/kg, the groups were evaluated by bronchoalveolar lavage (BAL) at 24 hours, 1 week, 5 weeks, and 3 months postexposure. Results showed that quartz particles produced sustained, lung inflammation. Exposures to TiO2 particles, sepellite or PBS produced transient lung inflammation. However, unlike the other particle-types, sepellite clay exposures produced macrophage-agglomerates or giant cells, recovered in BAL fluids at 1 wk, 5 wk and 3 month postexposure time periods. The biological significance of these findings is unknown. Following the bronchoalveolar lavage procedure, the lungs of randomly selected rats were then infused with formaldehyde fixative. Lung tissues from PBS control, high-dose Min-U-Sil quartz, negative control ultrafine TiO2, low and high dose sepellite-exposed rats at 3 months postexposure were then processed for lung tissue histopathological analyses.

Sectioned lung sections were prepared for histology and evaluated by light microscopy. Histopathology results revealed that quartz particles produced foamy alveolar macrophage accumulation and patchy evidence of alveolar tissue thickening, indicating incipient phases of lung fibrosis. Exposures to the ultrafine TiO2 particles resulted in accumulation of phagocytic alveolar macrophages but no tissue thickening. Exposures to sepellite particles produced evidence of multinucleated giant cells in airspaces and patchy evidence of minimal septal tissue thickening, indicating incipient phases of lung fibrosis. Exposure to the ultrafine TiO2 particles resulted in accumulation of phagocytic alveolar macrophages but no tissue thickening. Exposures to sepellite particles produced evidence of multinucleated giant cells in airspaces and patchy evidence of minimal septal tissue thickening, suggesting the development of lung fibrosis. The severity and frequency of tissue thickening with sepellite particle exposures appeared to be dose related. Follow-up, dedicated lung tissue histopathology studies currently are in progress to determine the reproducibility of this effect and the role of giant cells (if any) in producing minimal lung fibrosis following pulmonary sepellite exposures.

248  INFLUENCE OF PHYSICOCHEMICAL PROPERTIES OF NANO-SILICA PARTICLES ON IN VIVO TOXICITY.

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Nanometer-size amorphous silica nanoparticles (silica), which are used as functional additives in cosmetics and foods, have quickly become essential nanomaterials in our lives. With few exceptions however, there are insufficient data available on the risk assessment of nano-silica. We therefore analyzed the influence of the physico-chemical properties of nano-silica on acute toxicity. We have previously shown that injection of smaller nano-silica particles into mice induced acute toxicity as particle size decreased. In the present study, we used fluorescent-labeled nano-silicas whose surface was either unmodified or modified with -NH2 and -COOH (70 nm diam- etered, designated SP-plain, SP-NH2, and SP-COOH, respectively), to evaluate the influence of nano-silica surface properties on in vivo toxicity and distribution.

Methods) Acute toxicity tests were performed according to the following procedures. The mice were injected i.v. with 2 mg of SP-plain, SP-NH2, or SP-COOH. Survival of SP-injected mice was monitored, and histopathology tests were performed twenty-four hours after SP-injection. For image analysis, hairless mice (Hos: HK-1) were injected intravenously with nano-silicas. At indicated time points, post-injection imaging was performed using a Xenogen IVIS 200 imaging system.

Results and Discussions) A single intravenous injection of SP-plain induced acute lethal toxicity in mice. In contrast, injection of SP-NH2 and SP-COOH into mice caused little toxicity. In vivo optical imaging of the mice revealed that SP-plain had spread throughout the liver, whereas SP-NH2 and SP-COOH accumulated around the gallbladder. These results suggest that surface properties of nano-silicas were a critical factor for its liver accumulation and expression of toxicity.

249  SYSTEMICALLY-INTRODUCED NANOSCALE CERIA BIODISTRIBUTION AND TOXICITY.

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Objective: Characterize biodistribution and toxicity of nanoscale ceria that had entered blood. Rational: Ceria was chosen as a model insoluble, stable, metal oxide tracer with extensive engineered nanomaterial (ENM) applications. Materials: A 5% crystalline aqueous ceria dispersion, mean size ~ 30 nm (PSD); primary size ~ 3 to 5 nm (HR-TEM); surface area ~ 13 m2/g. Procedures: The effect of saline and 10% sucrose on ceria agglomeration was assessed. Ceria was given to un-anesthetized rats i.v. (0, 50, 250 or 750 mg/kg) terminated 1 or 20 hr later to assess its biodistribution, by ICP-AES/ICP-MS cerium analysis, and potential to produce neurotoxicity, assessed by 4-hydroxy-2-nonenal (HNE), 3-nitrotyrosine (3-NT), and protein carbonyls in frontal cortex (FC), hippocampus (HC) and cerebellum (CB). Five min prior to termination anesthetized rats were given i.v. Evans blue (EB)-albumin and Na fluorescein (Na2F) as blood-brain barrier integrity markers. Results: Blood incubated with ceria for 1 hr showed primary and agglomerated ceria by HR-TEM/STEM. Ceria was in the rat was << 1 hr. Brain EB and Na2F increased. Tissue [Ce] was ceria dose-dependent (spleen > liver > brain > blood serum) and accompanied ~ 50% of the dose. EB-NHE increased in the HC, 3-NT changed little in GC, HC or CB; and protein carbonyls decreased in the CB. Conclusions: Ceria was cleared by peripheral tissues. Much less entered the brain. The results provide a foundation to study the impact of physico-chemical properties of ENMs on peripheral organ distribution, brain entry and resultant toxicity. Supported by the Office of the VP for Research, UK and US EPA STAR Grant RD-833772.

250  FATE OF ULTRAFINE (UFTIO2) OR FINE TITANIUM DIOXIDE (FTIO2) FOLLOWING INTRATRACHEAL INSTILLATION IN RATS.

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Nanotechnology is expanding due to the unique properties of nanoparticles. Studies report that pulmonary exposure to nano-sized particles may produce greater pulmonary effects than their larger sized particle counterparts. However, differences in fate and clearance of inhaled nano-sized and fine-sized particles are unclear. This study explores differences in lung burden as well as translocation to the intestitium and the bronchial-associated lymphatic tissue (BALT) between rats exposed to UFTIO2 vs. FTIO2. Rats were intratracheally exposed to a PM_10 dose of UFTIO2 or FTIO2 (0.52 mg/rat or 10.7 mg/rat, respectively). Metal analysis indicates that the amount of UFTIO2 remaining in the lung decreased by 51% from 7 to 42 days post-exposure, while the lung burden of FTIO2 decreased by only 12% from 7 to 42 days. The amount of UFTIO2 present in the tracheo-bronchial (TB) and thymic lymph nodes (TLN) was also assessed. Lymph node burden for UFTIO2 from 7 to 42 days post-exposure increased by 246%, while a 134% increase was observed for FTIO2. Although relative migration of UFTIO2 to the lymph nodes exceeded that for FTIO2, this difference could not account fully for the much lower lung clearance of UFTIO2.
FTO2 relative to UFTO2, suggesting particle overload in rats exposed to FTO2. Lag burden of TeO2 was measured in both lavaged and unlavaged lungs at 7 and 42 days post-exposure. The amount of TeO2 present in the lavagable fraction represents particles phagocytosed by lavagable alveolar macrophages or present as free particles in the lavage. The non-lavagable fraction in the lung increased from 7 to 42 days post-exposure. With UFTO2, the majority of the particles were non-lavagable, suggesting migration to the interstitium. In contrast, the majority of FTO2 was found in lavagable alveolar macrophages. In summary, the results indicated that UFTO2 migrates more rapidly to the interstitium than FTO2 which is phagocytosed more avidly by alveolar macrophages.

**251 TOXICITIES OF NANOPARTICLES IN AN ENVIRONMENTALLY RELEVANT FISH MODEL.**

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Toxicities of aqueous suspensions of nanoparticles were tested in early life stages of an environmentally relevant fish species, Microgadus tomcod (Atlantic tomcod). Embryos were exposed to graded concentrations of nanoparticles which included: fullerenes, functionalized single-walled carbon nanotubes (SWNT) and metal oxides of silver, copper, iron, nickel and zinc (Ag, Cu, Fe, Ni and Zn). Also tested were nanoparticles consisting of a fullerenium shell with erbium or yttrium metal catalysts derived from various stages in their production process including soot, sludge and the finished material. Particles were dispersed in 5 ppt artificial sea water or DMSO and sonicated for one hour. Exposure began at 14 days post fertilization and continued until death or hatching. Survivorship and hatching success were evaluated. Fullerenes exposed in 100% mortality at 500 μg/l while SWNTs did not induce significant changes in mortality or hatching. Mortality increased with graded doses of Cu, Fe and Zn while no changes in mortality or hatching were detected for Au or Ni at concentrations up to 18 μg/ml. The finished manufactured nanoparticles caused dose responsive toxicities that were 100% lethal at 50 μg/ml. Soot and sludge particles with both erbium and yttrium resulted in dose dependent increases in mortality but erbium-containing particles were more toxic and caused 100% mortality at 10 and 50 μg/ml for soot and sludge, respectively. These results indicate that nanoparticles containing some metals (Cu, Fe, Zn) are toxic to fish embryos. Particles containing erbium and yttrium were more harmful than those containing only carbon. Fullerenes induced mortality in these particles but erbium-containing particles were more toxic and caused 100% mortality at 10 and 50 μg/ml for soot and sludge, respectively. These results indicate that nanoparticles containing some metals (Cu, Fe, Zn) are toxic to fish embryos. Particles containing erbium and yttrium were more harmful than those containing only carbon. Fullerenes induced mortality in these particles but erbium-containing particles were more toxic and caused 100% mortality at 10 and 50 μg/ml for soot and sludge, respectively. These results indicate that nanoparticles containing some metals (Cu, Fe, Zn) are toxic to fish embryos. Particles containing erbium and yttrium were more harmful than those containing only carbon.

**252 GOLD AND SILVER NANOMATERIALS REDUCE THE INFLAMMATORY RESPONSE TO INJURY.**

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The role of inflammation in rheumatoid arthritis (RA) is well known. Inflammation has also been implicated in the pathogenesis of other chronic diseases including cancer. The widespread role of inflammation in disease underscores an urgency to identify new and improved anti-inflammatory treatments. Chryotherapy, injection of gold (Au) salts, has been used for decades as a standard RA treatment despite an unclear mode of action. We tested the hypothesis that nanomaterials formed upon injection of Au ions are the primary agents responsible for reducing inflammation in patients undergoing chryotherapy. The anti-inflammatory potential of Au and silver (Ag) nanomaterials produced in organic media was measured using a larval zebrafish inflammation model. An inflammatory response can be induced in zebrafish by amputating the caudal fin. We tracked inflammation by exposing transgenic zebrafish with fluorescent neutrophils to inflammatory cells by exposing transgenic zebrafish with fluorescent neutrophils.
monary disease, it has been suggested that inhaled NPs may also induce similar effects on the cardiovascular system. This study was designed to determine if long-term exposure to inhaled nickel (Ni) NPs induced adverse effects on the cardiovascular system, resulting in exacerbation of atherosclerosis in a sensitive mouse model. Male ApoE knock-out mice were exposed either to filtered air or Ni NPs at 100 μg/m³, 10% of the current occupational guideline, for 5h/d, 5d/wk for either 1 week or 5 months. Various indicators of oxidative stress and inflammation were measured in the lung and cardiovascular tissue, and plaque formation on the aorta was determined after 5 months of exposure. The analyses of bronchoalveolar lavage fluid (BALF) revealed significant oxidative stress and pulmonary inflammation at both time points. This data was consistent with up-regulations of heme-oxygenase-1 (Hoe-1), interleukin-6 (Il-6) and monocyte chemotactic protein-1 (Mcp-1) genes in the lung tissues. Those three genes were also up-regulated in the heart tissue after 5 months of exposure, suggesting systemic effects induced by inhaled Ni NPs. Furthermore, Mcp-1 was over-expressed in the aorta tissue, along with C608 and vascular cell adhesion molecule-1 (Vcam-1), after the 5-month exposure. This phenomenon suggests that there is a potential to form atherosclerosis. These results suggest that inhaled Ni NPs, at occupationally relevant concentrations, can induce significant chronic adverse effects on the cardiovascular system, including exacerbation of atherosclerosis. Further studies are needed to investigate potential mechanisms in which inhaled NPs could affect the cardiovascular system.

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**PS 256 SILVER NANOPARTICLE AND FULLERENE STUDIES WITH ADULT AND EMBRYONIC OYSTERS, CRASSOSTREA VIRGINICA.**

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The fate and effects of nanoparticles on aquatic organisms are important environmental concerns that must be addressed as the production and uses of nanoparticles continue to increase. The purpose of these ongoing studies is to characterize the toxicity of various nanoparticle preparations (silver nanoparticles and fullerences) on oysters, Crassostrea virginica, a common estuarine species. As filter-feeders, oysters present a very valuable model species for characterizing nanoparticle bioavailability and interactions with basic cellular processes. Laboratory exposure studies were conducted with adult and embryonic oysters as well as with isolated hepatopancreatic cells. The potential for hepatopancreatic cell uptake was evaluated using a lysosomal destabilization assay, and lipid peroxidation assays were used to assess oxidative damage. For the embryo assays, newly-fertilized oyster embryos were exposed to the nanoparticles and then the percent normal development after 48 hours was assessed. We used these studies to address important issues, such as relative sensitivity of embryos compared to adults, tissue distribution, and cellular accumulation and effects. Generally, embryos tend to be slightly more sensitive than adults, and isolated hepatopancreatic cells were similar in toxicity to the whole oyster studies. Fluorescent confocal microscopy and atomic absorption spectrometry were used to verify the accumulation of the nanoparticles inside hepatopancreatic cells and embryos. Significant accumulation of fullerenes inside lysosomes was observed supporting the model that internalized nanoparticles are delivered to the lysosomal pathway. For these studies, gill and hepatopancreas tissues were removed from oysters 24 h after exposure. The effect of stirring time, cross-linker concentration, surfactant and sonication on nanoparticle size was systematically investigated. Additional experiments to grow the nanogels to micron-sized ANP were performed. Stability studies of ANPs were done at 37°C in rat plasma, PBS and PB. Male Sprague-Dawley rats were intravenously administered with either Hylure Fluorescent 750 dye or ANPs covalently labeled with Hylure 750 dye (DYE) or ANPs covalently labeled with Hylure 750 dye (DYE-ANP). Biodistribution of ANPs was determined using a Xenogen IVIS 100 Imaging System. Stability studies indicated ANPs were stable in rat plasma, PBS and PB. Male Sprague-Dawley rats were intravenously administered with either Hylure Fluorescent 750 dye (DYE) or ANPs covalently labeled with Hylure 750 dye (DYE-ANPs). Biodistribution of ANPs was determined using a Xenogen IVIS 100 Imaging System. DYE was quickly eliminated from the body, whereas DYE-ANPs accumulated in the lung within 30 minutes and the majority of the DYE-ANPs remained in the lung for 24 hours. In conclusion, these results show that it is feasible to design novel pegylated hydrogel nanoparticles and that these nanoparticles can form stable micron-sized aggregates. The micron-sized ANPs are retained in the body for more than 24 hours and are predominantly found in the lung.

Support: NIH/NIAMSD CounterACT Program (Award: U54AR055073)

**PS 257 INTEGRATION OF GENOMIC AND PROTEOMIC BIOSignatureS IMPROVES THE DISCRIMINATION OF RESPONSE TO NANOPARTICLES IN A MOUSE MODEL.**

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Health risks associated with contact and consumption of nanomaterials is largely unknown and a topic of considerable interest to both manufacturers and consumers. We compared the pulmonary response to repeated exposure to single-walled carbon nanotubes, crocidolite asbestos, and ultrafine carbon black in a mouse model. Biological signatures of exposure were derived using both genome and proteome profiling tools through high-content analytical platforms, including microarray, global mass spectrometry-based proteomics, and multiplexed protein ELISA microarray. The goal of this study was to derive biomarkers of response from these data streams and to evaluate the screening potential of integrated bio-signatures for hazard assessment. We developed a bio-signature integration approach that fuses the multiple data sources at a molecular level. Probability models were derived for individual datasets by using partial least squares discriminant analysis and transformed into likelihood values associated with the probability that a particular sample was exposed to one of the defined particles or the control group. The screening potential of the models was assessed by using the probability models to give insight into particle classes that biomarkers may be derived from each data source. Finally, Bayesian statistics were applied to the likelihood probability models to fuse all data streams into a single model. We find that the probability models associated with individual data types can only successfully separate less than 90% of the samples with cross-validation. However, the integrated probability model can nearly perfectly classify all samples. Using this approach, we identified a panel of biosignatures for each particle class with statistical power to predict response to particulates. Supported by Environmental Biomarkers Initiative and ESI16051

**PS 258 POLYETHYLENE GLYCOL (PEG) NANOGEL AGGREGATE DRUG DELIVERY SYSTEM FOR TARGETED SYSTEMIC DELIVERY.**

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The aim of this work is to develop a novel aggregated nanogel particulate (ANP) drug delivery system that targets the lung, liver or spleen using stable aggregates of a PEG nanogel. PEG nanogel particles were successfully formed by irreversibly cross-linking 8 Arm-PEG thiol polymer and a cross-linker (HBVS) in PB (pH 7.4). The effect of stirring time, cross-linker concentration, surfactant and sonication on particle size was systematically investigated. Additional experiments to grow the nanogels to micron-sized ANP were performed. Stability studies of ANPs were done at 37°C in rat plasma, PBS (pH 7.4) and PB (pH 7.4). Dynamic light scattering (DLS) and transmission electron microscope (TEM) were used for analyzing the size of ANPs. Among the parameters, sonication and the presence of surfactant (Tween 80, 0.1%/v/v) were found to be critical for forming hydrogel nanoparticles. DLS and TEM results showed that after stirring the reaction mixture for a long time, the nanogel particles form aggregates due to hydrophobic interactions. Both small (20 nm) and large (≥1 μm) particles were observed in the reaction mixture. Stability studies indicated ANPs were stable in rat plasma, PBS and PB. Male Sprague-Dawley rats were intravenously administered with either Hylure Fluorescent 750 dye (DYE) or ANPs covalently labeled with Hylure 750 dye (DYE-ANP). Biodistribution of ANPs was determined using a Xenogen IVIS 100 Imaging System. DYE was quickly eliminated from the body, whereas DYE-ANPs accumulated in the lung within 30 minutes and the majority of the DYE-ANPs remained in the lung for 24 hours. In conclusion, these results show that it is feasible to produce nanodized PEG hydrogel nanoparticles and that these nanoparticulates can form stable micron-sized aggregates. The micron-sized ANPs are retained in the body for more than 24 hours and are predominantly found in the lung.

Support: NIH/NIAMSD CounterACT Program (Award: U54AR055073)

**PS 259 TISSUE-SPECIFIC ANTIOXIDANT RESPONSES OF OYSTERS, CRASSOSTREA VIRGINICA, TO SILVER NANOPARTICLE EXPOSURES.**

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As nanoparticle technologies continue to grow, there is an increased potential of introduction of nanoparticles to aquatic environments. It is therefore important to characterize the potential risks to ecological receptors. Oxidative damage and impacts on antioxidant responses may impact the physiology and fitness of organisms directly, and may also increase their susceptibility to other stressors. Silver (Ag) nanoparticles are used extensively in clothing and other applications as an anti-microbial compound. The purpose of this ongoing work is to characterize the antioxidant-related responses of oysters (Crassostrea virginica) to Ag nanoparticle exposures. For these studies, gill and hepatopancreas tissues were removed from oysters following exposures to a range of Ag nanoparticle concentrations to evaluate tissue-specific antioxidant responses (e.g. glutathione and catalase concentrations). Gill and hepatopancreas tissues were also assessed for expression of metallothionein genes (MT I and II) using qT-PCR. Metallothioneins may play a dual role in mitigating the adverse effects of metal nanoparticles by scavenging excess metal ions, and by functioning as an antioxidant by removing oxyradicals. Lipid peroxidation assays were also conducted to assess for evidence of tissue damage associated with oxidative production. While the results indicated little direct tissue damage following short term exposures to Ag nanoparticles, tissue-specific alterations of antioxidant responses were observed.
Potential adverse health effects of nanotechnologies are of concern. Unlike other particles, NPs may gain access to the CNS causing toxic insult. Detection of antibiotic to bodies nervous system (NS) proteins in response to Ni-NPs following a 6 wk exposure was reported in mice. In the present study, 3 month old male C57 mice were exposed to 100 ng/cm³ of spark generated Ni NPs (GFG-100 Palas, Germany) or filtered air in a whole-body exposure system for 5h/d, 5d/week for 1, 6, 15, 17, 20 and 23 wks. In addition, mice subjected to hypoxia (n=5) were a positive control. Particle size was measured by a differential mobility analyzer- condensation nucleus counter system and exposure concentrations were determined gravimetrically. Facial vein bleeding was used for serum isolation. Antibodies, IgG, against neural proteins (neurofilaments (NF), glial fibrillary acidic protein (GFAP) and myelin basic protein (MBP)) were detected in sera using ELISA. Levels of IgG against neural proteins in controls (n=4-6/time point) were only marginally above the blocking-buffer blank. In Ni-NP exposed mice titer of IgG against NF proteins showed significant increases as early as 1 wk of exposure and were comparable to levels detected in hypoxic mice. IgG against GFAP were significantly elevated at 15 wks and for MBP at 15 and 17 wks. For anti-MBP these were comparable to hypoxic mice. Beginning at 17 wks, IgG against NF and MBP began to decline reaching control levels at 23 wks. This may reflect saturation of circulating IgG in the presence of elevated antigen. Declines in GFAP titer may reflect the occurrence of astrogliosis, rather than glocaloxicity after wk 15 and warrants investigation. The detection of neurotoxins confirms earlier findings and suggests that Ni-NP exposure induces neurotoxicity. Ni is known to induce oxidative stress and mitochondrial dysfunction in other tissues. These mechanisms are also involved in neurodegeneration. In contrast to Ni salts, where neurotoxicity has not been reported, NPs may pose a risk to the NS.

Titanium dioxide is widely used as pigment, sunscreen or photocatalyst. The present study investigated possible respiratory and cardiovascular effects of titanium dioxide on workers handling them. Ambient exposure level in a factory handling titanium dioxide on workers handling them. Ambient exposure level in a factory handling titanium dioxide was assessed using condensation particle counter (CPC). Personal samplers showed that the weight density of the particles larger than 1 μm was about 500 times greater than in the office. Granular shadows in the lung was found in one worker and aortic valve stenosis was found in another worker, but their association with exposure to titanium dioxide has not been fully evaluated. Recently, we have reported that manganese, silver and copper nanoparticles induce expression changes in dopaminergic system-related genes in PC-12 cells. We have also shown that these NPs are capable of producing free radicals and oxidative stress. In this study we evaluated the effects of manganese-30 (Mn-30) and aluminium-80 (Al-80) nanoparticles in mice. Adult male mice were injected (ip) with a 500 mg/kg single dose of Mn-30 or Al-80 and sacrificed 24 hours after the injection. Brains were quickly removed and dissected into caudate nucleus, frontal cortex and hippocampus, and real time RT-PCR analysis was performed using Mouse Oxidative Stress and Antioxidant Defense Arrays. In caudate, Al-80 up-regulated the expression of Aass, Als2, Apc, Aqr, Cat, Duox1, Ercc6 and Txnip; whereas Mn-30 up-regulated only the expression of Txnip. In frontal cortex, the expression of Nox1 and Txnip was up-regulated and the expression of Tnfr3 was down-regulated after Mn-30 treatment. Al-80 down-regulated the expression of Gpx1, Prdx1 and Prdx4. The naphthol selective estrogen receptor modulator (SERM) LY2066948 is in clinical trials for the treatment of uterine fibroids and myomas. LY2066948 is a novel selective estrogen receptor modulator (SERM) which is in development by Eli Lilly for the potential treatment of uterine fibroids and myomas. LY2066948, in simile with the equine estrogen equilin, contains a naphthol group expected to undergo oxidative metabolism to a naphthalene-3,4-di,ol. The 3,4-diol metabolite of equilin, 4OH-EN, is a carcinogen that autoxidizes to a genotoxic and rodox-cycling o-quinoine. LY2066948 was synthesized and assayed for antiangiogenic activity and was confirmed to be a more potent antiangiogenic than 4-hydroxymethoxamine, as measured by induction of alkaline phosphatase activity in Lysinibacillus endometrial cancer cells and ERE-luciferase expression in MCF-7 breast cancer cells. The naphthalene-3,4-diol metabolite of LY2066948 was indeed identified as an oxidative product in the model reaction with tyrosinase (phenol monooxygenase); interestingly, in the presence of ascorbate to prevent autoxidation, the 3,4-diol was formed quantitatively.
nase in the presence of GSH gave the expected GSH conjugates formed from trapping of the o-quinone, which were characterized by LC-MS-MS. In incubations with dexamethasone-induced rat liver microsomes, GSH conjugates were also observed, although in lesser amounts. In accordance, incubation with CYP3A4 superexpression gave only a very minor amount of naphthalene-3,4-diol metabolite. In similar with equillenin, LY286948 is oxidized to a naphthalene-3,4-diol metabolite, that readily autoxidizes to an o-quinone that is a potentially cytotoxic electrophile, and redox-cycling genotoxin. Further studies are warranted to study this metabolite and validate its formation in vivo.

**An Approach Based on LC/ESI-MS to Detect Diol Metabolites as Biomarkers of Exposure to Sterylene and 1,3-Butadiene.**

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Sterylene and 1,3-butadiene are important intermediates extensively used in the plastics industry. They are mainly metabolized through cytochromes P450 and NADPH oxidation to the corresponding epoxides, which are subsequently converted to diols by epoxide hydrolase or through spontaneous hydration. The resulting sterylene glycol and 3-buten-1,2-diol have been suggested as biomarkers of exposure to sterylene and 1,3-butadiene, respectively. Unfortunately, poor ionization of the diols in electrospray mass spectrometers becomes the obstacle to the detection of the two diols by LC/ESI-MS. We developed an LC/ESI-MS approach to analyze sterylene glycol and 3-buten-1,2-diol by means of derivatization with 2-bromopropylidene-5-boronic acid (BPBA), which not only dramatically increases the sensitivity of diol detection but also facilitates the identification of the diols. The analytical approach developed is simple, quick, and convincing without need of complicated chemical derivatization. In order to evaluate the feasibility of BPBA as a derivatizing reagent of diols, we investigated the impact of diol configuration on the affinity of a selection of diols to BPBA using the established LC/ESI-MS approach. We found that cis-diols showed higher affinity to BPBA than trans-diols and that the reactivity of vicinal diols to BPBA decreased with the increase in their molecular rigidity. In conclusion, BPBA may be used as a general derivatizing reagent for the detection of vicinal diols by LC/MS.

**Quinone Methide: Reactive Metabolite of Daucurine.**

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Daucurine (DRC), a bisbenzylisoquinoline alkaloid, has received great attention due to its wide range of pharmacological activities. Recently we found DRC produced selective lung injury in vivo. In addition, the pulmonary toxicity was reversed by pretreatment with ketocazone, a CYP3A4 inhibitor. This indicates that DRC-induced toxicity requires cytochrome P450-mediated metabolic activation. With the structure of para-methylenephene in DRC, we speculated that the alkaloid may be metabolized to a quinone methide intermediate. To clarify our speculation, we incubated DRC with mouse liver microsome in the presence of glutathione (GSH) as a trapping agent. A total of ten GSH conjugates were detected by LC/MS/MS using neutral loss, full scanning, and MS/MS scanning technique. One GSH conjugate with [M+1] 930 m/z was found to be the major one among the ten GSH conjugates detected. We chemically synthesized the GSH conjugate which is designated as 10-glutathionyl DRC confirmed by LC/MS/MS and NMR. The formation of the GSH conjugates was found to require NADPH, and the absence of NADPH in these microsome incubations failed to produce any GSH conjugates after exposure to DRC. To identify those cytochromes P450 responsible for the bioactivation of DRC, we incubated DRC with a selection of individual recombinant human cytochrome P450 enzymes. Among the P450 enzymes tested, CYP3A4 is the only one found to catalyze the formation of the quinone methide metabolites. In addition, we incubated human liver microsome with DRC mixed with individual recombinant P450 inhibitors. As expected, CYP3A4 inhibitor ketocazone dramatically suppressed the formation of the quinone methide metabolites. We have fully identified a quinone methide metabolite derived from DRC. This facilitates the understanding of the biochemical mechanism of pulmonary toxicity by DRC.

**Differential Localization of Flavin-Containing Monooxygenase Isoforms 1, 3, and 4 in Rat Liver and Kidney.**

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Flavin-containing monooxygenases (FMOs) play a significant role in metabolism of drugs and endogenous or foreign compounds. In this study, the regional distribution of FMO isoforms 1, 3, and 4 were investigated in rat liver and kidney using immunohistochemistry (IHC). Rabbit polyclonal antibodies to rat FMO1 and FMO4, developed using anti-peptide technology, and anti-human FMO3 antibody were used; specificities of the antibodies were verified using western blotting, immunoprecipitation, and IHC. The results show distinct localization patterns of FMO1, 3, and 4 in rat liver and kidney. In liver, the highest immunoreactivity for FMO1 and FMO3 was detected in the perivenous region and decreased in intensity toward the periportal region. In contrast, FMO4 immunoreactivity was detected with the opposite lobular distribution. In the kidney, the highest immunoreactivity for FMO1, 3, and 4 was detected in the distal tubules. FMO1 and FMO4 immunoreactivity was also detected in the proximal tubules with strong staining in the brush borders, whereas little FMO3 immunoreactivity was detected in the proximal tubules. Immunoreactivity for FMO3 and FMO4 was detected in the collecting tubules in the renal medulla and the glomerulus but little FMO1 immunoreactivity was detected in these regions. This data provides a compelling view of the specific localization patterns of FMO1, 3, and 4 in the rat liver and kidney.

In addition to rat tissues, the FMO4 antibody reacted with mouse and human tissues, providing the first evidence for expression of FMO4 at the protein level in these tissues.

**In Vitro Characterization of Hepatic Eugenol and Methyleneugenol Bioactivation Versus Detoxification Pathways.**

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Eugenol (CAS no. 97-53-0) is found in cosmetics, essential oils and herbal products and has been added to cigarettes as a constituent of cloves. The US NTP program reported “no evidence” of eugenol-induced carcinogenicity in rats, but “equivocal” evidence for tumor induction in female mice. Using in vitro tests for genotoxicity, eugenol has been demonstrated to be positive on occasions. In contrast, a variety of studies in animal models indicate that the structurally-related compound, methyleugenol (CAS no. 93-15-2), is consistently carcinogenic. To further understand the underlying mechanism of allylbenzene activation and detoxification, we have compared the oxidative and conjugative metabolism of eugenol and methyleugenol in vitro. Pooled human liver microsomes and S9 fractions were incubated with C14-eugenol and C14-methyleugenol (20 μM final, 1.5 μCi per compound) in the presence of phase I and phase II enzyme co-factors. Soluble metabolites were quantified by radio-HPLC and identified by co-elution with standards. Protein-bound metabolites were quantified following extraction of the insoluble fraction. CYP450-dependent formation of 1-hydroxy genotoxic precursors accounted for 19% and 5% of total eugenol and 30% and 37% of total methyleugenol in microsomes and S9 fractions respectively. Upon addition of glucuronide, eugenol was almost entirely conjugated and only traces of 1-hydroxy eugenol were observed (1% in the presence of S9). Glucuronidase treatment of these samples further confirmed that 1-hydroxy eugenol is a trace metabolite. In contrast, methyleugenol is not glucuronidated and formation of the 1-hydroxy metabolite remained at 57% of the initial dose. Our data indicate that methyleugenol metabolism leads to the accumulation of the 1-hydroxy genotoxic precursor, while eugenol metabolism to the 1-hydroxy compound is prevented by direct glucuronidation. The result supports the existing evidence for the genotoxicity of methyleugenol and the absence of genotoxicity for eugenol.

**Covalent Binding and Glutathione Depletion: Comparison of Non-Cytotoxic Diethyl Maleate with the Clara Cell Toxicant, Naphthalene.**

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Naphthalene (NA) is a polycyclic aromatic hydrocarbon (PAH) known to elicit a cytotoxic response in nonciliated bronchiolar epithelial (Clara) cells of the murine lung. Doses of 150 mg/kg i.p. or 2 ppm via inhalation will result in the depletion of glutathione (GSH) and the covalent binding of NA reactive metabolites to macromolecular protein targets but the precise mechanisms by which NA injures
Clara cells remain unclear. Diethyl maleate (DEM) is a non-cytotoxic compound known to deplete GSH in a dose-dependent manner. A dose of 1000 mg/kg i.p. depletes GSH in the airway epithelium from a starting concentration of 24.4 nmoles/mg protein to 3.32 ± 1.92 (N=4) within 15 minutes, and GSH levels remain significantly depressed (p-value < 0.05) for at least 2 hours after dosing. The extent of depletion seen following this dose is similar to the extent of depletion seen from a 250 mg/kg i.p. dose of NA. The similarities in GSH depletion caused by DEM and NA combined with the fact that NA is toxic but DEM is not, suggested that comparative studies might reveal additional fundamental insights into the mechanism of NA toxicity. The studies reported here were designed to be comparative—using an ex vivo system to isolate the lung—with NA or DEM instilled via the trachea to interact with Clara cells. Doses of DEM and NA showing similar GSH depletion (1000 M and 250 μM, respectively) in this ex vivo lung preparation show similar levels of covalent binding: 0.44 ± 0.08 nmoles/mg protein (n=6) for DEM and 0.64 ± 0.13 (n=6) for NA, despite the disparity in toxicity. Additional work comparing the epithelial protein targets should reveal whether these are key differences between DEM and the cytotoxic naphthalene. Supported by NIHES 04311 and 04699.

270 COMPARISON OF METABOLISM OF INHALED GLUCOCORTICOSTEROIDS BY CYTOCHROME P450 3A ENZYMES AND IDENTIFICATION OF POTENTIALLY TOXIC METABOLITES.

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Asthma is a chronic inflammatory disease of the airways estimated to affect 20.5 million Americans (6.2 million children under 18 years). Inhaled glucocorticoids are the bastion of asthma treatment while it is estimated approximately 30% of all asthmatics have some degree of steroid resistance. Glucocorticoids are metabolized by members of the cytochrome P450 (P450) 3A family of enzymes primarily in the liver and lung. The major goal of this research is to determine how metabolism of glucocorticoids by P450 3A enzymes could have an effect on their efficacy in asthmatic patients. We have used the metabolically relevant human P450 metabolizing systems and ex vivo human liver samples to determine the relative contributions of these isozymes. A comparison of the metabolism of the five drugs across the different isoforms identified potentially reactive intermediates that could be toxicologically significant. These intermediates were verified with glutathione (GSH) trapping. Additionally, comparing metabolism of the five drugs across the different isoforms showed significant differences between the rates at which metabolites are formed by each P450 3A isoform. These differences in metabolism could become important factors in determining the most effective glucocorticoid asthma treatment.

271 COMPARISON OF BIOCHEMICAL ANALYSIS TO GENE EXPRESSION AND LASER SCANNING CYTOMETRY FOR THE ASSESSMENT OF RAT HEPATIC CYTOCHROME P450 (CYP) ENZYME INDUCTION.


The purpose of this study was to compare 2 ex vivo methods, gene expression (GE) and laser scanning cytometry (LSC), against traditional biochemical (BC) methods to evaluate hepatic CYP induction in male rats exposed to xenobiotics. Sprague-Dawley rats were dosed with either 5-pregnen-3β-ol-20-one-16α-carboxylate (PCN, 50mg/kg, 5 days), ionizanid (ISO, 100mg/kg, 4 days), β-naphthoflavone (β-NF, 100mg/kg, 4 days), phenobarbital (PB, 100mg/kg, 5 days), dexamethasone (DEX, 50mg/kg, 5 days), or 1-aminobenzotriazole (AMB, 100mg/kg, 2 days). All compounds are known CYP inducers except for AMB which is an inhibitor of cytochrome P450. Corresponding control groups received equivalent volumes of the appropriate vehicle. Upon completion of the dosing phase, animals were sacrificed, livers were removed and stored, and either processed for histological examination and LSC or frozen for BC and GE analysis. The isoforms evaluated in all three procedures were CYP1A1, 2B, and 3A. CYP1A2 was evaluated by GE and BC only. Since CYP2P2 enzyme is regulated by post-translational modification, it was evaluated by BC and LSC. All procedures were CYP1A1, 2B, 2C, and 3A. CYP2P2 was measured by GE and BC only. Since CYP2P2 is regulated by post-translational modification, it was evaluated by BC and LSC. All procedures were CYP1A1, 2B, 2C, and 3A. CYP2P2 was measured by GE and BC only. Since protein expression and laser scanning cytometry have utility for CYP assessment in vivo, biochemical methods remain the gold standard.

272 MECHANISM-BASED INACTIVATION OF CYTOCHROME P450 3A4 BY RETRONECINE-TYPE PYRROLIZIDINE ALKALOIDS.

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Retrorsine and monocrotaline are two representative retronecine-type pyrrolizidine alkaloids known to be hepatic and pulmonary toxins, respectively. It has been documented that their toxicities are attributed to metabolic activation by cytochromes P450 to form dehydrogenated reactive intermediates which, in turn, produce toxicities possibly by covalent binding to macromolecules. The objective of this study is to determine whether retrorsine and monocrotaline irreversibly inactivate P450s as mechanism-based inactivators. We examined the inhibitory effects of retrorsine and monocrotaline on human P450s 1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, and 3A4. Rettorsine showed time-dependent inhibition on P450 3A4 in the presence of NADPH, but no such inhibition was observed on the other P450 isoforms tested. Unlike rettorsine, monocrotaline failed to inhibit P450 3A4. Further studies showed that the activity loss of P450 3A4 by rettorsine metabolism was in a concentration-dependent way, which was not recovered after equilibration dialysis. The presence of dextromethorphan, a P450 3A4 subtype, protected the enzyme from the inactivation by rettorsine. Exogenous nucleophile glutathione (GSH) and reactive oxygen species scavengers catalase and superoxide dismutase could not protect P450 3A4 from the inactivation. Both rettorsine and monocrotaline were found to be metabolized to the corresponding ethylenic intermediates trapped by GSH. In conclusion of this work, rettorsine is a mechanism-based inactivator of P450 3A4, but monocrotaline failed to show such inhibition, even it is metabolically activated.

273 IDENTIFICATION OF HUMAN CYTOCHROME P450 ENZYMES INVOLVED IN THE METABOLISM OF IN-1130, A NOVEL ALKS INHIBITOR.


We have investigated the pharmacokinetics and metabolism of 3-[(6- methylpyridin-2-yl)-4-(quinolin-6-yl)-1H-imidazol-2-yl]methyl]benzamide (IN-1130); a novel ALKS inhibitor, which suppresses renal and hepatic fibrosis, and also exerts anti-metastatic effects on breast cancer-bearing MMTV-neu mouse model. We have identified the cytochrome P450s (CYPs) responsible for metabolism of IN-1130 in liver microsomes of rat, mouse, dog, monkey and human, and in human CYP supernatomesTM, using specific CYP inhibitors. The order of disappearance of IN-1130 in various liver microsomal systems studied was as follows: monkey, mouse, rat, human, and dog. Five distinct metabolites (M1-M5) were identified in all the above microsomes and their production was substantially inhibited by CYP inhibitors such as SKF-525A and ketoconazole. Among nine human CYP supernatomesTM examined, CYP3A4, CYP2C8, CYP2D6, CYP2C19 were involved in the metabolism of IN-1130, and the production of metabolites were significantly inhibited by specific CYP inhibitors. IN-1130 disappeared fastest in CYP2C8 supernates. CYP3A4 produced four metabolites of IN-1130 (M1-M4), whereas supernatomes expressing human FMO cDNAs, such as FMO1, FMO3, and FMO5, produced no metabolites. Hence, we conclude that metabolism of IN-1130 is mediated by CYP3A4, CYP2C8, CYP2D6 and CYP2C19.

274 COMPARISON OF SEMIEMPIRICAL AM1, PM3, AND SAM1, AND A DENSITY FUNCTIONAL THEORY METHOD TO PREDICT ACTIVATION ENERGIES OF CYTOCHROME P450-MEDIATED HYDROXYLATION OF ALIPHATIC SUBSTRATES.

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Predicting the biotransformation of xenobiotics can be useful in estimating the toxicity and efficacy of drugs and other chemicals. Here, we present results extending the rapid methodology of Kotzekwa et al. (J. Am. Chem. Soc., 112, 7042-7046, 1990) in estimating the activation energy of hydrogen-abstraction by cytochrome...
P450 (CYP) enzymes, where the p-nitrosophenol radical is used as a simple surrogate for the CYP active oxygen species. The activation enthalpy is estimated with a linear regression model using the reaction enthalpy and ionization energy (of the substrate radical) as predictor variables, calculated by semiempirical (SE) quantum chemical methods. While Korzekwa et al used the SE method AM1, we apply PM3 and SAM1, and compare the results of the three methods. For 20 diverse substrates, the AM1-, PM3-, and SAM1-derived regression models showed R² values of 0.93, 0.95, and 0.96, respectively. The range of estimated activation enthalpies was similar for AM1 and PM3, but shifted toward lower values for SAM1, with substrates of different chemical classes sometimes ordered differently by the different methods. AM1, PM3, and SAM1 reaction enthalpies were substantially different from those determined by density functional theory (DFT), suggesting inaccuracies in the SE-calculated heats of formation. AM1, PM3, and SAM1 transition structures were compared and differences in geometry were found for certain substrates. Because AM1, PM3, and SAM1 calculations can differ from those of DFT for radical reactions, predictive models developed using semiempirically-derived predictors may be subject to further improvement. (This study was supported, in part, by NIEHS grant K25 ES012909.)

**FUNCTIONAL CHARACTERIZATION OF CYTOCHROME P450 3A37 FROM TURKEY LIVER WITH AFLATOXIN B1 OXIDIZING ACTIVITY.**

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In humans, hepatic cytochromes P450 (CYP) 1A2 and 3A4 bioactivate aflatoxin B1 (AFB1) to exo-AFBO, an 8,9-oxide (AFBO) which is a requisite step in the toxic and carcinogenic action of this mycotoxin. We previously demonstrated that the extreme sensitivity of turkeys to AFB1 is associated with efficient hepatic epoxidation by CYP3A4 and CYP1A2 homologues. Heterologously expressed turkey CYP1A5 metabolizes AFB1 to exo-AFBO and the detoxified metabolite aflatoxin Q1 (AFQ1). Formation of exo-AFBO was inhibited by the specific CYP3A4 inhibitor 17-α-ethynylestradiol (IC50 = 40.18 µM), while prototype inhibitors to other mammalian isoforms (1A2, 2D, 2E and 3A1) either slightly inhibited this activity, or not at all. Like its human homologue, CYP3A37 also had nifedipine oxidase activity. Polyclonal anti-CYP3A37 antibody directed against specific active-site peptides inhibited both AFB1 formation and nifedipine oxidase activity. The comparative kinetic constants for exo-AFBO formation of expressed CYP3A37 and CYP3A15 (Km and Vmax: 16.79 ± 5.13 µM and 1.17 ± 0.082 nmol min⁻¹ mol⁻¹ P450, 65 ± 12µM and 0.61 ± 0.037 nmol min⁻¹ mol⁻¹ P450, respectively), lead us to believe that CYP3A37 may predominate over CYP1A5 in AFB1 bioactivation in turkey liver. Supported by National Research Initiative Grants no. 2002-35204-12294 and 2006-04819 from the USDA.

**PHOSPHORYLATION OF SERINE-10 IN HISTONE H3 IS A CRITICAL MARK ESSENTIAL FOR INDUCTION OF THE CYP1A1 GENE BY THE ACTIVATED AH RECEPTOR.**

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Metabolic bioactivation of polycyclic and halogenated aromatic hydrocarbons, such as benzo[a]pyrene and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), is catalyzed by a cytochrome P450 monooxygenase encoded by the substrate-inducible Cyp1a1 gene. Cyp1a1 induction requires trans-activation by the heterodimeric transcriptional complex formed by the liganded Ah receptor (AHR) and its partner, ARNT. Previously, we have shown that AHR receptor ligands also activate signal transduction pathways, although the molecular mechanisms by which these pathways may contribute to AHR activation and the ensuing toxicity have yet to be defined. TCDD induces in Hepa-1 mouse hepatoma cells the immediate activation of extracellular signal-regulated kinases (ERKs) and Jun-N-terminal kinases (JNKs), two members of the mitogen-activated protein kinase (MAPKs) family involved in modulation of transcription factor activity and regulation of gene expression. Here we find that Cyp1a1 induction by the AHR/ARNT is associated with modification of specific chromatin marks, including hyperacetylation of histone H3K14 and JNK-mediated histone H3K4 and phosphorylation of H3S10. Chromatin immunoprecipitation studies show that the phosphorylation of Ser-10 in histone H3 is linked to the recruitment of AHR to the Cyp1a1 promoter. Specific pharmacological inhibitors of ERKs (extracellular signal regulated kinase) and JNK (Jun N-terminal kinase), but not as much of the p38 MAP kinase, block the AHR-dependent phosphorylation of this serine residue and the transcriptional induction of the Cyp1a1 gene. These results show that by modification of histone marks, MAP kinases play a central role in the regulation of Cyp1a1 expression that does not involve phosphorylation events directed to the AHR itself, underscoring the requirement for a concerted series of chromatin remodeling events to complete the initial steps of gene trans-activation by the Ah receptor(Supported by NIH R01ES6273, NIH R01ES10807, and NIH P30 ES06096).
approach HepG2 cells were transiently transfected with CYP3A4 or CYP2E1. Both assay systems were used to study CYP substrates known for their potential to form metabolites that exhibit higher toxicity than the parent compounds. Results demonstrated that both assay systems are capable to metabolize the test compounds leading to increased toxicity, compared to their respective control systems. The co-incubation with CYP inhibitors confirmed that the formation of reactive metabolites was CYPs dependent. These in vitro tools to screen for metabolic activation, or deactivation are considered useful, rapid and relatively inexpensive for the prediction of CYPs metabolism-mediated toxicity.

280 \textbf{IN VITRO AND IN VIVO METABOLISM OF ARQ 501 (β-LAPACHONE) IN HUMANS.}


ARQ 501 is a fully synthetic version of the natural product β-lapachone and has shown promising anti-cancer activity in clinical trials. The studies presented here were designed to characterize phase I and phase II metabolism of ARQ 501 in humans. LC/MS/MS analysis was employed to profile metabolites in vitro from human blood, microsome, and hepatocyte samples as well as in vivo from plasma samples obtained from treated patients. Where feasible, metabolites were confirmed with synthetic standards. Human metabolite profiles obtained from patient plasma revealed that hepatic phase II metabolism was the dominant pathway; both sulfation and glucuronidation products being present. However, in vitro metabolism of ARQ 501 by human hepatocytes occurred solely via glucuronidation. Additionally, a novel in vivo glucuronosyl metabolite of ARQ 501 which was not present in vitro was identified. Studies exploring extrahuman metabolism as a potential cause for these differences are ongoing. Results from in vitro experiments of ARQ 501 in human blood demonstrated evidence for metabolism of ARQ 501 by red blood cells (RBC).

281 \textbf{CHARACTERIZATION OF A NOVEL CYTOCHROME P450, CYP2S1 USING A PROTEIN EXPRESSED FROM A SYNTHETIC GENE.}

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CYP 2S1 was recently identified in the human and mouse and shown to be inducible by dioxin, polyacrylic hydrocarbons (PAHs), and hypoxia. Induction by dioxin and PAHs is mediated by the AhR Hydrocarbon Receptor (AHR). In the human, CYP2S1 is highly expressed in epithelial cells of tissues that are exposed to the environment, such as the skin, and the respiratory and intestinal tracts. However, the biological function of CYP2S1 has yet been determined. We are investigating its possible role in xenobiotic and carcinogen metabolism using an in vitro approach. Obtaining a large quantity of active enzyme for in vitro substrate screening has been challenging. We encountered difficulties in obtaining heterologous expression of CYP2S1 in mammalian cells and bacteria. For example, the bacterially expressed material contained large amounts of inactive CYP2S1 (more than 70% of CYP2S1 was present as P420, an inactive form of P450) that compromised both sulfation and glucuronidation products being present. However, in vitro metabolism of ARQ 501 by human hepatocytes occurred solely via glucuronidation. Additionally, a novel in vivo glucuronosyl metabolite of ARQ 501 which was not present in vitro was identified. Studies exploring extrahuman metabolism as a potential cause for these differences are ongoing. Results from in vitro experiments of ARQ 501 in human blood demonstrated evidence for metabolism of ARQ 501 by red blood cells (RBC).

282 \textbf{MICE LACKING THE GENE FOR CYTOCHROME P450 (CYP1A2) DISPLAY INCREASED LEVELS OF F2-ISOPROSTANES, OXIDATIVE DNA ADDUCTS, AND AUGMENTED SUSCEPTIBILITY TO HYPEROXIC LUNG INJURY.}

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Supplemental oxygen therapy is frequently administered to infants having pulmonary insufficiency. However, hypoxia contributes to bronchopulmonary dysplasia (BPD) in infants. In this study, we tested the hypothesis that mice lacking the gene for CYP1A2 would display increased susceptibility to hyperoxic lung injury in vivo, a phenomenon that would be accompanied by augmented levels of F2-isoprostanes and oxidative DNA adducts in liver and lungs of these animals. Twelve week-old male wild type (WT) (C57BL/6j) mice or mice lacking the gene for CYP1A2 were exposed to hyperoxia (greater than 95% O2) or room air for 24-72 h. The CYP1A2 (-/+) mice were more susceptible to lung damage than similarly exposed WT animals. Seventy-two hours of hypoxia caused significant (1.5-2 fold) increases in F2-isoprostanes in liver and lung of WT animals, compared to room air controls, as determined by GC/MS. In the CYP1A2 (−/−) mice exposed to hyperoxia, the F2-isoprostane levels in lung and liver were enhanced 5-fold compared to room air controls. Oxidative DNA adducts, as determined by 32P-postlabeling, were augmented in the lungs of WT and CYP1A2 (−/−) mice exposed to 72 h of hypoxia, compared to room air controls, with CYP1A2 (−/−) mice showing a greater degree of enhancement. The major DNA adducts were derived from cyclopropene dimers (e.g., Ac4, Ge4) or from F2-isoprostanes. Our results indicate that hepatic CYP1A2 enzyme plays a protective role in hyperoxic lung injury. The positive correlation between lipid peroxidation product levels (e.g., F2-isoprostanes), oxidative DNA adducts, and extent of lung injury suggests that both lipid peroxidation and oxidative DNA damage contribute to lung injury, and that CYP1A2 enzyme protects against injury by detoxifying lipid hydroperoxides. Further studies could lead to the development of novel strategies for the prevention/treatment of BPD in infants. (Supported by HL087174.)

283 \textbf{ETHANOL METABOLISM AND RELATED CYTOTOXICITY IN A431 CELLS.}

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Alcoholic chronic pancreatitis (ACP) is one of the major public health problems that causes significant morbidity and mortality. Due to the lack of a suitable model, the underlying mechanism of ACP is poorly understood. Using alcohol dehydrogenase (ADH)-deficient rat pancreatic tumor (A431) cells and human hepatocellular carcinoma (HeP2 cells), we have reported earlier that cytotoxicity of ethanol is associated with the formation of nonoxidative metabolites of ethanol, fatty acid ethyl esters (FAEEs). To determine the role of ethanol metabolism in ethanol induced acinar cell injury, we incubated ethanol (800 mg%) with A431 cells pre-treated with inhibitor of all the ADH isozymes or class I and II ADH [1,10-phenanthroline (10 μM), 4-methylpyrazole (4-MP), respectively], cytochrome P450 2E1 (sodium azide) or catalase [1,2-dichloroethylenol], and evaluated the formation of total FAEEs and resultant cytotoxicity after incubation at 37°C for 6 h. Significant increases in the formation of FAEEs were observed in the cells incubated with 1,10-phenanthroline (-2-fold) and 4-MP (-1.4-fold) vs. control. A significant cell death by ethanol was observed in the cells pre-incubated with 1,10-phenanthroline vs. 4-MP. Inhibition of ADH isozymes by 1,10-phenanthroline probably impairs ADH-catalyzed ethanol oxidation and facilitates the formation of FAEEs as an alternative mechanism for ethanol disposition. Our results suggest that impaired ADH (as reported in chronic alcohol abuse) could be a key factor for the ethanol metabolism via nonoxidative pathway(s) and resultant cytotoxicity. Further studies to determine the underlying mechanism(s) of ethanol-induced pancreatic acinar cell injury are in progress.
physiological roles for this gene in fish. Higher levels of CYP1B1 mRNA were observed in heart, eye and brain, and the highest levels of the CYP1Cs were observed in testis. PCB126 induced expression of CYP1A and 1B1 in all organs, while 1C1 was induced in all organs except testis. Although the CYP1Cs had similar transcript profiles in the different organs of control fish, CYP1C2 was less responsive to PCB126 compared to CYP1C1. Interestingly, CYP1D1 was not induced by PCB126 in any of the organs. The most significant changes in CYP1 expression in response to PCB126 was CYP1C1 (1000-fold) in liver, followed by induction of CYP1A (+500-fold) in testis. CYP1B1 in liver and gut. CYP1A1 in gill and CYP1C1 in gill were also strongly induced (-100-fold). The differential response of CYP1s in response to PCB126 could improve sensitivity in the use of CYP1s as biomarkers of aquatic contamination employing F. heteroclitus. (NIH P42ES007381 and 1R01ES015912)

285 CELL PROGRESSION AND PROGRAMMED CELL DEATH: INTERRELATION OF CYTOTOXIC PATHWAYS ACTIVATED BY AMIODARONE AND ITS MAJOR METABOLITE DESETHYLAMIODARONE.

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Normal cells progress through the cell cycle free of anomalous events affecting cell growth and proliferation. In contrast, when cells suffer a toxic insult or stress, the cell cycle is often stalled while the stressed cell recovers from the damage. However, if the damage is too severe to rescue the stressed cells, cell death can occur. Because cell proliferation and apoptosis processes are closely linked, we propose that the simultaneous monitoring of these events in cells will provide insight on specific mechanisms involving cell cycle arrest and cell death progression. We developed a quantitative cell-based imaging assay that simultaneously monitors multiple indicators of both cell cycle (cell number, BrdU incorporation, DNA content) and cell death (cell loss, nuclear morphology, p53 induction, and caspase 3 activation). To test our hypothesis, we quantitatively measured these different cell cycle and apoptosis features in a range of cancer cells treated with Actinomycin D. We found that Actinomycin D causes cell cycle arrest as an early event and then induces apoptosis through caspase 3 activation. Because the multiplexed assay enables identification of drugs that either induce cell cycle arrest or induce cell death, we also tested our hypothesis on other compounds that may result in either outcome. We found that okadaic acid, arsenite, camptothecin and etoposide inhibit cell cycle and activate cell death mechanism through p53 and caspase 3. In conclusion, we simultaneously quantified cell cycle and apoptosis features under different conditions and drug treatments and found that the cell cycle arrest and apoptosis progress occur as interrelated events in response to various cytotoxic insults.

286 CHARACTERIZATION OF THE CYTOTOXIC PATHWAYS ACTIVATED BY AMIODARONE AND ITS MAJOR METABOLITE DESETHYLAMIODARONE.

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Amiodarone (AM) is a potent antiarrhythmic agent associated with several adverse effects, one of which is potentially life-threatening pulmonary fibrosis. The mechanisms involved in amiodarone-induced pulmonary toxicity (AIP) have yet to be fully elucidated. Desethylamiodarone (DEA) is a primary metabolite of AM and may contribute to the pathogenesis of AIP. The objective of this study was to characterize the cytotoxic pathways activated by AM and DEA using the HPL1A human peripheral lung epithelial cell line as a model of target cells in AIP. Determination of apoptotic cell death was achieved by annexin-V-FITC staining with flow cytometric analysis and detection of activated caspase-3 was measured by immunoblotting. Necrotic cell death was determined by propidium iodide staining, followed by flow cytometric analysis. Treatment with 20 μM AM for 24 h increased the percentage of necrotic cells from 10.8% (control) to 81.4%, but decreased the percentage of apoptotic cells from 9.1% (control) to 1.9% (p<0.05). In contrast, treatment with 3.5 μM DEA for 24 h increased the percentage of cells exhibiting necrosis from 10.8% (control) to 20.5%, and increased the percentage of apoptotic cells from 9.1% (control) to 21.5% (p<0.05). Treatment with DEA, but not AM, increased intracellular levels of activated caspase-3. In addition, DEA induced cell death at an earlier timepoint and at lower concentrations than AM, confirming that DEA is a more potent cytotoxicant than AM. Hence, despite a difference of only one ethyl group in chemical structure, AM activates primarily necrotic pathways, whereas DEA activates both necrotic and apoptotic pathways. In view of these results, therapeutic treatments targeting AIP will have to address cell death pathways initiated by both AM and DEA. (Funded by CIHR Grant No. MOP-13257)

287 PROFILING ENVIRONMENTAL CHEMICALS IN APOPTOSIS PATHWAY USING A HIGH-THROUGHPUT FORMAT.

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Caspases, a family of cysteine proteases, play a critical role in apoptosis, a form of programmed cell death involved in embryonic development, homeostasis, and the pathology of many diseases. There are two characterized apoptotic pathways, extrinsic and intrinsic; the target of a toxic insult determines the pathway activated. Caspase 9 is an initiator of the extrinsic pathway, while caspase 8 is an initiator of the extrinsic pathway. Both these caspases activate downstream caspases 3 and 7, which then cleave other protein substrates within the cell, resulting in apoptosis. To evaluate the potential for environmental chemicals to activate apoptosis, we screened an NTP library of 1408 compounds in assays measuring the activity of caspases 3/7, 8, and 9. The assays were optimized and validated in Jurkat cells in a 1536-well plate format using quantitative high-throughput screening (qHTS)(1). Screening results confirmed the activity of several compounds known to induce caspase 8 or 9. For example, staurosporine stimulated caspase 9, while Trail and human Fas ligand activated caspase 8, all in a concentration-dependent manner. We also identified several other compounds that stimulate these caspases. Some compounds activated caspase 3/7 through the caspase 8 pathway, others through caspase 9, and others through both caspases 8 and 9. The data generated from this screening provide important information for further understanding the ability of environmental chemicals to stimulate the apoptosis pathway. Inglese et al. 2006. Proc Natl Acad Sci USA 103:11473.

288 FAS AND TRAIL GENE-DEFICIENT MICE SHOW ALTERED SPERMATOGENESIS AND DIFFERENTIAL SENSITIVITY TO MEHP-INDUCED GERM CELL APOPTOSIS.

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Fasl and TRAIL are ligands of the TNF protein superfamily and are expressed in the testis by bertioli cells and implicated in the control of germ cell apoptosis. However, neither the physiological role of these two members of the TNF ligand superfamily nor the involvement of these ligands in toxicant-induced germ cell apoptosis is clearly established. Here we report the testicular phenotype of Fasl and TRAIL gene-deficient mice (FasL−/−, TRAIL−/−, respectively). Evaluation of young peri-pubertal (28-day-old) FasL−/− and TRAIL−/− mice show increased testicular weight (26.58% and 21.54%) as compared to the C57BL/6J wild-type strain (5.16%). The testis of Fasl−/− mice show a higher level of TRAIL protein expression by western blot analysis, while TRAIL−/− mice show an increase in Fasl expression, suggesting that these proteins may compensate for each other. Histological analysis of testis cross sections reveals abnormal germ cell development in young Fasl−/− mice. The testis of young TRAIL−/− mice show an overt meiotic arrest of spermatocytes. In adult, 44-day-old Fasl−/− and TRAIL−/− mice, a decrease in mature spermatid formation occurs (reduced to 51% and 39%). Surprisingly, exposure of young Fasl−/− mice to the Sertoli cell toxicant mono-(2-ethylhexyl) phthalate (MEHP) caused a decrease in the basal germ cell apoptotic rate, whereas a slight increase was observed in TRAIL−/− mice. Interestingly, the expression of e-FLIP, a protein modulator of death receptor activation, was rapidly and strongly induced after MEHP exposure in young Fasl−/− mice, while its expression was only gradually increased in the testis of TRAIL−/− mice. Taken together, these data point to disparate functional roles for Fasl and TRAIL in spermatogenesis and in triggering germ cell apoptosis after MEHP injury. It is hypothesized that e-FLIP serves as the mechanism that distinguishes the biological pathways influenced by Fasl and TRAIL.

289 A MAVERICK ROLE FOR HAIRLESS ON THE REGULATION OF APOPTOSIS.

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Although hairless (hr) is widely expressed throughout the body its effects have only been well characterized in the skin and hair follicle. It has been observed that hr KO mice exhibit accelerated brain aging, but the mechanism behind this increased vulnerability has not been elucidated. This study explores the role of hr both in vitro in...
P19 cells and in vivo in the brains of wildtype (hr+/hr+), heterozygous (hr+/hr−) and KO mice (hr−/hr−). Exposure of the P19s to cadmium, mercury and apoptosis inducers (staurosporine and thapsigargin) increased the expression of hr. Transfection of the P19s with a plasmid which over expresses hr significantly increased the proportion of cells which survive these chemical insults. Cytochrome C was also retained within the mitochondria and the expression of BCL2 was increased when compared to control transfected cells. In situ hybridization revealed widespread endogenous expression of hr in the brains of 7 day old wildtype mice. The highest levels were in the cerebellum and hippocampus but lower levels were also detected in the cortex and optic nerve. Subcutaneous injections of ethanol (5 mg/kg) were administered to 7 day old mice to induce apoptotic neurodegeneration. Postmortem examination of the brain tissue revealed increased apoptosis in both the heterozygous and KO mice compared to the wildtype mice, this increased apoptosis was confirmed by western blotting, caspase activity assay and immunocytochemistry. These findings indicate that hr plays a greater regulatory role in cell cycle and apoptosis than was previously recognized.

EFFECTS OF THE COFFEE DITERPENE KAHEWLOL ON THE INDUCTION OF APOPTOSIS IN HUMAN LUNG ADENOCARCINOMA A549 CELLS.

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The coffee-specific diterpene kahweol have been reported to be protective against various type of cancer. Animal data support such a chemopreventive effect of coffee. However, the precise underlying protective mechanisms are poorly understood. In this study, we investigated the effects of kahweol on cell cycle progression and induction of apoptosis in human lung adenocarcinoma A549 cells. Our results show that treatment with kahweol results in a significant inhibition of cell proliferation in a dose-dependent manner and causes apoptotic death in human lung adenocarcinoma A549 cells. We found that kahweol inhibits considerably the expression of Bcl-2 family, Bax, a pro-apoptotic member of this family, accelerates cell death, while the pro-survival member, Bcl-xL, can antagonize the pro-apoptotic function of Bax to promote cell survival. In the present study, we have evaluated the effect of Bcl-xL on Bax-induced alterations in mitochondrial respiration and calcium release. We found that in primary cultured astrocytes, recombiant Bcl-xL is able to antagonize Bax-induced decrease in mitochondrial respiration when stimulated with an ATP-regenerative system. We also confirm its ability to inhibit Bax-induced calcium release. The present result in favor of an interaction between the two proteins and indicate that this interaction may contribute to the anti-apoptotic property of Bcl-xL.

HEAT SHOCK-INDUCED CELL DEATH.

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Heat shock therapy is currently undergoing clinical trials, alone or in combination with other therapies for the treatment of various cancers, including gastric and metastatic colon cancers. Unfortunately, though intense heat shock induces apoptosis, the underlying mechanisms remain controversial and unclear. We have recently shown that heat shock does not require any of the known initiator caspases or their activating complexes in order to induce apoptosis (Milleron and Bratton, J. Biol. Chem. 281, 16991–17000). Herein, we demonstrate that heat shock-induced cell death does require mitochondrial outer membrane permeabilization (MOMP), as cells deficient in the BIM3-only proteins, Bid and Bim, or the prosapotic Bcl-2 family members, Bax and Bak, were resistant to cell death. Remarkably, heat shock also activated c-Jun N-terminal kinases (JNKs) and induced MOMP through a novel tumor necrosis factor (TNF) receptor-associated factor 2 (TRAF2)-dependent pathway, but TNF receptor 1 (TNFR1)-independent mechanism. Thus, we speculate that JNKs may sensitize cancer cells to heat shock-induced MOMP by activating Bid and Bim. Studies are currently underway to further delineate the pathway responsible for JNK activation following heat shock (The American Cancer Society, RSG-05-029-01-CCG; NIH, CA129521).

MECHANISMS OF LEAD-INDUCED APOPTOSIS IN HUMAN LEUKEMIA (HL-60) CELLS.

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Lead is a multi-targeted toxicant that affects many organ systems including; the gastrointestinal tract, hematopoietic system, cardiovascular system, central and peripheral nervous systems, immune system, and reproductive system. There are many published studies that have documented the adverse effects of lead in children and the adult population. Previous in vitro studies in our laboratory have shown that lead nitrate induces cytoxicity to HL-60 cells in a dose-dependent manner. However, the molecular mechanisms of toxicity and carcinogenicity are still largely unknown. In this research, we hypothesized that oxidative stress plays a key role in lead nitrate-induced toxicity and cell death in human Leukemia (HL-60) cells. To test this hypothesis, we performed lipid hydroperoxide assay for assessing the levels of the degradation products of polyunsaturated fatty acid (PUFA) hydroperoxide in lead nitrate-treated HL-60 cells and the flow cytometric analysis of phosphatidylserine externalization to detect the percentage of death cell. Data generated from lipid hydroperoxide assay indicated a significant increase (p < 0.05) in the production of hydroperoxides (degradation products of lipid peroxidation) with increasing doses of lead nitrate in treated cells. Upon 24 h of exposure, the hydroperoxide concentrations in the sample [μM] (mean ± SE, n = 5) compared to untreated controls were 6.7 ± 2, 7.1 ± 1, 14.7 ± 2, 15.7 ± 6, 16.2 ± 4, and 15.2 ± 1 in 0, 10, 20, 30, 40, and 50 μg/mL of lead nitrate, respectively. The results of flow cytometric assessment (Annexin V) also showed a strong dose-response relationship between lead nitrate exposure and early stage apoptosis in HL-60 cells. In summary, these data show that lead nitrate represent an apoptotic inducing agent in human Leukemia (HL-60) cells and its apoptotic mechanism is mediated through oxidative stress and phosphatidylserine externalization.
METALLIC NICKEL PARTICLES INDUCE CELL APOPTOSIS THROUGH A CASPASE-8-AIF-MEDIATED CYTOCROME C-INDEPENDENT PATHWAY.

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Numerous studies have described the pathogenic and carcinogenic effects of nickel compounds, but little has been published on the biologic effects of metallic nickel. The present study investigates metallic nickel particle-induced apoptosis and the signal pathways involved in this process in JB6 cells. Using MTT assay, we found that metallic nickel nanoparticles (80 nm) exhibited higher cytotoxicity than fine particles (3 μm). Both metallic nickel nano- and fine particles induced JB6 cell apoptosis in a dose-dependent manner. Western-blot analysis showed an upregulation of proapoptotic factors, including Fas, FADD, caspase-8, DR3 and BID, following exposure to metallic nickel particles. Metallic nickel particles showed only a very slight activation of caspase-3, -6 and -9. IP western blot analysis demonstrated the formation of the Fas-related death-inducing signaling complex (DISC) during the apoptotic process. Furthermore, during this process, lamin A, β-actin and PARP were cleaved. Moreover, we found that apoptosis-inducing factor (AIF) was upregulated and released from mitochondria into cytoplasm in nickel- treated cells. However, no cytochrome c release from mitochondria into the cytoplasm was found in nickel-treated cells. In addition, activation of anti-apoptotic factors, including phospho-Akt and Bcl-2, was detected. In conclusion, we report for the first time that metallic nickel nanoparticles caused higher cytotoxicity and apoptotic induction than fine particles in JB6 cells. Apoptotic cell death induced by metallic nickel nanoparticles in JB6 cells is through a caspase-8/AIF-mediated cytochrome c-independent pathway. Activation of Bcl-2 and Akt may play an important role in preventing cytochrome c release from mitochondria into the cytoplasm and may also be important in the carcinogenicity of metallic nickel particles. The data obtained from this study will be of benefit for elucidating the pathogenic and carcinogenic potential of metallic nickel particles.

Key words: metals; in vitro study; cytotoxicity; pathogenicity; carcinogenicity

P53-DEPENDENT AND P53-INDEPENDENT APOPTOSIS IN CHROMIUM CARCINOGENESIS.

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In multicellular organisms, cells with genomic damages have arrest-of-cell-cycles (senescence) and apoptosis and become cancerous. This carcinogenic experiment has been demonstrated in animals. Using hexavalent chromium as an example, we show in vitro how the metal can affect at the p53-dependent, p53-independent-apoptosis and senescence. Cr (VI) is converted to Cr (V) and Cr (IV) intracellularly. Both of these can cause changes in the genome such as DNA double-strand breaks. We have demonstrated in vivo with Drosophila wing-spot screening that Cr (V) induces both somatic recombination and somatic mutation without apoptosis while Cr (V) induces apoptosis and the somatic mutation event. Cancer occurs with increased Cr-induced genetic plasticity not accompanied by cell death or oncogenic-senescence. With Raji and a colon cancer cell-line we show how chromium can affect p53-dependent and p53-independent-apoptosis. Apoptosis can occur via a direct activation of caspase-2 at the time of exposure to the disruption of the phosphodiester bond. It can also occur with additional changes of p53/HAUSP/BAX and SMAC. Cr also caused oncogenic-senescence through p21WAF1/CIP1 and subsequent to the expansion of the multistage/multipathway of cancer progression at the organismic level as first defined by Leslie Foudil is now demonstrated experimentally at the subcellular level with chromium exposure. This is consistent to virus-induced (e.g., Maloney murine-leukemia-virus) cancer with shortened latency from losses of the functions of p53/p21/H2AX (independently, or in combination) in targeted knockout mice. This multistage/ multipathway carcinogenic mechanism now can be related to the genetic modifications of p53, p21, or H2AX at the cellular level both in subcellular level and mathematically. Disclaimer: The opinions and conclusions in this abstract are only those of the authors and do not reflect the institutions they represent.

MT ATTENUATES CARDIAC CELL DEATH V44 SUPPRESSION OF ER STRESS IS ONE OF THE MECHANISMS AGAINST DIABETIC CARDIOMYOPATHY.

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Our previous studies showed that cardiac MT overexpressing mice (MT-TG) are highly resistant to diabetic cardiomyopathy. Since apoptosis plays a critical role in the development of diabetic cardiomyopathy, and ER stress is one of the intrinsic apoptosis pathways, the present study was to test whether ER stress exists in the diabetic hearts, and whether MT prevents diabetic cardiomyopathy by blocking ER stress-induced cell death. Diabetes was induced by streptozotocin (one dose of 150 mg/kg) in both MT-TG and wild-type(WT) mice. Cardiac cell death was detected by in situ TUNEL staining and Western blotting of the activated form of caspase-3. Representative makers of ER stress were detected by Western blotting assay. We found that cardiac apoptosis in the WT, but not MT-TG, diabetic mice was significantly increased 2 weeks, and slightly increased 2 and 5 months after diabetes onset, with a detailed with apoptotic cell death, Western blotting revealed the hallmark of ER stress, including GRP78 and GRP94, ATF6, and phosphorylated eIF2α all significantly increased in the hearts of WT, but not MT-TG, diabetic mice 2 weeks, and slightly increased 2 months after the onset of diabetes. To further define the direct link of ER stress to apoptotic cell death and also confirm the direct cardiac protection of MT against ER stress-induced apoptotic effect, ER stress animal model was generated by was induced by direct administration of ER stress inducer(tunicamycin at 1 mg/kg and 1.5 mg/kg). Treatment with tunicamycin for 12 hr significantly increased cardiac cell death, detected by TUNEL staining and Western blotting of activated caspase-3, in the hearts of WT mice, but not MT-TG mice. These results suggest that ER stress exists in the diabetic heart and is involved in induction of cardiac cell death. MT prevented both diabetes and ER stress-induced cardiac cell death, implying that MT protection of diabetic ER stress-induced cardiac cell death is at least one of the mechanisms against diabetic cardiomyopathy (Supported, in parts, by ADA grants).

ANGIOTENSIN II-INDUCED CARDIAC APOPTOSIS IS MEDIATED BY P53-DEPENDENT AND -INDEPENDENT PATHWAYS.

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Our previous studies showed that angiotensin II (Ang II)-induced cell death plays a critical role in the development of diabetic cardiomyopathy. Tumor suppressor gene p53 was implicated in the pathogenesis of Ang II-induced cardiac cell death. However, the direct causative role of p53 in Ang II-induced cardiac apoptosis and its signaling pathways remain unclear. Therefore, H9C2 cells were exposed to Ang II at 100 nM for 7 hr and 24 hr, which significantly induced cardiac apoptotic cell death, along with significant increases in the total and phosphorylated p53 expressions. Interestingly, Ang II induced a time-dependent translocation of p53 from cytosol to mitochondria and nucleus. Furthermore, both mitochondrial permeability, detected by fluorescent staining and flow cytometry, and mitochondrial cytochrome c release, detected by Western blotting, significantly increased at 7 hr. P53 expression and activation were inhibited by pretreatment with p53 inhibitor pifithrin-a (PFTA-a). However, pretreatment of the cells with PFTA-a only inhibited apoptosis at 24 hr, but not at 7 hr after Ang II treatment. Pretreatment of cells with PFTA-a also inhibited Ang II-induced caspase-8 activation. In vivo, FVB mice were given subcutaneously injection of Ang II at the dose of 1 mg/kg body weight with and without PFTA-a at 2.2 mg/kg body weight twice at 30 min before and 1 hour after Ang II treatment. Expression of both total and phosphorylated p53 were increased in Ang II-treated mice, but not in Ang II/PFT-a-treated mice. Ang II-induced caspase-3 activation in the hearts was prevented in Ang II/PFT-a-treated WT mice only at 24 hr, but not at 7 hr after Ang II administration. These results suggest that Ang II-induced cardiac apoptosis is mediated by both p53-dependent and -independent pathways. The activation of mitochondrial cytochrome c release mediated apoptotic signaling pathway plays a critical role in Ang II-induced p53-dependent pathway (Supported, in parts, by ADA and AHA grants).

INHIBITION OF APOPTOSIS IN PRIMARY RAT HEPATOCYTES BY 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN.

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a highly toxic pollutant ubiquitously present in the environment. Most of the toxic effects of TCDD are believed to be mediated by high-affinity binding to the aryl hydrocarbon receptor (AhR) and subsequent effects on gene transcription. TCDD was classified as a class I human carcinogen. It causes cancer in multiple tissues in different animal species. In initiation-promotion studies TCDD was shown to be a potent liver tumor-promotor. Among other hypotheses it has been suggested that TCDD acts as a tumor-promotor by preventing initiated cells from undergoing apoptosis. In rat hepatocytes in primary culture TCDD (1 nM) inhibits UVC light induced apoptosis.
This effect is seen with intercellular DNA-fragmentation as well as with chromatin condensation and fragmentation and appears to be mediated by the AKR. Apoptosis induced by UV-C light in these cells is caspase-dependent and is accompanied by alterations in apoptosis-related gene expression such as up-regulation of pro-apoptotic bcl-2 family genes like bak and ban, and a marked down-regulation of the expression of the anti-apoptotic bcl-2. TCDD treatment of irradiated hepatocytes does not alter these changes. Upstream apoptotic events, namely caspase-activation and cleavage of poly (ADP-ribose) polymerase (PARP) and inhibitor of caspase-activated DNase (ICAD) are not inhibited by TCDD treatment; neither does TCDD affect CAD and ICAD gene expression. Furthermore, we performed an in vitro CAD activity assay. TCDD did neither act on CAD-catalyzed intracellular cleavage of DNA in TCDD-treated cells nor did it affect the degradation of isolated DNA. Since TCDD does act only on down-stream nuclear events of apoptosis we believe necleases to be putative targets of this pollutant. We hypothesize that TCDD inhibits late-stage apoptotic events maintaining chromosomal integrity probably in order to sustain metabolic capacity and hepatic elimination of substrates despite of an initiation of apoptosis.

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**300 SATRATOXIN G-INDUCED APOPTOSIS IN MOUSE OLFACTORY SENSORI NEURONS IS NOT MEDIATED BY DOUBLE-STRANDED RNA-ACTIVATED PROTEIN KINASE R (PKR).**


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Satratoxin G (SG) is a macrocyclic trichothecene mycotoxin produced by Stachybotrys chartarum. Acute intranasal exposure of mice to SG specifically induces apoptosis in olfactory sensory neurons (OSNs) of the nose. We have previously reported mRNA expression of double-stranded RNA-activated protein kinase (PKR), is increased in SG-treated mouse ethmoid turbinates and the neural crest-derived rat pheochromocytoma cell line, PC-12. PKR inhibition blocked SG-induced apoptotic gene expression and apoptosis in PC-12 cells suggesting a role for this widely expressed serine/threonine kinase. We tested the hypothesis that PKR mediates SG-induced OSN apoptosis in vivo and in vitro. To test the in vivo role of PKR, we generated type I/II and homozygous PKR knockout (KO) C57Bl6 mice were intranasally exposed to SG (250 µg/kg bw) and then ethmoid turbinates examined 24 h later for expression of apoptosis-related genes (Fas, Fasl, p53, BAX, PKR, caspase-3 and caspase-activated DNase) and histopathological effects. SG induced robust apoptotic gene expression in WT mice and these responses were not suppressed in PKR-KO mice. OSN cell death and TUNEL positivity were also similarly observed in SG-treated WT and PKR-KO mice. In addition, when C57Bl6 mice were pretreated with vehicle or the PKR inhibitor C16 (75 µM) towards FaDu cells. Gene expression analysis suggested that JS-K was able to suppress the expression of efflux transporters ABCG1 and ABCG2 transcripts and decreased the immunostaining for ABCG2 protein. JS-K also increased the expression of TNFR and enhanced immunostaining of caspase-3, suggesting the activation of efflux transporters and apoptosis in treated cell death pathways in FaDu cells. JS-K appeared to impact the expression of genes involved in metastasis, including a decreased expression of vascular endothelial growth factor (VEGF) and increased expression of tissue inhibitor of metalloproteinase-1 (TIMP). Taken together, these data indicate JS-K is effective in killing FaDu cells when used alone and in combination with other anticancer drugs. The combined effects appear to be due, at least in part, to reduced expression of efflux transporters and activation of TUNEL-mediated cell death pathways.

**302 CARDIOLIPIN OXIDATION, HYDROLYSIS AND ACCUMULATION OF MONOLYSOCARDIOLIPINS AND OXIDIZED FREE FATTY ACIDS DURING APOPTOSIS: ROLE OF CYTOCHROME C.**


Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA, Centro Interdisciplinar de Investigacion Biomolecular-CIB, Universidad de Magi de las Cruces, Madrid, Spain, Mogi das Cruzes-SP, Brazil and Center for Free Radical and Antioxidant Health, University of Pittsburgh, Pittsburgh, PA.

Oxidized phospholipids are important signaling participants of the mitochondrial stage of apoptosis. Accumulation of oxygenated molecular species of cardiolipin, CL and its hydrolysis products – monolysos-CL and free fatty acids – has been detected during early apoptosis in different cell lines. Apoptosis-associated selective oxidation of CL is due to the formation of complex between CL and cyt c with a peroxidase activity triggered by oxidized equivalents generated by disrupted electron transport of mitochondria. Using ESI-MS analysis we demonstrated that hydroperoxy-, hydroxy- and hydroperoxy-hydroxy-derivatives were the most dominant oxidation products of CL. Now, we discovered that cyt c itself is able to hydrolyze oxidized CL with the formation of two major products – non-oxidized monolysos-CL and oxidized free fatty acids; the identity and structure of the products were confirmed by 2D-HPTLC followed by fluorescence HPLC and LC-ESI-MS analysis. Non-enzymatic oxidation of CL by Fe/H2O2, Fe/ascorbate or by a lipid-soluble azo-initiator, AMVN, did not result in accumulation of CL hydrolysis products. CL hydrolysis products were not detected after incubation of non-oxidizable TOCL with cyt c/H2O2. Further, incubation of isolated mouse mitochondria with an organic hydroperoxide, tBOOH, also caused accumulation of monolysos-CL and oxidized free fatty acids. Finally, apoptosis induced by actinomycin D in cyt c/-/ but not in cyt c+/+ mouse embryonic cells resulted in CL oxidation and accumulation of CL hydrolysis products. Thus, cyt c displays a CL-specific phospholipase A activity directed towards hydrolysis of oxidatively modified polyunsaturated acyls in sn-2 position. Support: U19AI068021, HL70755, PA Dept of Health SAF 4100027294.

**303 MULTIPLEXED ASSAY PANEL OF CYTOTOXICITY IN HK-2 CELLS FOR DETECTION OF RENAL PROXIMAL TUBULE INJURY POTENTIAL OF COMPOUNDS.**


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Proximal tubules of the kidneys are common targets of nephrotoxic compounds. Screens to predict nephrotoxic potential of compounds with insights to mechanisms of toxicity facilitate lead optimization, guide structure-activity relationships, minimize risks of clinical nephrotoxicity and therefore are valuable in the process of drug discovery. In vitro cytotoxicity assays routinely measure mitochondrial function; although a good marker of effect, it seldom provides insight into mechanism. We developed an in vitro assay multiplexed to measure several endpoints of cytotoxicity using HK-2 cells in 96-well format. Assays for lactate dehydrogenase leak as a marker of necrosis, cellular caspase 3/7 activation as a marker of apoptosis, resazurin dye reduction as a marker of mitochondrial function and Hoechst 33342 staining for double-strand DNA were multiplexed to maximize the ability to detect cell death. Assays were performed for 5 or 24 hour incubations to further enhance the sensitivity of detection of toxicity. Individual assays were optimized for cell density, assay linearity and assay performance under multiplexed conditions. Inducers of apoptosis (staurosporine) and necrosis (perhexiline) were used to validate the mechanistic aspects of cell death (apoptosis, necrosis). Nephrotoxic compounds were also tested using the assay. This multiplexed assay panel allows for more reliable prediction of renal proximal tubule injury potential of compounds.

**THE CYTOLETHAL EFFECTS OF JS-K, AN ANTI-TUMOR NITRIC OXIDE RELEASING PRODRUG, IN HUMAN PHARYNGEAL CARCINOMA FADU CELLS.**


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Pharyngeal cancer is very common in certain regions of East Asia and Africa, and effective chemotherapy is presently unavailable. JS-K is a nitric oxide (NO) releasing prodrug, which has been shown to be effective against several cancer cells and cancer xenografts. This study was conducted to further examine the effect of JS-K on the human FaDu pharyngeal carcinoma cell line. Cells were treated with JS-K or JS-K plus other anticancer agents for 48 hours. Cell viability was then determined by the MTS assay, JS-K-induced alterations in gene expression were determined at the transcriptional level by real time RT-PCR, and at the protein level by confocal imaging, JS-K was cytolethal to FaDu cells in a concentration-dependent manner, with an IC50 of approximately 10 µM. JS-K at 3 µM additively enhanced the cytolethal effects of other anticancer agents including cisplatin (10 µM), Fluorouracil (100 µM), or arsenic (10 µM) towards FaDu cells. Gene expression analysis suggests that JS-K was able to suppress the expression of efflux transporters ABCG1 and ABCG2 transcripts and decreased the immunostaining for ABCG2 protein. JS-K also increased the expression of TNFR and enhanced immunostaining of caspase-3, suggesting the activation of efflux transporters and apoptosis in treated cell death pathways in FaDu cells. JS-K appeared to impact the expression of genes involved in metastasis, including a decreased expression of vascular endothelial growth factor (VEGF) and increased expression of tissue inhibitor of metalloproteinase-1 (TIMP). Taken together, these effects are likely to be due, at least in part, to reduced expression of efflux transporters and activation of TUNEL-mediated cell death pathways.
pounds (5-fluorouracil, gentamicin, cisplatin, acetaminophen, p-aminophenol, potassium dichromate, ibuprofen, doxorubicin, cyclosporine A, citrin, puromycin) with known direct or indirect association to injury to proximal tubules were used to determine the potential of this method to detect proximal tubule toxicity. Overall, this cost-effective multiplexed platform is more sensitive than a single endpoint assay, provides mechanistic cues of toxicity and is amenable for higher throughput screening. Limitations of the assay in predicting in vivo toxicity, such as those seen in nonspecific cytotoxicity and the importance of in vivo disposition are also highlighted.

304 MULTI-PARAMETER CYTOMETRY TO DETECT DRUG-INDUCED MITOCHONDRIAL TOXICITY.

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Mitochondrial dysfunction has been increasingly implicated as a mechanism for drug-induced toxicity. Screening for mitochondrial dysfunction prior to lead optimization is being conducted by several pharmaceutical companies in lieu of cytoxicity assays. In this study, we selected 30 drugs/compounds that were known to induce various organ-toxicities related to mitochondrial dysfunction in both humans and animals. Compounds were categorized into four major groups: mitochondrial respiratory chain inhibitors, oxidative stress inducers, mitochondrial polymerase inhibitors, and various kinase inhibitors. In addition, 10 drugs/compounds that did not induce mitochondrial toxicity were chosen as negative controls. A high-throughput multiparameter flow cytometry-based platform using human proxymelyocytic leukemia cell line (HL-60 cells) was set up to monitor mitochondrial membrane potential (MMP) change (using potentiometric dye JC-1), intracellular reduced glutathione (GSH) level (using monobromobimane) and cell viability (using calcine-AM) simultaneously. Results obtained (IC50 values for each parameter per compound) demonstrated that disruption of MMP is the earliest indicator for the onset of mitochondrial damage. Over 60% of compounds screened triggered MMP change within 6 hours, progressing to 90% at 24 hours accompanied by depletion of intracellular GSH and decreased viability at 24 hours. Our results also demonstrated that a comparison of drug-induced changes in glucose as opposed to galactose/glutamine-enriched medium and vice versa is essential to separate mitochondrial dysfunction from frank cytotoxicity. In conclusion, a comprehensive analysis of MMP/GSH/viability could increase the probability of predicting mitochondrial toxicity in a cell-based screening system. Cytometry-based screening assays with human cell lines have excellent correlations (~90%) with in vivo human toxicity.

305 NADPH CYTOCHROME P450 REDUCTASE MEDIATES REDOX CYCLING BY 2- AND 4-HYDROXYESTRASTRODIOL CATECHOL METABOLITES.

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Estrogen exposure is a major risk factor for the development of breast-cancer. Estrogens are known to modulate cellular proliferation by binding to nuclear receptors, a process that alters transcription of estrogen-responsive genes. Previous studies demonstrated that the estrogenic estrogen equilenin, a component of hormone replacement therapy, and diethylstilbestrol are converted to catechol metabolites; subsequent oxidation reactions generate redox-active ortho-quinones. One-electron reduction of the quinones by NADPH-dependent oxidoreductases to semi-quinones and their subsequent reaction with molecular oxygen generates reactive oxygen intermediates (ROI). In the present studies we compared redox cycling of two endogenous catechol estrogens, 2- and 4-hydroxyestradiol, with estradiol and 2-methoxyestradiol. Using Superoxide that overexpress human cytochrome P450 reductase we found that the estradiol (1-300 μM) readily generated ROI as measured by the formation of hydrogen peroxide and hydroxyl radicals. Redox cycling was found to be NADPH- and cytochrome P450 reductase-dependent and inhibited by diphenylethylenosimdiamine, confirming that the reaction was mediated by cytochrome-P450 reductase flavoenzyme. The Km’s of the catechol estrogens were both approximately 1 μM, indicating similar binding efficiency with the enzyme. However, the Vmax’s varied from 0.5-5 moles min-1 mg-1 protein in the rank order 4-hydroxyestradiol > 2-hydroxyestradiol > estradiol and 2-methoxyestradiol. The finding that the Km’s were similar yet the Vmax’s different, suggests that 4-hydroxyestradiol is a more efficient generator of ROI than 2-hydroxyestradiol. Taken together, these data indicate that NADPH-cytochrome P450 reductase mediated redox cycling by catechol estrogens, implying that NADPH-cytochrome P450 reductase has a role in endogenous estradiol redox cycling and potentially in the etiology of breast cancer. Supported in part by NIH grants E505022, AR055073, CA100994 and CA093798.

306 EXAMINATION OF GENETIC DAMAGE IN GLUTATHIONE (GSH)-DEPLETED CELL CULTURES USING THE MICRONUCLEUS TEST: SINGLE CELL GEL ELECTROPHORESIS ASSAY (COMET), AND 8-HYDROXY-DEOXYGUANOSINE (8-OH-DG) LEVELS IN NUCLEAR DNA.


David Geter, Fagen Zhang, Melissa Schuler, Amanda Wood, Lynn Kan, Yo-Chan Jeong, Michael Barrels, Gallapudi, Bhaskar, Toxicology and Environmental Research & Consulting, The Dow Chemical Company, Midland, Michigan, 48674. Depletion of GSH in cells exposed to certain xenobiotics has been implicated in oxidative stress leading to damage of cellular macromolecules such as proteins, lipids, and DNA. Diethyl maleate (DEM), is known to conjugate with GSH and rapidly lower cellular GSH levels. The objective of these studies was to investigate the influence of DEM on various genotoxicity endpoints in mouse lymphoma L5178Y (TK+/-) cell cultures. Cells were exposed to DEM for 4 hours at concentrations of 0.6, 13.5, 26.9, 53.8, 107.6, and 215.3 μg/ml. Genotoxicity was evaluated by examining the induction of 1) micronuclei (50 h post-treatment), 2) DNA strand breaks (immediately following treatment), and 3) 8-OH-dG levels in nuclear DNA (immediately following treatment), and correlating these end points to cellular GSH levels. A significant increase in micronuclei was observed at exposure concentrations starting at 53.8 μg/ml. However, induction of DNA strand breaks as measured by the alkaline comet assay was only evident at concentrations of 107.6 μg/ml and higher. Quantifiable levels of 8-OH-dG (2 adducts per 1 x 10^6 NT) were not detectable at any exposure concentration under the experimental conditions despite the fact that GSH levels were significantly reduced at all test concentrations in a dose-dependent manner. Taken together, these results demonstrate an association between DEM-induced genotoxicity and GSH depletion in cell cultures. Although the mode of action for the observed genotoxicity is not discernible from these studies, it is postulated that oxidative stress resulting from GSH depletion is partly responsible for the effect.

307 IDENTIFICATION OF AN FAD-INDEPENDENT REDOX CYCLING ACTIVITY FOR 9,10-PHENANTHRENEQUINONE IN MOUSE LUNG EPITHELIAL CELLS.


9,10-Phenanthrenequinone (PQ) is a component of airborne particulate matter and can cause protein oxidation and cytotoxicity via a one or two-electron reduction. One electron reduction of PQ results in a semiquinone radical; oxidation of the radical leads to the formation of reactive oxygen species including superoxide anion, hydrogen peroxide and hydroxyl radicals. In the lung, this can cause alveolar inflammation, epithelial cell damage and pulmonary fibrosis. Using mouse MLE-15 lung epithelial cells, we discovered a novel NADPH-dependent redox cycling activity in cytosolic cellular fractions. Purification and sequencing identified this material as sepiapterin reductase (SPR), an enzyme catalyzing the final step in the biosynthetic pathway of tetrahydrobiopterin. Human SPR was cloned and its enzymatic activities characterized. In addition to reducing sepiapterin, the enzyme readily mediated PQ redox cycling as measured by hydrogen peroxide formation in the Amples Red assay. Dickomorol was found to be a noncompetitive inhibitor (IC50 = 200 μM) of sepiapterin reduction without inhibiting redox cycling. Similar selective inhibition of SPR was found with other inhibitors (2.5 μM), indomethacin (8.1 μM), ethacrynic acid (22.9 μM), and rutin (24.0 μM) while PQ was a noncompetitive inhibitor of SPR activity. Sepiapterin reduction and redox cycling activity were not inhibited by diphenylethylenosimdiamine, a finding consistent with the fact that SPR does not contain flavin cofactors. Taken together, our data identify a novel enzyme in lung epithelial cells that mediates chemical redox cycling. Redox cycling via SPR may be an important mechanism by which contaminants in airborne particulate matter redox cycle and initiate toxicity. Supported by CA100994, CA132624, CA093798, ES004738, ES005022, GM034130 and AR055073.
0.1-1.2 mM. Hydroxyl radical formation was dependent on FeCl2, but not FeCl3, terephthalate in reaction mixes using HPLC with fluorescence detection. We found formation of GSH. Hydroxyl radicals were detected by quantifying formation of 2-OH Cys-Gly sulfenic acid, which may be cytotoxic to cells. In the present studies we compared chemical redox cycling by NADPH-cytochrome P450 reductase (EC 1.6.4.4, CPR), a microsomal NADPH-dependent oxidoreductase, and sepiapterin reductase (EC 1.1.1.35, SPR), a nonfavin-containing cytoplasmic oxidoreductase important in the biosynthesis of tetrahydrobiopterin. Using Amplex Red to quantify hydroquinone production during redox cycling, 9,10-phenanthrenequinone was found to be the most potent redox cycle for CPR, followed by menadione, nitrofurantoin, paratquat, mitomycin c and diquat. The Vmax ranged from 4.4 to 10.4 μmol hydrogen peroxide/mg protein/min, while the Km varied from 1.2 μM to 434 μM. In contrast, SPR only catalyzed redox cycling of 9,10-phenanthrenequinone and menadione (Vmax = 2.0 μmol hydrogen peroxide/mg protein/min vs. 5.0 nmol hydrogen peroxide/mg protein/min, respectively), with little or no activity for nitrofurantoin, mitomycin c, parquat and diquat. Redox cycling of 9,10-phenanthrenequinone mediated by CPR was inhibited by hemin, diphenyleneiodonium and chlorpromazine, while no inhibition was found in SPR catalyzed redox cycling of this compound. These findings are consistent with the fact that only CPR is a flavoenzyme. Taken together, our results demonstrate distinct substrate specificities for the one electron reduction of redox active compounds by CPR and SPR. Depending on enzyme tissue distribution for SPR and CPR, this may be an important mechanism mediating selective toxicity of redox active chemicals. Supported by CA100994, CA135264, CA093798, ES004738, ES005022, GM034310 and AR055073.

Glutathione (GSH) is an important intracellular antioxidant. However, it can also undergo non-enzymatic autooxidation and form reactive oxygen intermediates (ROI) including superoxide anion, hydrogen peroxide and hydroxyl radicals. These processes can generate a variety of GSH degradation products including GSH- and Cys-Gly sulfenic acid, which may be cytotoxic to cells. In the present studies we characterized the formation of hydroxyl radicals during non-enzymatic autooxidation of GSH. Hydroxyl radicals were detected by quantifying formation of 2-OH tertephthalate in reaction mixes using HPLC with fluorescence detection. We found that autooxidation of GSH was time- and concentration-dependent in the range of 0.1-1.2 μM. Hydroxyl radical formation was dependent on FeCl2, but not FeCl3, and FeCl3, and blocked by diethylthiocarbamate (DDM) or sodium azide. These findings suggest a role for oxidative stress in GSH autoxidation in the presense of transition metals can be a source of highly toxic hydroxyl radicals. Moreover, the dual pro- and antioxidant effects of reduced GSH may have significant biological importance. Supported by CA100994, CA135264, CA093798, ES004738, ES005022, GM034310 and AR055073.

Currently, no antidote exists for SM exposure but recent studies, using the SM analog 2-chloroethyl ethyl sulfide (CEES), have focus on the ability of antioxidants to prevent toxicity. Though antioxidants can prevent CEES-induced toxicity, it is largely unknown why these compounds are effective against these types of alkylating agents. Using human bronchial epithelial (16HBE) cells and primary small airway epithelial (SAE) cells we show that CEES causes a significant increase in mitochondrial ROS peaking 12h after exposure. We have also identified a catalytic antioxidant metalloporphyrin that can rescue airway cells from CEES-induced toxicity when added 1h after CEES exposure. In addition, the catalytic antioxidant can prevent increases in ROS, DNA oxidation, and decreases in intracellular GSH. These findings suggest a role for oxidative stress in CEES toxicity and provide a rationale to investigate antioxidants as rescue agents.

4-Hydroxynonenal (4-HNE), an end product of lipid peroxidation, is generated in cells in response to oxidative stress. This highly reactive aldehyde readily forms adducts with proteins, lipids and DNA which can be detected in many tissues including the lung and liver in response to chemical toxicants. In the present studies we characterized 4-HNE metabolism in 59 post-mitochondrial fractions from liver and lung. Metabolites were analyzed using HPLC with fluorescence detection. These findings suggest a role for oxidative stress in CEES toxicity and provide a rationale to investigate antioxidants as rescue agents.

Glutathione (GSH) is an important intracellular antioxidant. However, it can also undergo non-enzymatic autooxidation and form reactive oxygen intermediates (ROI) including superoxide anion, hydrogen peroxide and hydroxyl radicals. These processes can generate a variety of GSH degradation products including GSH- and Cys-Gly sulfenic acid, which may be cytotoxic to cells. In the present studies we characterized the formation of hydroxyl radicals during non-enzymatic autooxidation of GSH. Hydroxyl radicals were detected by quantifying formation of 2-OH tertephthalate in reaction mixes using HPLC with fluorescence detection. We found that autooxidation of GSH was time- and concentration-dependent in the range of 0.1-1.2 μM. Hydroxyl radical formation was dependent on FeCl2, but not FeCl3, and FeCl3, and blocked by diethylthiocarbamate (DDM) or sodium azide. These findings suggest a role for oxidative stress in GSH autoxidation in the presense of transition metals can be a source of highly toxic hydroxyl radicals. Moreover, the dual pro- and antioxidant effects of reduced GSH may have significant biological importance. Supported by CA100994, CA135264, CA093798, ES004738, ES005022, GM034310 and AR055073.

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313 DETECTION OF CIGARETTE SMOKE-INDUCED REACTIVE OXYGEN SPECIES (ROS) USING A CM-H2DCFDA FLUORESCENCE INDICATOR.

T. Carr1, T. Tai1, S. Faux1 and M. Gaca1. 1Group RE-D, British American Tobacco, Southampton, United Kingdom and 2Toxicology group, Advanced Technologies (Cambridge) Ltd, Cambridge, United Kingdom. Sponsor: C. Meredith.

Reactive oxygen species (ROS), thought to be generated by cigarette smoke, may potentiate the total physiological oxidative stress load and may be a factor in development of various smoking related diseases. To establish the role of ROS generation induced by cigarette smoke total particulate matter (PM), we investigated the fluorescence probe, 5-(and-6)-chloromethylene-2,7-dichlorodihydrofluorescein diacetate (CM-H2DCFDA), a fluorophore which reacts with ROS, in in vitro cultures of lung epithelial cells. Monolayers of human lung epithelial cells (NCl-H292) were initially primed with CM-H2DCFDA for 1 hour at 37°C followed by treatment with oxidative stress positive controls; potassium bromate, tert-butyly hydroperoxide or hydrogen peroxide. The levels of oxidative stress caused by incubation with the positive controls, potassium bromate (0.1-2.8M), tert-butyly hydroperoxide (0.1-400μM) or hydrogen peroxide (0.78-200μM), for 1 hour at 37°C were compared to incubation with TPM (1.56-200μg/ml). Relative fluorescence was measured using a microplate fluorescence spectrophotometer at excitation and emission wavelengths of 485nm and 530nm respectively. The concentrations of agents that induced formations of ROS at levels significantly higher than the media control were; potassium bromate - 14μM, tert-butyly hydroperoxide - 7.8μM, with saturation for human therapies. Experiments were conducted to elucidate the status of glutathione in the oxidized (GSSG) and reduced (GSH) forms in mouse ES (mES) cells after treatment with tertiary-butyl hydroperoxide (tBH) to cause oxidative stress. Glutathione was measured at picomolar levels by fluorometric HPLC after derivatization of existing mES cells with dansyl chloride. GSH content decreased significantly from 1.92 ± 0.22 (mean ± SEM) fmol per cell to 1.10 ± 0.16 fmol per cell (p < 0.02) following 15 minutes exposure to 1.06 mM tBH in culture medium. GSSG increased significantly from 0.25 ± 0.04 fmol per cell to 0.91 ± 0.15 fmol per cell (p < 0.0001) following the same exposure time. By 60 minutes, both GSH and GSSG had returned to control levels. The loss of GSH was accounted for by increases in GSSG. By contrast, mES cells exposed to 10.6 mM tBH for 15 minutes were unable to recover GSH levels after 60 minutes. Previous studies in this laboratory have shown that mouse blastocyst stage preimplantation embryos were unable to recover from 15 minutes exposure to tBH at 0.026 mM. These data suggest that mES cells are less sensitive to reactive oxygen species than preimplantation embryos.

317 5-CARBOXYAMIDO-5-FORMAMIDO-2-MINOHYDANTOIN (2-IH), A NEW OXIDATION PRODUCT IN DNA.

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Oxidation of DNA bases by reactive oxygen species results in adverse outcomes including cancer, ageing and other degenerative processes. The products of single-electron oxidants (such as 8-oxoguanine) have been extensively characterized, however the outcome of two-electron oxidation is less well documented. We show here that oxidation of short strands of DNA by peracids and other model two-electron oxidants results in the formation of an oxidation product that is not a keto/enol tautomer and that shows spectroscopic properties that are not consistent with existing 8-oxoguanine or 8-hydroxyguanine models. This is evidence that the chemistry of oxidation products of DNA is complex and may not be limited to the keto/enol tautomer that is the predominant product of single-electron oxidants.
oxidants converts guanine bases into 5-carbomethoxy-5-formamido-2-iminohydantoin (2-Ih). The product has been characterized by mass spectrometry (ESI-MS, ESI-MS/MS and MALDI-MS) and 1H and 13C nuclear magnetic resonance spectroscopy both in the intact oligonucleotide sequence and as the deoxynucleoside obtained by enzymatic digestion of the DNA. Labeling studies with base and deoxyribonucleoside showed that the reaction proceeds by initial epoxidation of the C4–C5 bond of Guanine followed by a 1,2-shift of C6 of Guanine to give 2-Ih. One of two added oxygen atoms is derived from the oxidant, the other from water; molecular oxygen is not directly required. This lesion is stable through DNA digestion and chromatographic purification, suggesting that it will be detectable if formed in vivo, for instance by peroxides formed from lipid peroxidation. No 8-oxoguanine derivative was seen in these oxidations. The 2-Ih lesion therefore appears to be a pathway-specific lesion and holds promise as a potential biomarker.

318 HEMOGLOBIN/HAPTOGLOBIN AGGREGATES PRODUCED BY PEROXIDASE ACTIVITY ARE TAKEN-UP AND INJURE MACROPHAGES.

A. Kapralov1,2, W. Feng1,2, I. Vlasova2, A. Maeda1, J. A. Caricillo1, H. Bayir1 and V. E. Kagan1, 2. 1Center for Free Radical and Antioxidant Health, University of Pittsburgh, Pittsburgh, PA, 2Departments of EOH, University of Pittsburgh, Pittsburgh, PA and Departments of Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA.

As a hemoprotein, Hb can, in the presence of oxidizing equivalents such as H2O2, act as a peroxidase with very high oxidizing potential. In red blood cells, this dangerous activity is strictly regulated by reducing environment and lack of oxidizing equivalents. The ferric form of Hb is short-lived and the hemoprotein is effectively converted into ferro-Hb by metHb reductase. For stroma-free Hb this regulation is lost and a potential for Hb to become a peroxidase is very high. This potential may be markedly increased by inflammatory cells generating oxygen radicals, superoxide. The latter can be converted into H2O2 – a fuel for Hb capable of feeding its peroxidase activity. It has been proposed that extra-cellular Hb is bound by its haptoglobin (Hpt) (types 1-1 or 2-2) resulting in apparent peroxidase activity. We demonstrate that: 1) Hb peroxidase activity is significantly increased by binding with Hpt; 2) H2O2/Hpt use complexes undergo self-inflicted cross-linking provided H2O2 is available; 3) inflammatory cells generate superoxide->H2O2 at levels sufficient to maintain the peroxidase activity of Hb/Hpt complexes; 4) Hb/Hpt aggregates are taken-up by inflammatory cells; 5) oxidative stress weakens the peroxidase activity. We demonstrate that: 1) Hb peroxidase activity is increased by its binding with Hpt (types 1-1 or 2-2) resulting in apparent peroxidase activity; 2) peroxidase activity of Hb/Hpt complexes is lost and a potential for Hb to become a peroxidase is very high. This potential may be used in evaluating the prognosis of SLE as well as in elucidating the mechanism of disease pathogenesis, especially in relation to exposure to environmental agents that are known to cause oxidative stress.

320 INVOLVEMENT OF OXIDATIVE AND NITROSATIVE STRESS IN SYSTEMIC LUPUS ERYTHEMATOSUS.

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Pathology UTMB, Galveston, TX.

Environmental factors, including chemical exposure, are known to contribute to the development of systemic lupus erythematosus (SLE) and other autoimmune diseases (ADs). Previous studies in our laboratory using an autoimmune-prone MRL+/+ mice exposed to trichloroethene suggested an association between oxidative/nitrosative stress and autoimmune response. However, relevance and involvement of oxidative/nitrosative stress in disease progression and pathogenesis in humans are not understood. To investigate the status of oxidative/nitrosative stress in ADs, sera from 72 SLE patients with various SLE scores (SLE disease activity index; SLEDAI) and 40 age- and gender-matched healthy controls were evaluated for oxidative/nitrosative stress markers. Serum analysis showed significantly higher levels of both anti-malondialdehyde (MDA)- and anti-4-hydroxynonenal (HNE)-protein adduct antibodies in SLE patients. Interestingly, our data showed not only increased number of subjects positive for anti-MDA- or anti-HNE-protein adduct antibodies, but also greater increases in both these antibodies in SLE patients with greater SLEDAI (6), which were significantly higher than the group with lower SLEDAI (6). Data also showed a good correlation between anti-MDA and anti-HNE antibodies and SLEDAI (r = 0.734 and 0.647, for anti-MDA and anti-HNE antibodies, respectively). Serum Cu/Zn superoxide dismutase levels were reduced in SLE patients, suggesting a compromised antioxidant balance. Furthermore, sera from SLE patients had higher levels of both iNOS and nitrotyrosine, suggesting increased nitrosative stress. These findings support an association between oxidative/nitrosative stress and SLE, and suggest that oxidative/nitrosative stress markers may be useful in evaluating the prognosis of SLE as well as in elucidating the mechanisms of disease pathogenesis, especially in relation to exposure to environmental agents that are known to cause oxidative stress.

321 THE ROLES OF OXIDATIVE STRESS AND PHAGOCYTIC ACTIVATION IN THE SUBACUTE TOXICITY OF THE WATER CHLORINATION BY PRODUCTS, DI- AND TRI-CHLOROACETATE.

E. Hassoun, J. Ceerfoss and J. Spildener. Pharmacology, The University of Toledo, Toledo, OH.

Dichloroacetate (DCA) and trichloroacetate (TCA) are byproducts produced dur- ing the process of water chlorination and were found to be hepatotoxic and hepatocarcinogenic in rodents. The roles of oxidative stress and phagocytic activation in the toxicity of the compounds have been studied in B6C3F1 male mice. Groups of mice were administered daily doses of 7.7, 77, 154, and 410 mg/kg of each of DCA and TCA, by gavage, for 4 weeks. Mice were sacrificed at the end of the treatment period and hepatic tissues were collected and studied for the production of superoxide anion (SA) and lipid peroxidation (LP). Peritoneal lavage cells were also collected and studied for SA production, myeloperoxidase (MPO) activity and tumor necrosis factor alpha (TNF-alpha) production. While DCA treatment resulted in dose-dependent increases in SA production in the hepatic tissues, TCA treatment was associated with significant SA suppression in those tissues, as compared with the controls. Also DCA resulted in Dose-dependent increases in hepatic LP, however, TCA treatment was associated with significant increases in hepatic LP, only with doses above 77 mg/kg/day. Treatmentwith DCA and TCA also resulted in significant modulations in MPO activity, SA production and TNF alpha levels in the peritoneal lavage cells. These results indicate the involvement of oxidative stress and phagocytic activation in the subacute toxicity of the compounds. (Supported by NIH/NIAMS # 1R15ES013706-01A1).

322 THE CHEMICAL SECURITY ANALYSIS CENTER: KNOWLEDGE MANAGEMENT AND CHEMICAL THREAT AWARENESS.

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The Chemical Security Analysis Center (CSAC) was established within the Department of Homeland Security to develop and implement a capability for providing chemical threat awareness and assessment as applied to issues related to homeland security and to serve as the central repository of chemical threat characterization data. In support of this mandate, the CSAC develops knowledge man-
agrement capabilities related to chemical threats that include toxic industrial chemicals, chemical warfare agents, pesticides, pharmaceuticals, and other threats as they are identified. The objective of the CSAC knowledge management project is to provide verifiable chemical threat information with a capability for an efficient chemical data search, review, analysis and output. This effort involves identification and collection of chemical data sources, incorporation of the data into computer electronic formats, the proper training and ranking of the data contained in the sources, and the development of a computer assisted workbench graphical user interface for searching, reviewing, analyzing and outputting data. Interagency collaboration is required to identify data sets, information sources, and subject matter experts. The CSAC utilizes this extensive data repository to develop comprehensive science and technology based assessments of specific chemical hazards and threats, to compile scientific information related to single topics in a series of source books, and to provide expert analytical support through a centralized 24/7 reachback capability to respond to chemical threat-related inquiries from federal agencies.

323 STEERING UNDERGRADUATE ENVIRONMENTAL SCIENCE RESEARCH IN NORTH DAKOTA.

D. A. Sens, Pathology, University of North Dakota, Grand Forks, ND. The University system of North Dakota has made a concerted effort to introduce research into the undergraduate curriculum. A major part of this effort has been to provide undergraduate research in the area of environmental science and human health. The Short Term Educational Experiences for Research (STEEER) program of the National Institutes of Health Sciences has provided this opportunity to undergraduate at the University of North Dakota (UND), one of the State's two research intensive universities. The STEER program was further enhanced at UND by an administrative commitment for 10 to 15 additional undergraduate summer research opportunities. Undergraduate research was stimulated at the State's four primarily undergraduate universities (PUI) through support from the INBRE program sponsored by the National Center for Research Resources of the NIH. At least one laboratory at each PUI is active in environmental based research and overall 8 of 11 laboratories have an environmental component to their undergraduate-based research. Currently, over 50 undergraduates are actively engaged in undergraduate research during the academic year or summer months. Current efforts are centered on adding undergraduate research to the Community-based Tribal Colleges within the borders of North Dakota.

324 TOXICOLOGY IN THE TEXTBOOKS USED IN THE LOWER LEVELS OF FINNISH COMPREHENSIVE SCHOOLS.

R. O. Juvonen and M. A. Rimpiläinen, Pharmacology and Toxicology, University of Kuopio, Kuopio, Finland. Sponsor: M. Vilkkula.

Toxicology is a multidisciplinary science that examines the adverse effects of chemicals in biological systems. Its major task is to protect human beings against these adverse effects. In that respect, it is important that basic aspects of toxicology are taught at home and during the first school years. The aim of this study was to find out how many and what kind of items of toxicology are found in the textbooks used at lower levels of Finnish comprehensive schools. For that purpose 25 toxicology related items, pictures and texts were analyzed in 18 textbooks devoted to environmental and natural sciences in the topics of biology, geography, physics, chemistry and health education. The total number of toxicological related subjects was found to be 1422. Their number increased from 32/book in the first grade to 167/book in the sixth grade. Chemicals were the largest group of toxicology related subjects (46 %), followed by poisonous organisms (27 %), intoxicants (17 %), environmental pollutants and hazardous waste (8 %) and warning labels of chemicals (4 %). Pictures and textual subjects were found in equal amounts in the textbooks used in first and second grade but the amount of the text increased to being more than 80 % by the sixth grade. It is concluded that toxicology related subjects have been incorporated in all of the textbooks used by the lower level of Finnish comprehensive schools. However, the amount of toxicology related material should be enlarged and expanded to include different groups of chemicals, pharmaceuticals, intoxicants and hazardous environmental agents.

325 JOHN SNOW, THE FATHER OF EPIDEMIOLOGY, WAS A TOXICOLOGIST: THE NEED FOR SYSTEMS APPROACHES TO CHEMICAL CAUSATION.

R. D. Godstein, Univ Pittsburgh Graduate School of Public Health, Pittsburgh, PA.

Ideally, toxicology and epidemiology are cooperative disciplines in evaluating the causation of human disease by chemical and physical agents. Unfortunately, toxicological and ecological sciences, including insights into disease mechanisms, are being assigned a lesser role in establishing causation by various regulatory bodies and by state and federal courts. The diminishing role for toxicology in part comes from advocates of the Precautionary Principle (PP) who insist that only an epidemiological association need be the basis for regulatory action. As many PP advocates contend that John Snow, known as the father of epidemiology for his work on cholera, epitomizes the PP, it is pertinent to evaluate the scientific background and methodology used by Snow. In fact, Snow was a superb toxicologist. His pulmonary studies in laboratory animals led to innovative approaches to the then new field of anesthesiology, including studies relating chloroform levels in air and inhalation rate to chloroform blood concentrations and anesthetic effect. He developed devices to improve inhalation and was one of the first to consider structure–activity relationships in CNS toxicity. His understanding of the respiratory system led him to discount the prevailing view that cholera was caused by inhalation. He also reasoned from the absence of blood abnormalities in early cholera patients that this gastrointestinal disease could not have been transmitted from lung to GI tract through the blood. His groundbreaking study of a specific London cholera epidemic was not a chance observation but rather was the result of a fully conceived hypothesis which the epidemic allowed him to test. Rather than being consistent with a call to act without basic scientific understanding, Snow’s approach to the challenge of cholera causation epitomizes the importance of toxicological science working collaboratively with epidemiology and exposure assessment to understand and effectively prevent human disease caused by external agents.

326 DEVILS LIE IN THE DETAILS: PRACTICES AND PROBLEMS IN NEUROPATHOLOGY - SIGNIFICANCE FOR NEUROTOXICOLOGY.

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The pathologic examination of the nervous system is an important component of experimental and regulatory neurotoxicology and in studies of neurodegenerative disease. Given the significance of the scientific and public health assessments, the ease with which histologic artifacts can be introduced into the process, and the possibility that the latter may be interpreted as representing toxicant-induced changes it is important to highlight these issues. Recent publications so interpreting such artifacts underscore the need for a review of this subject within the toxicology community. Mark Butt will provide an overview of the proper practice of neuropathology as it relates to study design, tissue fixation and specimen preparation. Robert Garman will describe the artifacts found in histologic preparations of the central nervous system, including their genesis, morphology and potential interpretative problems while Bernard Joynter will review similar aspects of artifacts of the peripheral nervous system. This session will be of interest to pathologists, toxicologists, and neuroscientists involved in toxicologic investigations.

327 THE USE OF ENGINEERED NANOMATERIALS IN FOOD AND FOOD-RELATED PRODUCTS: IS THIS A CONCERN FOR HUMAN AND ENVIRONMENTAL SAFETY?

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The advent of nanotechnology has brought with it questions related to human and environmental safety. The application of nanotechnology in food packaging and as food or color additives has generated questions on the safety of nanomaterials in biological systems. Thus, it is important that we consider concerns expressed in the literature, press, and general toxicology community for unforeseen human and environmental health effects that may be associated with engineered nanomaterials in food and food-related products. In order to understand these potential concerns we need to consider both general and specific questions. Does the current regulatory framework adapt well to engineered nanomaterials in food as it is designed to do for other new materials manufactured for use in foods? Are there knowledge gaps and/or research needs for regulators that when filled may better prepare us to assess human health and environmental risks of food-related engineered nanomaterials? Can toxicology data generated on nanomaterials via dermal or pulmonary exposure be of use in informing us in the assessment of risks from oral exposure to nanomaterials in food packaging or food additives? Does nanoscapsulation of a dietary or nutritional supplement as a means to alter bioavailability also increase its potential for toxicity? In exploring these types of questions, we will also take the opportunity to identify planned and ongoing research efforts in the area of food-related nanomaterials safety to facilitate discussion.


B. Mahadevan1 and M. Soni, 1Mechanistic and Predictive Toxicology, Schering-Plough Research Institute, Summit, NJ and 2Soni & Associates Inc., Vero Beach, FL.

Bhopal was the site of one of the worst industrial disasters in history. During the night of December 2, 1984, nearly 30 metric tons of methyl isocyanate from the Union Carbide India Limited (UCIL), pesticide factory in Bhopal, India, leaked...
into the surrounding environment. On that fateful night over 2,000 individuals died immediately and more than 200,000 were directly affected. What followed this incident was the devastating impact of the chemical on the eyes, lungs and gastro-intestinal systems. Gynecological and obstetric complications soon became apparent, as did neurological disorders, immunological changes, emotional and mental stress. Twenty-five years later, the impact of the gas leak is evidenced by continuing medical and environmental issues. Besides safety challenges, the sheer scope of the Bhopal incident made it an extremely complex problem of public communication. The post-Bhopal era also witnessed a worldwide regulation on chemicals and toxicity and a demand by communities to the right to information. The story of the Bhopal gas disaster demonstrates the complexity of the interaction of science, public reaction and government in forming the regulatory policy. The historical, scientific and global impact of the disaster will be explored to enable us to develop better public and environmental health and safety policies, to provide the current status of health effects from the disaster, and a review of the lessons learned from the disaster.

PS 329 PEER REVIEW OF TOXICOLOGY, EXPOSURE, AND RISK DATA: ENSURING THE BEST SCIENCE.


The Toxicology and Environmental Health Information Program, National Library of Medicine, Bethesda, MD, 1European Centre of Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium, 2Agency for Toxic Substances and Disease Registry, Atlanta, GA, 3Toxicology for Excellence for Risk Assessment, Cincinnati, OH, 4Technical Resources International, Bethesda, MD, 5Office of Liaison, Policy, and Review, National Toxicology Program, Research Triangle Park, NC, 6Retired, Gulf Breeze, FL, 7NCEA, ORD, U.S. Environmental Protection Agency, Washington, DC and 8Center for Substances and Integrated Risk Assessment, National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands.

Toxicology data bases are increasingly important tools in the regulatory and risk assessment process. While most toxicologists are well-versed in the intricacies of peer review in relation to journal publications and grants, there is considerably less understanding of how this process works even in the evaluation of chemical toxicities and risk values as reflected in certain databases and monographic series. Therefore it is important to present examples of the scientific peer review process within the context of real databases and publications focusing on the toxicity and risk assessment of chemicals. Issues such as panel selection, impartiality and conflicts of interest, funding, transparency in the conduct of meetings, procedure for reaching consensus, opposing views, and public involvement will be discussed for a number of high profile tools widely consulted in the toxicology community. Our panel of experts will refererence many of these databases used including the National Library of Medicine’s Hazardous Substances Data Bank, Toxicology Excellence for Risk Assessment International Toxicity Estimates for Risk, the International Agency for Research in Cancer’s IARC Monographs and the European Centre of Ecotoxicology and Toxicology of Chemicals. Evaluating chemical toxicity when confronted with either a paucity of data or a bewildering array of sometimes conflicting data can be a particular challenge. High quality peer-reviewed databases can play a critical role in supporting the Global Harmonized System (GHS) of Classification and Labeling of Chemicals and be relevant to ethical concerns such as the reduction of animal testing, by offering consolidated and vetted information.

With an increasing insistence that the regulatory framework be supported by the best science, this session will delve into ways of reaching consensus and credible decisions on chemical toxicity and human health. It should appeal to a broad cross-section of toxicologists.

PS 330 NEUROTOXIC EFFECTS OF TIN COMPOUNDS: FROM ION CHANNEL TO BEHAVIOUR.


1Toxicology and Environmental Health Information Program, National Library of Medicine, Bethesda, MD, 2European Centre of Ecotoxicology and Toxicology of Chemicals, Brussels, Belgium, 3Agency for Toxic Substances and Disease Registry, Atlanta, GA, 4Toxicology for Excellence for Risk Assessment, Cincinnati, OH.

Copper (Cu) is an essential trace element vital for brain function; excess or deficiency of Cu can result in neurological malfunction. Cu ions are transported across cellular membranes by ATP7A, a membrane ATPase translocating Cu with concurring ATP hydrolysis. The homeostasis of Cu in the cerebrospinal fluid (CSF) is believed to be regulated by the blood-CSF barrier (BCB) in the choroidal plexus (CP). While it is known that ATP7A regulates Cu transport in the gastrointestinal tract, the mechanisms underlying Cu transport in the BCB and the impact of Fe on these processes were largely unknown. This study was designed to test the hypothesis that (1) ATP7A plays an important role in transporting Cu ions through the BCB and (2) Fe status may alter the expression and/or localization of ATP7A in this tissue. The 64Cu/Fe efflux study was conducted in an immortalized choroidal epithelial Z310 cell line. Incubation of the cells with Cu and Fe at a concentration of 10 μM each showed a marked influx of Cu into the cells (0.75, 6, 15 μmol/l) or TMT (0.75, 3, 6 μmol/l) produced a significant impairment of retention in the PAR. The results show that the organotin compounds, especially DMT and TMT, disturb the function of glutamatergic receptors and, consequently, affect the hippocampal Schaffer collateral-CA1-synapse in a receptor specific manner. Although the organotin compounds clearly affect excitatory synaptic processes, the learning behaviour was not affected in the tested animals in the concentrations used.

PS 331 NEUROLOGICAL CA CHANNEL SUBTYPES DIFFERENTIALLY MODIFY SENSITIVITY TO METHYLMERCURY-INDUCED CELL DEATH.


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Voltage gated Ca channels (VGCC) are a sensitive target of methymercury (MeHg). MeHg blocks current carried through VGCC, but VGCC also contribute to the MeHg-induced elevation of intracellular [Ca2+], suggesting that MeHg facilitates the GPCR system. This has been difficult to test objectively. The objective of this study was to determine if expression of a single phenotype of VGCC in a non-neuronal cell would increase its susceptibility to cytotoxicity induced by MeHg, and to what extent is the subtype of VGCC important? HEK293 cells were transiently transfected with cDNAs for human P-Qα (α1A), N- (α1B), and L-type (α1C) VCC subunits. Following expression of the recombinant protein, cells were exposed in culture to MeHg (1.2 or 5 μM) for 1 or 3 hrs. Cytotoxicity was determined 24 hrs later using a commercial “live/dead” assay. MeHg caused a concentration-dependent increase in cytotoxicity following expression of any of the three VGCC α1 subunits. Further, cytotoxicity following 3-hrs exposure was greater than that following 1-hr. However, the three VGCC subtypes differentially affected the relative sensitivity of the cells to MeHg. Cells containing α1A were most sensitive and exhibited 15 - 30% cell death after 1, or 3 hrs exposure to 5 μM MeHg, respectively. In contrast, α1C-containing cells were relatively insensitive, showing between 5-13% cytotoxicity after 1 and 3 hrs exposure respectively: α1B-containing cells were intermediate in their sensitivity. The ability of a VGCC antagonist to protect cells against MeHg-induced cytotoxicity was tested using 1 μM nimodipine. MeHg induced cytotoxicity was significantly reduced after 3-hrs exposure to 1 μM MeHg. Nimodipine did not reduce cytotoxicity following only 1-hr exposure to MeHg. In conclusion, VGCC clearly contribute to the cytotoxicity produced by MeHg even in a non-neuronal cell, however there is differential sensitivity depending upon the VGCC subtype. Furthermore L-type VGCC are less sensitive in this regard. Supported by NIEHS grant R01ES03299 and an ASPEL SURF Fellowship to B.P.G.
mM Fe in an artificial CSF. Interestingly, the ATTPA proteins in the control tissue were primarily located toward the apical membrane facing the CSF; Fe treatment caused the relocation of ATTPA from the apical membrane to the cytoplasm. Taken together, these data suggest that ATTPA in the BCB plays an important role in Cu transport through the blood and CSF and that Fe status significantly alters the function of ATTPA and thereby affects Cu transport at the BCB. (Supported by NIH/NIEHS ES08186).

These data are consistent with other work suggesting preferential striatal Mn accumulation, with some distribution throughout the entire brain. More importantly, our data suggest that low-level chronic Mn exposure may lead to increased cellular metabolism, perhaps through mitochondrial uncoupling.

333 DIVERGENT CATION-MEDIATED CELL DEATH INDUCED BY METHYL MERCURY IN A MOTOR NEURON CELL LINE (NSC34).

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In Amyotrophic Lateral Sclerosis-ALS, and especially its prevalent form, Sporadic-ALS (SALS), interaction between genetic factors and environmental exposures has strongly been implicated, but never tested directly. Metals including lead and mercury such as methylmercury (MeHg) have been regularly suggested to contribute to ALS, but again, this has not been tested directly. Glutamate-mediated excitotoxicity contributes to motor neuron degeneration in ALS. This may be due to impaired astrogliotic glutamate uptake, or enhanced Ca2+ and Zn2+ influx through Ca2+-permeable kainate/AMPA receptors. We tested the sensitivity of a motor neuron cell line (NSC34) to increases in divalent cations (Me2+ and Ca2+) mediated cytotoxicity induced by acute exposure to MeHg. Changes in intracellular [Me2+ ] were monitored using Ca-sensitive fluorescent fura-2, and were compared to those occurring in a well-described neuronal cell model, the NGF-differentiated PC12 cell. Bath exposure to 5 μM MeHg caused two patterns of response in NSC34 cells: both a single phase, and a biphasic increase in fura-2 fluorescence. In comparison to a non-motor neuron (PC12 cells), the response to MeHg in NSC34 cells was more variable, and occurred at higher concentrations. TPEN (20 μM), a divalent cation chelator, decreased the MeHg-induced response suggesting that a Me2+ such as Zn may also increase. Cytotoxicity was determined in NSC34 cells 24 hrs after exposure to MeHg for 1 h using a live/dead assay. MeHg (0.1-10 μM) caused a concentration-dependent increase in cytotoxicity. The Ca2+- chelator BAPTA significantly reduced the % of cells which died in response to MeHg at 5 and 10 μM. Thus in a motor neuron cell line, acute exposure to MeHg causes an increase in both [Ca2+]i and an endogenous Me2+. Chelation of the [Ca2+]i reduced the incidence of MeHg-induced cytotoxicity in this cell line. Supported by NIEHS grant R01-ES03299, ST55RO17491-07, R25GM59549-10 to the UPR-Cayey and the MSU McNair-SROP Program.

334 BRAIN GLUCOSE UTILIZATION FOLLOWING CHRONIC MANGANESE EXPOSURE IN MALE SPRAUGE-DAWLEY RATS.

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Manganese (Mn) is toxic at high concentrations, and prolonged exposure may result in manganism, a neurological disorder similar to Parkinson’s disease. Data suggest Mn accumulates in mitochondria, which may result in toxicity. Unfortunately, few studies have characterized neuronal changes resulting from low-level, chronic exposure. Using non-invasive μPET (micro positron emission tomography), we quantified brain glucose uptake, an indirect measure of cellular respiration, in male Sprague-Dawley rats. Rats were injected (iv) with isotonic saline (CN) or MnCl2 (3 mg/kg/week; TX), and routinely imaged using fluorodeoxyglucose (FDG), a radioactive μPET tracer. Each rat also underwent μCT (micro computer tomography) to aid in identification of brain nuclei in μPET images. Raw data was reconstructed using ordered subsets expectation maximization 2D (OSEM2D), and dose-corrected for each scan. μPET and μCT images were registered the Ruben Rat Brain atlas for analysis of various regions of interest (ROI; striatum, cortex, thalamus, cerebellum, hippocampus and whole brain). ROI were evaluated for standard uptake value (SUV) of FDG.

By week 2, treated rats (n = 7) had decreased body mass (p < 0.001) compared to controls (n = 6). Both striatum (p = 0.0074) and hippocampus (p = 0.0072) were also smaller (by mass) in TX compared to CN. Whole brains from TX animals scanned during weeks 2-7 demonstrated a small trend towards increased FDG uptake in (11%; p = 0.58), which increased to 17% (p = 0.40) by weeks 8-11. ROI values normalized to whole brain values, to identify areas responsible for this trend, showed increased striatal FDG uptake in weeks 6-7 (4.6%; p = 0.14) that was statistically significant by weeks 8-11 (15%; p = 0.00015).

335 EFFECT OF PERINATAL AND CONTINUOUS LEAD EXPOSURE ON HISTONE MODIFICATIONS IN THE HIPPOCAMPUS OF YOUNG ADULT RATS.


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Lead (Pb2+) is a ubiquitous neurotoxicant that affects the intellectual capacity of children. Recent evidence suggests that epigenetics play an important role in cognitive function. Modifications in the N-terminal tail of histones change the structure of chromatin and regulate gene transcription. In this study, we investigated whether different Pb2+ exposure paradigms altered histone modification in the hippocampus of young adult rats. Adult female rats were fed a 0 or 1500 ppm lead acetate diet prior to and during gestation and lactation and pups were removed from the Pb2+ exposure at postnatal day 21 (PN21) or PN50. Rats were euthanized at PN50, the hippocampus dissected and histones extracted to assess histone modification. We measured acetylation of H3 (lys9 and lys14), acetylation of H4 (lys5), and monomethylation of H3 (lys4) by western blot. Phosphorylation of H3 (ser10) was not detectable by western blot, therefore, it was measured by immunohistochemistry. Blood Pb2+ levels at PN21 were 29.4 ± 3.9 μg/dl (n=12). For animals that were removed from the exposure at weaning, blood Pb2+ levels at PN50 were 1.2 ± 0.1 μg/dl (n=12) while animals with continuous exposure had 13.7 ± 0.6 μg/dl at PN50 (n=13). Control rats had blood Pb2+ levels of 0.6 ± 0.1 μg/dl (n=40). Western blots of hippocampal histone extracts showed no significant effect of Pb2+ or any of the histone modifications tested. However, counting of cells that stained for phosphorylation of histone H3 (ser10) in the dentate gyrus of PN50 rats exposed to Pb2+ perinataly was different (p=0.056) from controls. No significant effect was measured with rats continuously exposed to Pb2+. These preliminary findings suggest a selective effect of perinatal Pb2+ exposure on H3 Ser10 phosphorylation in the dentate gyrus. This histone modification is associated with initiation of transcription of the early gene c-fos and depends on NMDA receptor signaling (Chandramohan et al., J. Neurochem. 101: 845, 2007). (Supported by ES06189 to TRG).

336 MANGANESE STABILIZES CELLULAR PRION PROTEINS AND ALTER THE RATE OF PROTEOLYSIS-DEPENDENT LIMITED PROTEOLYSIS.


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Prion diseases are fatal neurodegenerative diseases in both animals and humans. The key event in the pathogenesis of prion diseases is the conversion of normal cellular prion proteins (PrPc) to the proteinase K (PK) resistant, abnormal form (PrPSc); however, the cellular mechanisms underlying the conversion remain enigmatic. Binding of divalent cations such as copper to the octapeptide repeat regions of PrP has been shown to be important for the stability of the protein. Nevertheless, the roles of other divalent cations in the normal processing of cellular PrP are not well understood. In the present study, we examined the effect of manganese (Mn) on PrP expression and degradation in neuronal cells expressing mouse prion proteins with a genetically altered novel epitope (mAb ST4). Exposure of Mn (100μM) over the course of 24 hr increased PrP levels in both cytosolic and membrane-rich fractions in a time-dependent manner. Interestingly, Mn treatment neither increased PrP mRNA transcripts as measured by qRT-PCR nor altered the mRNA stability as determined in actinomycin D treated cells, indicating that the effect of Mn may be at the level of post-translation and/or degradation levels of PrP. In order to determine whether the accumulation of PrP is due to impairment of the proteasomal degradation pathway by Mn, protein (PR) degradation was measured. The results showed no significant alteration of the proteasomal degradation pathway. Notably, pulse-chase analysis showed that the PR turnover rate is significantly decreased with manganese treatment. Furthermore, limited digestion with PK also revealed that manganese treatment decreased the digestion rate of the PrP. These data together clearly suggest that the divalent metal manganese can alter the normal processing of PrP, resulting in the accumulation of PrP with altered susceptibility to PK (supported by DoDH/MHRP grant 4871XWH/05-10239 and NIH grant ES10586).
PS 337 ACUTE EXPOSURE TO LEAD (PB) RESULTS IN AN INCREASED ACCUMULATION OF βAMYLOID IN THE CHOROID PLEXUS.

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β-Amyloid (Aβ) proteins are known to participate in the etiology of Alzheimer’s disease (AD) and are cleared by the choroid plexus (CP), a barrier between the blood and cerebrospinal fluid that plays a critical role in transport of materials between these two body fluids. Cumulative evidence from human and animal studies shows that Pb accumulates in the CP in an age-dependent fashion. The aim of this study was to investigate whether and how Pb exposure disrupted Aβ transport in the CP. Rats received a single ip injection of 50 mg/kg Pb acetate or Na acetate and 24 h later, were anesthetized and infused with FAM labeled Aβ40 (200 pmol) for 15 mins in the lateral ventricle. The CP was then examined by confocal microscopy for five Aβ uptake. Data showed a visible increase in intracellular Aβ in the Pb-exposed group compared to controls. In vitro studies were conducted by pre-incubating choroidal epithelial Z310 cells with 10 μM Pb for 24 h, followed by 1-h incubation with Aβ2 (2 μM). ELISA analysis demonstrated a 25% increase (p<0.05) in intracellular Aβ in the Pb treated cells. To explore a mechanism for this finding, we assessed whether the expression and function of LRP1, an Aβ efflux transporter, was altered by Pb exposure. Conofal microscopy revealed that LRPI was re-distributed from the cytosol in control animals towards the apical surface of the CP in Pb-exposed ones (50 mg/kg ip, 24 h). Quantitative Real time RT-PCR and Western blot analyses revealed a significant decrease in LRPI mRNA and protein levels (p<0.05) following Pb exposure in rats. When Z310 cells were exposed to Pb (10 μM, 24 h), a similar reduction of LRPI mRNA and proteins (p<0.05) was also observed. These results suggest that Pb accumulation in the CP leads to increased levels of intracellular Aβ; this effect seems to be associated, at least in part, with the altered clearance of Aβ via its efflux transporter LRPI. Our finding supports the possibility that Pb exposure eventually exacerbates AD by potentially interfering with Aβ clearance at the CP. (Support in part by ES08146 and ES013118)

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Copper (Ca) is essential to a variety of normal body metabolic processes; yet elevated Cu levels can cause systemic or neurological diseases. Our previous study indicates that exposure to lead (Pb) results in a greater accumulation of Pb in the choroid plexus (CP), a tissue where the blood-CSF barrier (BCSB) is located. The current study was designed to test the hypothesis that acute Pb exposure disrupted Cu transport at the CP resulting in an increased Cu levels in the CSF. Rats received an injection of 50 mg Pb/kg daily for 5 days. At the end of exposure, rats were subjected to an in situ ventriculo-cisternal perfusion of [64Cu] in artificial CSF along with a space marker [14C]sucrose. At steady state, [64Cu] in the CSF outflow in Pb-treated animals (58.2±4%) was significantly increased by 13% as compared to controls (51.6±2%) (p<0.05). Pb treatment, however, did not affect the outflow of [14C]sucrose, suggesting a decreased Cu clearance by the CP in Pb-exposed rats. Choroidal epithelial Z310 cells were used to further verify this effect. Cells were incubated with a combination of 2 μM Cu and 0.5 μCi/mL [64Cu] for 1 h followed by treatment with 10 μM Pb for 24h. A 65% increase in intracellular Cu levels was observed in Pb-treated cells. To study the mechanism, Z310 cells were grown on Transwell membranes simulating an in vitro BCB model. [64Cu] along with [14C]sucrose was added to the donor chamber and the radioactivity was determined in the acceptor chamber. Pb treatment did not affect the Cu transport from the basolateral (blood) side to apical (CSF) side. Interestingly, a decreased transport of Cu from the apical to the basolateral side with an elevated intracellular Cu accumulation was observed in Pb-exposed cells (p<0.05). The same treatment did not affect [14C]sucrose transport, suggesting that Pb treatment did not cause non-specific leakage across the barrier. Taken together, it appears likely that Pb exposure decreases Cu clearance from the CSF by altering Cu transport across the BCB. (Support by ES08146)

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SOT 2009 ANNUAL MEETING

PS 339 NEUROTOXIC EFFECTS OF ARSENIC COMPOUNDS: FROM ION CHANNELS TO BEHAVIOUR.


Lateral cilia of gill of Crassostrea virginica are controlled by a dopaminergic-seotoninergic innervation. Dopamine is an excitatory neurotransmitter within ganglia, but an inhibitory transmitter at gill, causing cilia-inhibition. High levels of manganese (Mn) are neurotoxic to people, causing Parkinsonism-like disease. Clinical interventions for Parkinsonism have not been very successful. Recently, p-
Manganese (Mn) exposure at high doses produces cognitive, psychiatric and motor dysfunctions with symptoms reminiscent of Parkinson’s disease. Several basal ganglia nuclei, including the substantia nigra pars reticulata (SNr), have been implicated in the pathogenesis of manganism. Increasing evidence suggests that microglial activation is involved in the development of neurodegenerative disorders. Thus, we investigated whether Mn-induced neurotoxicity is associated with microglial changes in the SNr and pars compacta (SNc). We examined microglia in the midbrain of Cynomolgus macaques exposed to different weekly doses of Mn (3.3–5.0, 5.0–6.7, 8.3–10 mg Mn/kg; ip). In controls, we observed sparse LN3-positive cells with fine ramified processes characteristic of resting microglia. On the other hand, in Mn-treated animals, microglial cells were increased in number and frequently fused into rod-like cell aggregates. Reactive microglial cells exhibited dystrophic changes including beading, bulbous spheroids and hypertrophic cytoplasm. Microglia activation was more prominent in the SNr than in the SNc. We also observed microglia engulfing pyknotic bodies potentially representing either phagocytized cellular remnants and/or apoptotic cells. The SNr of animals that received the highest weekly doses of Mn, we observed that dystrophic cellular compartments of activated microglial cells were usually co-labeled with increased iNOS expression and ferric iron. Therefore, it appears that in the SNr of Mn-exposed animals, microglia become activated, increase iNOS levels and produce iron potentially leading to iron release from ferritin. These events can produce neurotoxic effects as well result in microglial injury reducing their capacity to support normal neuronal functions. Similar microglial dystrophic changes have been described in the aging brain and in human neurodegenerative diseases. [Supported by ES010975 to TRG]

**345 VANADIUM CAN SYNERGISTICALLY INCREASE MANGANESE-INDUCED NEUROTOXICITY IN DOPAMINERGIC NEURONAL CELLS VIA A CAPSASE MEDIATED PATHWAY.**

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Chronic exposure to manganese (Mn) is known to cause Parkinson’s disease (PD) like symptoms. Consequently, there is a growing concern that chronic exposure to welding fumes may be associated with increased incidences of PD. Welding fumes contain many different metals, including Mn and vanadium which is typically present as vanadium pentoxide (V2O5). Unfortunately, the possible neurotoxic effects of V2O5 are not well characterized. In this study, we examined the effect of V2O5 on dopaminergic cell culture model (N27 cells) and characterized the oxidative signaling events. Our results revealed that exposure to 3-300μM V2O5 for up to 12 hr resulted in a time- and dose-dependent increase in cell death with the EC50 value determined to be 37μM. Exposure to 40μM V2O5 resulted in a significant increase in reactive oxygen species generation, followed by cytochrome c release from the mitochondria, and activation of mitochondrial-dependent proapoptotic events such as sequential activation of initiator caspase-9, and executioner caspase-3. We also found that V2O5 exposure induced proteolytic cleavage of native protein kinase Cβ (PKCβ) to yield catalytically active and regulatory fragments with increased PKCβ kinase activity. Pretreatment with caspase inhibitors blocked V2O5-induced proteolytic cleavage of PKCβ, demonstrating the role of caspase-3 in kinase proteolysis. Pharmacological attenuation of caspase-3 and PKCβ activity prior to V2O5 treatment significantly suppressed increases in DNA fragmentation. The use of siRNA-mediated knockdown of PKCβ effectively blocked V2O5-induced apoptosis, further confirming the role of PKCβ in the neurotoxicity of V2O5. We also found that non-toxic doses of Mn potentiated V2O5-induced neurotoxicity. Together, our results demonstrate V2O5 alone and synergistically with Mn exert...
neurotoxic effects in a dopaminergic cell model of PD via caspase-3 dependent pro-
teolytic activation of PKCδ, suggesting a possible role of vanadium neurotoxicity in 
environmentally linked PD (supported by NIH grant ES01586).

346 METHYLMERCURY-INDUCED DEATH IN C. ELEGANS 
AND ITS ISOLATED EMBRYONIC NEURONS.

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The nematode Caenorhabditis elegans (C. elegans) is a valuable model organism that 
has been used extensively in neurobiology to study neuronal development, and cell 
dearth. C. elegans may provide new insights in studying early developmental and 
chronic effects, or cell-specific effects of neurotoxicants. Effects of methylmercury 
(MeHg) on lethality and growth in C. elegans were examined. MeHg (5-300 μM) 
was applied to the liquid culture medium, and exposure was continuous for 6-48 
hrs. Cultures were synchronized so that toxicity could be examined in the larval 
(1.1-2.2) stages. In some experiments, aged worms (L4) were also examined. There 
was both a concentration- and time-dependent increase in whole organism toxicity 
with MeHg. After 6 hrs, an approximate LC50 in the L1-L2 worms was 220 μM; 
this was reduced to ~ 100 μM after 12, 24, and 48 hrs (approximately 1 generation 
time) exposures. Significant differences in body length, an effective measure of 
growth, were seen after 6-hr of exposure to MeHg > 100 μM, and at 48 hrs with 
exposure to MeHg > 5 μM. No difference was seen in lethality between L1-2 and 
L3-4 populations. Viability on whole worm populations, even when age-synchronized, 
depends on factors other than direct neurotoxicity. Thus, we then assayed viability 
using acutely dissociated embryonic neurons in culture. The C. elegans Nw1229 
line constitutively expresses GFP tagged to the DPY20 gene in all neurons. After 2-4 
days, when the GFP expression is robust, cells received 1hr MeHg treatment 
in the culture medium. Following replating, GFP-tagged cells were assayed for viability 
using ethidium homodimer. Sensitivity to MeHg-induced cell death was markedly 
increased in the GFP-labeled neurons, with a significant increase in mortality 
already seen at 1.2 μM. Thus isolation of pan-GFP-expressing neuronal cells permits 
examination of MeHg-induced neurotoxicity in C. elegans at concentrations 
approximately equal to those of mammalian neurons. Supported by NIH grant 
R01ES03299, a grant from MSU OVPGRS, the RISE Program at UPR-C and 
MSU McNair-SROP.

347 ESTROMEN ANTAGONIZES THE PRO-
INFLAMMATORY EFFECTS OF MANGANESE ON 
EXPRESSION OF NITRIC OXIDE SYNTHASE 2 (NOS2) 
in ASTROCYTES.

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Degenerative movement disorders affecting the basal ganglia, including Parkinson’s 
and Huntington’s diseases, are debilitating diseases that affect more men than 
women. It is well documented that animal models show the same sex-dependent 
neurodegeneration and most evidence indicates that estradiol (E2) is the key neu-
roprotectant influencing gender susceptibility. However, the mechanism by 
which E2 is protective is poorly understood. Manganism, a degenerative movement 
disorder caused by excessive accumulation of manganese (Mn) in basal ganglia, 
presents with clinical features similar to Parkinson’s disease, but the relative gender 
susceptibility to Mn is not well understood. Additionally, epidemiological evidence 
suggests that children exposed to moderately high levels of Mn in drinking water 
suffer from cognitive deficits (Woolf, Wright et al. 2002) but the gender-specific 
risk is similarly unknown. Because astrogliaisis and expression of inducible nitric 
oxide synthase (NOS2) is a known neuropathological feature of Mn neurotoxicity, 
we postulated that E2 might antagonize Mn-dependent induction of NOS2 in as-
astrocytes at the level of gene transcription. Astrocytes exposed to 50 μM MnCl2 and 
the inflammatory cytokines TNFα (10 pg/ml) + INFγ (1 ng/ml) had a significant 
increase of NOS2 mRNA and protein expression compared to control. Upon 24hr 
pre-incubation with 10 μM 17-β-estradiol, Mn- and cytokine-induced NOS2 mRNA 
and protein expression was significantly decreased. Additionally, live-cell 
fluorescence imaging indicated that E2 effectively prevented activation of NF-κB in 
Mn- and cytokine-treated transgenic astrocytes expressing an NF-κB-GFP reporter 
construct. Previous studies from our laboratory indicated that NF-κB is a critical 
transcription factor mediating expression of NOS2 in response to Mn in astrocytes. 
The capacity of E2 to block inflammatory activation of astrocytes and to decrease 
expression of NOS2 suggests a potential mechanism for neuroprotection that may 
also influence gender susceptibility.

348 DIFFERENTIAL GENDER AND STRAIN 
SUSCEPTIBILITY TO THE MOTOR EFFECTS 
INDUCED BY CHRONIC LOW-LEVELS OF ARSENIC 
EXPOSURE IN C57BL/6J AND CD-1 MICE.

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Although other authors have discussed the differential susceptibility to tumor for-
mation among different mouse strains after As exposure, there is no information re-
lated to differential behavioral and gender susceptibility. Thus, it is important to 
study the differential effects of As exposure according to gender and mouse strain in 
order to define a better animal model to evaluate the neurotoxic effects of chronic 
low-level arsenic exposure. In the present study, male and female C57BL/6J and 
CD-1 mice were exposed to environmentally relevant doses of 0.05, 0.5, 5.0 or 50 
mg As/l of drinking water during four months. Spontaneous locomotor activity was 
assessed every month. C57BL/6J male mice exposed to 0.5 mg As presented hyper-
activity, while the group exposed to 50.0 mg As showed hypactivity after four 
months of exposure. In contrast, C57BL/6J female mice exposed to 0.05, 0.5 and 
5.0 mg As presented hyperactivity after one, two, three and four months of As 
exposure. CD-1 male mice exposed to 5 and 50 mg As presented hyperactivity after 
two, three, and four months of As exposure; while the group exposed to 0.05 mg 
As showed hyperactivity after four months of exposure. No differences in sponta-
neous locomotor activity were observed in female CD-1 mice at any time point. 
These results show important differences in spontaneous locomotor activity associ-
ated to gender and mouse strains. Female C57BL/6J mice are more susceptible than 
male mice; while male CD-1 mice are more susceptible to chronic low-level expo-
sure to As than female mice. This work was supported by grants CONACYT 
60662, PAPIIT 214608-19, CONACYT 46-161-M, U. P. Bardallas (228586) and 
J.H. Limón-Pacheco (435533) received a fellowship from CONACYT.

349 MAGNETIC RESONANCE IMAGING (MRI) AND 
1H-MAGNETIC RESONANCE SPECTROSCOPY (MRS) IN 
THE MANGANESE-EXPOSED NON-HUMAN 
PRIMATE BRAIN.

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This study is part of a multidisciplinary effort investigating the behavioral, in vivo 
neuroimaging and neurotoxicological effects of chronic Mn exposure in non-
human primates. It comprises one of the largest cohorts studied using MRI and 
MRS. Male research naïve Cynomolgus macaques received baseline MRI and MRS 
studies prior to Mn administration and at two different time points during the 
administration of weekly doses of Mn. For the MRI studies, a Pallidal Index 
Equivalent [PIE; (signal from a brain region/frONTAL white matter signal)] was meas-
ured in several brain regions including the cerebellar white matter, globus pallidus, 
caudate, putamen, thalamus and substantia nigra. The putidary gland and muscle 
surrounding the skull were also measured. A comparison of the PIE indicated in-
creases in the globus pallidius, substantia nigra and putamen as well as the 
putidary gland relative to baseline indicative of increased Mn accumulation. Unexpectedly, 
a significant decrease in PIE was measured in the thalamus as a result of Mn expo-
sure. However, if a PIE is calculated using muscle, rather than frontal white matter as 
the denominator, all brain regions demonstrate increased PIE during Mn expo-
sure relative to baseline. This observation confirms our previous findings (Guillarte 
et al., Toxic Sci 94: 351, 2006) that the commonly used Pallidal Index, where the 
frontal white matter signal is used as the reference region (denominator), is not an 
accurate measure of regional brain Mn accumulation. Preliminary analysis of the 
MRS data in post-frontal white matter suggests a transient increase in the 
choline/creatine ratio (Cho/Cr). Elevated Cho/Cr ratio is indicative of inflamma-
tion or demyelination. The latter suggests white matter involvement in Mn neuro-
toxicity. [Supported by ES010975 to TRG]

350 RESPONSE OF NEURAL STEM CELLS TO 
TRIMETHYLTIN INJURY IN THE MURINE 
HIPPOCAMPUS.

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Stem cells in the developing mammalian brain migrate from the ventricular zone to 
form secondary sites of neurogenesis that persist in the adult: the subventricular 
zone (SVZ), and the hippocampal subgranular zone (SGZ). SVZ cells occupy the
and E. J. Catapane

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The molecular basis of MeHg toxicity. We will also describe our current work in coexposure with selenium. Gene expression studies also indicate a significant increase in protein levels following MeHg exposure, and this upregulation can be suppressed by the expression of specific heat shock proteins and glutathione transferases following exposure to MeHg. Whole genome microarray and real-time PCR results show a very strong induction of MeHg show a significantly decrease in brood size. Exposure of younger larvae to MeHg-induced death, and this effect is dependent on a live bacteria food source. Methylmercury (MeHg) exposure from occupational, environmental and food sources is a significant threat to public health. The toxin easily passes the blood brain barrier and can cause severe neurological problems.

We show that MeHg exposure confers animal death at low concentrations, and that the sensitivity is partially dependent on group size. Consistent with vertebrate studies, growth in the presence of selenium dramatically rescues the MeHg-induced death, and this effect is dependent on a live bacteria food source. Developmental defects are also observed as older larvae exposed to non-lethal doses of MeHg show a significantly decrease in brood size. Exposure of younger larvae to similar concentrations of MeHg results in over a 2-fold delay in development. Whole genome microarray and real-time PCR show a very strong induction of specific heat shock proteins and glutatione transferases following exposure to MeHg. In particular, gsp-38 is significantly upregulated at both the mRNA and protein levels following MeHg exposure, and this upregulation can be suppressed by coexposure with selenium. Gene expression studies also indicate a significant induction of genes involved in electron transport, cell growth and protein folding. Overall these studies suggest that C. elegans is a powerful model system to explore the molecular basis of MeHg toxicity. We will also describe our current work in mammalian neuron cultures that builds on our in vivo studies, as well as our preliminary results from a novel genome-wide screen to identify mediators and suppressors of the MeHg neurotoxicity.

Assessment of Tellurium Toxicity in Rat Hippocampal Astrocytes.

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Tellurium (Te), a trace element, is present in the earth’s crust, seawater, soil and also in body fluids such as blood and urine. Plants can also accumulate Te from the soil. This element is widely used in the electronics industry, photography, in the vulcanization of rubber and in pesticides. Occupational and environmental exposure is common, and yet little is reported about cytotoxic effects of tellurium compounds. Several studies have been reported that both organic and inorganic tellurium compounds exhibit toxicity in rats by causing peripheral neuropathy resulting from demyelination. This effect is attributed to inhibition of squalene epoxidase, an enzyme necessary for cholesterol biosynthesis. Teratogenic and hematologic disorders have also been reported. Limited studies have addressed its potential adverse effects to the central nervous system. The purpose of this study was to evaluate toxic effects of diphenyl ditelluride and tellurium tetrachloride in rat hippocampal astrocytes. Cells were grown to 90% confluence and treated with 24 hrs. with the following concentrations of each compound: 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.90, 1.95, 0.98, 0.49, 0.24 μM. Cell viability was measured by MTT assay. Significant decrease in cell viability were seen at all concentrations tested for both compounds. Viability studies were confirmed with light and scanning electron microscopy. Cellular distortion involving loss of processes and plasmalemmal alterations including cytoplasmic blebbing were observed in concentrations of 125, 62.5, 31.25, 15.625 μM. In order to investigate whether cell injury was accompanied by lipid peroxidation, a thiobarbituric acid reactive substance (TBARS) assay was performed using concentrations of 125, 62.5, 31.25, 15.625 μM for both compounds. Lipid peroxidation was not observed in astrocytes treated with either of the compounds and cellular injury does not appear to involve peroxidative processes.

Methylcyclopentadienyl Manganese Tricarbonyl (MMT) was used as an antinoxidant agent and as an octane booster in Canadian unleaded gasoline. Its combustion leads to Mn emissions. Considering that MMT is no longer used in the Canadian gasoline, the objective of this research was to examine the distribution of inorganic Mn in Canada from 2001 to 2007, covering the period prior to and following MMT use. Three sampling stations were selected because their proximity to roads with widely differing and well-known traffic. Results from 2001 to 2007 were obtained. The first sample of each month was selected and Mn analysis was performed by neutron activation analysis. TSP (total suspended particulates) was calculated by weighing the filters before and after dust collection. Results show a significant decrease of Mn over time at each station, while TSP decreased significantly in two stations, with one showing a trend. Comparing atmospheric Mn during and after the period of use of MMT 2001 – 2003 vs 2005 – 2007 showed a significant decrease at all stations. TSP, only one station showed borderline significant difference between these two periods. The difference between the two periods shows 41% and 17% of decrease for Mn and TSP respectively. These data suggest that the combustion of MMT led to an increase of airborne Mn of approximately 24%. These results should help in decision-making processes leading to the acceptance or rejection of the use of MMT in gasoline in other countries.

**Methylenecycloptadependyl Manganese (MCPM) was used as an antinoxidant agent and as an octane booster in Canadian unleaded gasoline. Its combustion leads to Mn emissions. Considering that MMT is no longer used in the Canadian gasoline, the objective of this research was to examine the distribution of inorganic Mn in Canada from 2001 to 2007, covering the period prior to and following MMT use. Three sampling stations were selected because their proximity to roads with widely differing and well-known traffic. Results from 2001 to 2007 were obtained. The first sample of each month was selected and Mn analysis was performed by neutron activation analysis. TSP (total suspended particulates) was calculated by weighing the filters before and after dust collection. Results show a significant decrease of Mn over time at each station, while TSP decreased significantly in two stations, with one showing a trend. Comparing atmospheric Mn during and after the period of use of MMT 2001 – 2003 vs 2005 – 2007 showed a significant decrease at all stations. TSP, only one station showed borderline significant difference between these two periods. The difference between the two periods shows 41% and 17% of decrease for Mn and TSP respectively. These data suggest that the combustion of MMT led to an increase of airborne Mn of approximately 24%. These results should help in decision-making processes leading to the acceptance or rejection of the use of MMT in gasoline in other countries.**
in reducing Cd accumulations, especially at low concentrations. The study shows the chelating agents are effective in reducing Mn and Cd accumulations and supports the hypothesis the mechanism of action of PAS in treatment of Manganism is related to its chelating abilities.

**NEUROTOXIC EFFECTS OF ARSENITE IN ASTROGLIAL AND NEURONAL MODELS: MECHANISMS INVOLVED IN DIFFERENTIAL SUSCEPTIBILITY.**

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Chronic exposure to inorganic arsenic produces toxic damage in CNS, disrupting neurological functions. Glutamate (Glu), being the main excitatory neurotransmitter is critically involved in these events. Glu levels are tightly regulated through Na+-dependent Glu transporters in order to avoid its well-characterized excitotoxic effects. In order to gain insight into the molecular mechanisms of arsenite toxicity, we analyzed the effects of arsenite exposure in Glu transport. To this end, arsenite was applied in epidemiologically relevant doses (≤5 μM) to confluent neuronal and glial cultured cells, and Glu transport was evaluated through a [3H]-D-aspartate uptake assay. The human neuroblastaoma SH-SY5Y and the rat glioma C6 cell lines as well as a Bergmann glia primary culture were used. Arsenite exposure reduced the uptake activity in glial cultures whereas in the neuronal cell line an increase in uptake was found. It has been reported that oxidative stress can severely affect Glu transporters; therefore we evaluated lipid peroxidation in glial cells; however no correlation was found since arsenite did not induce lipidperoxidation in these cells, although we did observe an increase in glutation levels. Proliferation and activation of p53 were also evaluated under arsenite exposure. An increase in p53 protein levels and its DNA binding activity was found both in neuronal and glial cells in a dose-dependent manner. Interestingly, arsenite exposure significantly decreased the proliferation rates in C6 and SH-SY5Y cells. These results strongly suggest that Glu accumulation and p53-dependent transcriptional regulation participate in the molecular mechanisms of arsenite neurotoxicity. Supported by CONACyT 50414.

**RELATIONSHIP BETWEEN BLOOD MANGANES- IRON RATIO AND EARLY ONSET NEUROBEHAVIORAL ALTERATIONS.**

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A biomarker for early onset neurobehavorial alterations in manganism remains unknown. The purpose of this study was to use a neurobehavioral test battery to identify subtle changes in Mn-induced motor and memory dysfunction and to relate the quantitative neurological dysfunction to an established Mn exposure index such as blood Mn:Fe ratio (MIR). A total of 323 subjects were recruited to control (n=106), low (122), and high (95) exposure groups. The test battery consisted of standard procedures including the nine-hole and groove-type steadiness tester, Benton test, and Purdue pegboard. No health problems or clinically diagnosed neurological dysfunctions were observed except for increased blood pressure in Mn-exposed smelters. Benton test did not reveal any abnormal memory deficits among Mn-exposed smelters, nor did the groove and nine-hole tests detect any abnormality in dynamic and static steadiness in tested subjects. Purdue pegboard test showed a remarkable age-related decline in fine movement coordination among all study participants regardless of the Mn exposure condition. Mn exposure significantly exacerbated this age-related decline. Statistical modeling revealed that the plasma and erythrocyte Mn:Fe ratio (pMIR and eMIR, respectively) were associated with Purdue pegboard scores. Among all age groups, pMIR were above the cut-off value (COV), pMIR was significantly correlated with pegboard scores (r=0.261, p=0.002), whereas for those whose age were above 40 years the eMIR, but not pMIR, was associated with the declined pegboard performance (r=0.219, p=0.069). When both factors were taken into account (age >40 and MIR > COV), only pMIR was inversely associated with pegboard scores. Combining their usefulness in exposure assessment, we recommend that the blood Mn:Fe ratio may serve as a reasonable biomarker not only for judgment of Mn exposure but also for health risk assessment.

**DECREASED DMT1, TF AND HEPcidIN GENe EXPRESSIONS IN LEUCOCYTE OF MANGANESE EXPOSED WORKERS.**

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Manganese (Mn) is widely used in several industries including the manufacture of steel. Increased uptake of Mn to the brain, mainly resulting in progressive neurological disorders, similar to Parkinson’s disease. Previous literature suggests that there are a set of common genes involved in the absorption, transportation and metabolism of Mn and iron (Fe) and Mn exposure alters Fe metabolism. This study was designed to test the hypothesis that workers exposed to Mn had an altered expression of genes involved in Fe metabolism in the leucocyte. A Mn exposed group (n=62), working in a Mn-Fe alloy factory was compared to non-Mn-exposed (control) workers (n=39) from a pig iron plant in Zunyi City, China. The working environment of both groups were similar. Mn concentrations in the plasma and in the erythrocytes were analyzed by AAS. Results revealed a significant decrease in the expression of DMT1, TF and hepcidin genes in Mn-exposed workers by 77.9%, 80.7% and 90.1%, respectively, as compared to controls. There was no significant difference observed in TIR levels. By adjusting for age, sex and cumulative exposure, the expressions of DMT1, TF and hepcidin genes were inversely correlated with the Mn concentrations in the erythrocytes: their Spearman r are -0.22, -0.18, -0.24, respectively (all p values were <0.01). In summary, Mn exposure decreases the expressions of DMT1, TF and hepcidin in the leucocytes. Further studies are warranted to elucidate the mechanism of Mn action. (J DOD W81XWH-05-1-0239, NIH/NIEHS ES-08164, and China NSF 30702029).

**MANGANESE MODIFICATION OF GLUTAMINE TRANSPORT IN PRIMARY ASTROCYTES.**

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Glutamine (Gltn) plays an important role in the CNS as an energy metabolite and as a precursor of neurotransmitters: excitatory glutamate (Glu) and inhibitory γ-aminobutyric acid (GABA). Previous studies have shown that astrocytic Gltn transport is impaired following manganese (Mn) exposure. The present studies were performed to investigate the hypothesis that Gltn transporter expression dysregulation by Mn plays a role in neurotoxicity. mRNA expression and protein level of Gltn transporters belonging to the systems: N (SNAT3, SNAT5), ASC (ASCT2), A (LAT2) and M (SNAT2) were studied in rat neonatal primary astrocyte cultures exposed to Mn. Significant (p<0.01) inhibition of SNAT3’s mRNA expression was observed after incubation of cells with 100, 500 and 1000 µM Mn for 4h and with 500 and 1000 µM Mn for 8h and 24h. Expression of the mRNA coding for SNAT2 and LAT2 was inhibited at 100, 500 and 1000 µM Mn after 8h and 24h treatment. Mn exposure didn’t affect expression of mRNA coding for SNAT5 and ASC2 transporters. 500 µM and 1000 µM Mn caused a protein level after 1h and 4h degradation after 8h and 24h of treatment. Incubation of astrocytes with Mn resulted also in decreased protein level of SNAT2 (after 4h, 8h and 24h exposure with 1000 µM Mn and 8h and 24h treatment with 500 µM Mn) as well as LAT2 (after 8h and 24h exposure for 500 µM and 1000 µM Mn). Treatment of the cells with Mn didn’t change neither SNAT5 nor ASC2 protein levels. Taken together, these studies demonstrate that Mn causes disability of astrocytic Gltn transport by decrease in Gltn transporter genes expression. These alternations can diminish the availability of precursor for synthesis of Glu and GABA in neurons, leading to decrease in these neurotransmitters neuronal pools, a phenomenon known to be associated with Mn toxicity. This work was supported by Public Health Service of National Institute of Health Grants ES007531.

** METHYLMERCURIC INDUCED OXIDATIVE STRESS IN CULTURED ASTROCYTES LEADS TO NRF2 ACTIVATION AND NUCLEAR TRANSLLOCATION.**

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Methylmercury (MeHg) is a significant environmental contaminant that will continue to pose great risk to human health. A number of studies suggest that oxidative stress mediates MeHg-induced neuronal damage. NF-E2-related factor 2 (Nrf2)
was identified as a major regulator of cytotoxic reactive oxidative stress in oxidative damage. Under normal conditions, Nrf2 in the cytoplasm is bound to Keap1. In the presence of oxidative stress, the Keap1-Nrf2 complex dissociates and Nrf2 translocates into the nucleus. Upon activation, Nrf2 upregulates the expression of genes associated with antioxidant responses. In our study, we investigated the protective effects of Nrf2 in response to MeHg exposure in astrocytes. Primary astrocytes were prepared from cerebral cortices of one-day-old Sprague-Dawley rats. MeHg toxicity in astrocytes was assessed by lactate dehydrogenase (LDH) leakage into the culture medium. Cytoprotection was determined by measuring the conversion of a tetrazolium salt (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, MTT) to a formazan product. Apoptosis was assessed by measuring caspase-3 activity. Cells were treated with MeHg at 1 or 10 μM for 1, 8, or 16 h, then whole-cell, or nuclear and cytoplasmic Nrf2 expression levels were assessed by western blot analysis; nuclear translocation of Nrf2 was examined by immunocytochemistry staining with laser confocal microscopy. Our results showed that MeHg led to a significant increase in LDH release, MTT reduction and activation of caspase-3 (p<0.05 by ANOVA). These effects were significantly attenuated by antioxidants such as ebselen or trolox (p<0.05). MeHg treatment (8 and 16 h) significantly (p<0.01) amplified Nrf2 levels by 3-5 fold and Nrf2 translocated to the nucleus. These results indicate that MeHg exerts its neurotoxicity through oxidative stress and activation of the Nrf2-ARE pathway in astrocytes is likely associated with protection against oxidative damage caused by MeHg.

**360 ANTIOXIDANTS INFLUENCE METHYLMERCURY-INDUCED OXIDATIVE REACTION IN PRIMARY CULTURED ASTROCYTES.**

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Methylmercury (MeHg) is a well-documented neurotoxin in animals and humans. It is well established that MeHg preferentially accumulates in astrocytes, affecting their ability to induce and/or exacerbate neuronal dysfunction. The present study was designed to test that MeHg activates stress related proteins and that its effects can be attenuated by antioxidants. Astrocyte oxidative damage and inflammatory reaction were detected by measuring protein expression using antibodies to DJ-1, Caspase-3, TRPM2, and PPAR. DJ-1 is an antioxidant protein or a redox sensor in oxidative stress. TRPM2 is a membrane protein that can be activated by oxidative stress and confers susceptibility to cell death. PPARs are nuclear hormone receptors that can be triggered by various compounds including prostaglandins in response to inflammatory reaction. Western blotting analysis and immunofluorescent assays were employed. The potentiometric dye tetramethylrhodamine ethyl ester (TMRM) was used to probe mitochondrial membrane potential (ΔΨm). Results showed a significant (p<0.05) reducton of ΔΨm expression and caspase-3 activation in cultured astrocytes exposure to MeHg at 10 μM. Pre-treatment of ebselen or trolox partially reversed the MeHg effects, maintaining DJ-1 expression and caspase-3 activation at levels indistinguishable from controls. A significant (p<0.05) increase in TRPM2 immunoreactivity was also observed upon exposure to MeHg, consistent with activation of oxidative reaction. The presence of PPAR, an anti-inflammatory reagent, significantly (p<0.05) reduced the activation of TRPM2. MeHg significantly (p<0.05) increased PPAR expression, suggesting increased inflammatory response. The administration of PPAR maintained the expression of PPARα indistinguishable from controls. TMRM imaging demonstrated that MeHg resulted in collapse of ΔΨm (p<0.05 vs. control), ebselen or trolox partially reversed this effect of MeHg. These results indicate that induction of oxidative damage, inflammatory reaction and associated mitochondrial dysfunction represent important mechanisms of MeHg neurotoxicity.

**361 CAENORHABDITIS ELEGANS AS A MODEL TO STUDY MECHANISMS OF METHYLMERCURY TOXICITY.**

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Methylmercury (MeHg) is a toxicant having detrimental effects on the mammalian nervous system, but the mechanisms of toxicity are not well understood. Humans are exposed to MeHg in seafood and the toxicant is able to traverse the blood-brain and placental barriers, making it harmful to the nervous system, particularly that of the developing fetus. Caenorhabditis elegans is an organism amenable to use in toxicological research. Therefore, C. elegans was used as a model to dissect pathways involved in MeHg developmental neurotoxicity. Previous results indicated that although MeHg was lethal to C. elegans, surviving animals show no defects in locomotion, birthing or neuronal morphology. However, MeHg accumulated in treated animals in a dose and time-dependent manner (after 15 hour treatment) L4 worms treated at 0.1 and 0.4 mM MeHg had 20- and 150-fold increases in MeHg content, respectively) and induced a dose-dependent delay in development and pharyngeal pumping rate (after 15 hour exposure of L4 worms, at 0.1 and 0.4 mM MeHg, pumping rate was reduced 27% and 70%, respectively) indicating an effect of MeHg on the pharyngeal nervous system. GFP reporters will be used to determine if MeHg affects the differentiation or morphology of neurons that control pharyngeal pumping. Following initial MeHg insult, worms were less sensitive to subsequent MeHg exposure, shifting the lethality dose-response curves of MeHg rightward with increasing initial MeHg exposure. These results indicate that exposure to non-lethal concentrations of MeHg induces factors in C. elegans that protect it from additional insults and candidates are being studied to assess involvement in this process.

**362 ASSESSMENT OF MANGANESE EXPOSURE BY 3D HIGH-RESOLUTION T1-WEIGHTED MRI.**

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Exposure to excessive manganese (Mn) leads to psychological and motor disorders, indicating a permanent damage to certain brain structures. Yet, there has been no reliable means for early, pre-symptomatic diagnosis and assessment of Mn intoxication. The aim of this study was to use magnetic resonance imaging (MRI) as a non-invasive method to distinguish Mn-exposed subjects from the control subjects. A group of 10 smelting workers as well as a group of 10 control subjects with no history of Mn exposure were recruited from a Mn-Fe alloy manufacturer in Zunyi, China. All study subjects were subjected to physiological examination, blood testing, and MRI examination. High-resolution 3D T1-weighted isotropic images (15x15x15 mm) were acquired in addition to conventional axial T1-weighted 5mm thick slices and analyzed by visual inspection and region of interest (ROI) analysis. The pallidal index (PI) was calculated based on (1) the signal ratio between a ROI within the globus pallidus (GP) and white matter in the frontal cortex (PIwm) and (2) the signal ratio between the same ROI in the GP and a ROI in the neck muscle (Plmu). Results showed that 3D T1-weighted MRI was much more sensitive than the axial T1-weighted method in identifying Mn deposit in brain. Plmu and PIwm, based on the 3D T1w-MRI, showed 90% and 70% success in predictability, respectively, in identifying the Mn exposed smelters out of the 20 subjects scanned. Thus, it appeared that Plmu was more accurate than PIwm in distinguishing Mn exposed smelters from the control workers. In addition, 3D imaging allowed us to trace the regions of Mn accumulation in high detail to the most interior parts of the GP. Further analysis of changes in brain metabolites as seen by MRS is in progress. (Supported by DoD USAMRMC W81XWH-05-1-0329)

**363 PRENATAL EXPOSURE TO BENZO(A)PYRENE IMPAIRS LATER-LIFE CORTICAL NEURONAL FUNCTION.**

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Prenatal exposure to environmental contaminants, such as Benzo(a)pyrene (BaP) has been shown to impair brain development. We hypothesize that glutamate receptor subunit expression is vital for cortical evoked responses and that prenatal BaP exposure modulates the temporal developmental expression of glutamatergic receptor subunits in the somatosensory cortex. To characterize prenatal BaP exposure on the development of cortical function, pregnant Long Evans rats were exposed to 500μg/kg BW BaP(α) by oral gavage on gestational days 14 to 17. At this dose, BaP had no effect the number of pups born per litter, the pre-weaning growth curves and initial and final brain to body weight ratios. Control and BaP(α) exposed offspring were profiled for BaP(α) metabolites in plasma and whole brain during the pre-weaning period. A time-dependent decrease in total metabolite concentration was observed in BaP(α)exposed offspring. On PND100-120, cenoencephalic mRNA expression was determined for the glutamatergic NMDA receptor subunit NR2B and neural activity was recorded from neurons in primary somatic sensory cortex. Semiquantitative PCR revealed a significant reduction in NR2B mRNA expression in BaP(α)exposed offspring relative to controls. Recordings from
B(a)P-exposed offspring revealed that N-methyl-D-aspartate (NMDA) receptor-dependent evoked neuronal activity in barrel cortex was also significantly reduced as compared to controls. Analysis showed that the greatest deficit in cortical neuronal responses occurred in the shorter latency epochs post-stimulus. This suggests that in utero exposure to B(a)P results in reduced expression of the NR2B receptor subunit which results in later deficits in cortical neuronal activity in the offspring. Supported in part by U54NS041071, S11ES014156, T32MH065782, and RO35032.

### 364 MEHG-INDUCED OXIDATIVE STRESS IN MICROGLIAL CELLS AND ITS ROLE IN NEUROTOXICITY.

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MeHg poisoning in Minamata Bay and recent studies in human cohorts have provided ample evidence for the neurodevelopmental effects of MeHg and its wide range of neurological symptoms. Microglial cells, accounting for 12% of the brain cells are "professional phagocytes" and their activation contributes to neuronal damage. Given their prominent role in the maintenance of CNS homeostasis, we initiated a project to investigate effects of MeHg on microglial cell function, testing the hypothesis that these cells mediate MeHg effects. Primary microglial cells were prepared from cerebral cortices of 1-day Sprague-Dawley rats. Cytotoxicity was determined by measuring conversion of tetrazolium salt (MTT) to formazan product and quantification of the leaked LDH into the culture medium. Nrf-2, p-AKT and p-ERK were quantified by Western blot using anti-red dye conjugated secondary antibodies. Immunostaining was carried out with a Nikon eclipse microscope. Both the MTT and LDH assays confirmed the toxicity of MeHg to microglial cells. MeHg caused microglial death in a concentration-dependent manner and increased COX2 expression after 6-hour treatment, suggesting the existence of MeHg-induced inflammation. This was further confirmed by observing a concentration-dependent increase in microglia glutathione (GSH) levels after MeHg treatment, indicating the accumulation of free radicals. Nrf-2, a nuclear transcription factor, binds with KEAP1 in the cytoplasm in the absence of oxidative stress. In response to the oxidative stress, Nrf-2 is released from KEAP1 and undergoes nuclear translocation. Immunostaining studies support Nrf-2 nuclear translocation after 24h MeHg treatment at both 1h and 3h of MeHg exposure. It has been documented that AKT and ERK are phosphorylated as a result of oxidative stress. Consistent with these observations, MeHg significantly increased phospho-AKT after 30min treatment. Our results suggest that oxidative stress promotes the nuclear translocation of Nrf-2 and selectively activates downstream AKT pathways, implicating the involvement of microglia in MeHg-induced neurotoxicity. Supported by NIEMES10563.

### 365 OXIDATIVE DAMAGE AND NEURODEGENERATION IN MANGANESE-INDUCED NEUROTOXICITY.

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Exposure to excessive manganese (Mn) levels in certain occupational or dietary conditions results in neurotoxicity to the extrapyramidal system and the development of Parkinson’s disease (PD)-like movement disorders, called manganese toxicity. Although the mechanisms by which Mn induces neuronal damage are not well defined, its neurotoxicity appears to be regulated by a number of factors, including mitochondrial dysfunction and oxidative injury. To investigate the mechanisms underlying this neurotoxicity, we studied the effects of Mn on reactive oxygen species (ROS) formation, neuroinflammation, changes in high-energy phosphates (HEP) and associated neuronal dysfunctions in mouse brain and neonatal rat primary neurons. Biomarkers of oxidative damage, F2-isoprostanes (F2-isoPs) and pro-inflammatory prostaglandins E2 (PGE2) were significantly (p<0.05) increased (145% and 125%, respectively) in brain of C57Bl/6 female mice exposed to Mn (100 mg/kg, sc) for 24 hours. At the same time, quantitative morphometric analysis of striatal medium spiny neurons (MSNs) revealed significant decreases (p<0.05) in F2-isoPs and decrease (67%) in HEP (ATP), as determined by GC-MS and HPLC, respectively. These results are consistent with oxidative stress, neuroinflammation, mitochondrial dysfunction and consequent neurodegeneration as major mechanisms in Mn-induced neurotoxicity (Supported by DoD W81XWH-05-1-0239).
369 DETECTION OF MITOCHONDRIAL TOXICITY IN HEPG2 CELLS USING SOLUBLE OXYGEN AND PH SENSORS.

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Mitochondrial Toxicity has been implicated as a major contributor to drug-induced toxicity (Wallace KB. Mitochondrial off targets of drug therapy. Trends Pharmacol Sci. 2008). We have developed a screen to measure oxygen consumption using soluble sensors in 96 well format, using isolated rat liver mitochondria (Will Y. Hynes J, Ogurtsov VI, Papkovsky DB. Analysis of mitochondrial function using phosphorescent oxygen-sensitive probes. Nat Protoc. 2006). In addition to general cytotoxicity screening via ATP depletion, this assay has become part of our routine screening paradigm. Performing these measurements in whole cells would be advantageous, as isolated organelles could potentially overpredict and might not be suitable for in vitro /in vivo correlations. However, in cells oxygen consumption measurements alone would not delineate between true mitochondrial toxicity and general cytotoxicity unless combined with subsequent measurements of pH changes, indicative of glycolytic compensation. True mitochondrial toxicity would result in a decrease in oxygen consumption with subsequent increase in acidification due to compensation by glycolysis. In contrast, cytotoxicity resulting from other than mitochondrial insult, would lead to a decrease in oxygen consumption without subsequent acidification. Here we show that classical mitochondrial toxins such as rotenone, antimycin, oligomycin and FCCP follow the predicted paradigm. Drugs, previously described by us to have mitochondrial liabilities, such as nefazodone, flutamide, phenformin, usnic acid and sorafenib also follow the pattern of oxygen depletion and pH increase. In contrast, drugs that where mitochondrial liabilities are not thought to be the primary reason for observed toxicity, such as tamoxifen, ketcodazol, troglitazone, showed decreases in oxygen consumption without pH changes. Both parameters could be monitored in real time and long before ATP depletion could be observed, which makes this screen a much more sensitive readout with additional mechanistic insights.

370 METAL TOXICITY IN DANO RERIO.

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The zebrafish, Danio rerio, is an attractive model for toxicogenomic studies because of its low maintenance costs and sequenced genome. To develop rapid screening methods for leading genomic indicators of toxicant exposure and effect, we exposed 25 male zebrafish for 24 h to NiCl2, CdCl2 and Na2CrO4 at the 96 h LC50, LC100 and LC20 concentrations for each metal. Five fish from each group were preserved in RNA later and the histopathology. For all conditions, four pools of liver RNA from five fish each were used for gene expression studies on custom microarrays. We observed no marked histological changes in fish exposed to Ni. Fish exposed to Cd showed gill hyperplasia and lamellar fusion of minimal severity. The Cr exposed fish displayed gill hyperplasia, lamellar fusion, and hemorrhage in the gills. These Cr exposed fish also experienced atrophy of the intestinal mucosal folds and pharyngeal epithelium. We found no evidence of liver pathology at the histological level in any case. However, we did observe alterations in gene expression in the liver. Increased expression was observed for genes involved in copper metabolism. As concentrations of metal increased, the number of genes affected also increased. Many of the changes in gene expression correlate well with known effects of the metals. Thus, transcriptional analysis of the zebrafish liver appears to be a feasible approach for studying toxicological effects and identifying potential biomarkers of exposure for a wide variety of chemicals. Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army. Research presented complies with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals (NRC 1996) in facilities that are fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International. The research described herein was sponsored by the U.S. Army Medical Research and Materiel Command, Military Operational Medicine Research Program.
AN IN VITRO MODEL FOR ASSESSING THE EFFECTIVENESS OF ANTIOXIDANTS IN COSMETICS.


Oxidative stress in skin has been linked to the development of wrinkles, the aging process and other more serious effects. In vitro cell-based systems that can rapidly and efficiently evaluate whether a new chemical or formulation has antioxidant properties are important in the development of new products for the cosmetic industry. The aim of this study was to induce oxidative stress in normal human epidermal keratinocyte (NHEK) cells and then demonstrate protection in the presence of Trolox (Vitamin E) and test products. Oxidative stress was induced with two model compounds: menadione, which requires metabolism to reactive quinone intermediate, and cumene hydroperoxide, which does not require metabolism in order to produce oxidative stress. Formation of reactive oxygen species was monitored with 5-(and-6)-chloromethyl-2-7-dichlorodihydrofluorescein diacetate (CM-H2DCFDA) and glutathione (GSH) status. Cell viability was measured with propidium iodide. NHEK cells were seeded into 96-well culture plates (10,000 cells/100 μL) and cultured with human keratinocyte growth medium and 10% BSA. Exposures to menadione (20 μM) and cumene hydroperoxide (100 μM) were for 3, 6, or 24 hr. Menadione increased DCFDA signal by approximately 2- to 3-fold after 6 hr and reduced GSH to levels less than 50% of controls after 6 and 24 hr. These oxidative stress effects were also significantly reduced in the presence of Trolox (50 μM). Cumene hydroperoxide (20 and 100 μM) also produced significant oxidative stress, increasing DCFDA signal by approximately 8- to 10-fold and reducing GSH levels after a 6 hr exposure. These oxidative stress effects were also significantly reduced in the presence of Trolox. When the NHEK model was used to evaluate the antioxidant properties of new products, it was possible to demonstrate clear antioxidant properties.

DOMINANT NEGATIVE ZEBRAFISH AHR2 PROTECTS AGAINST TCDD TOXICITY.

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Zebrafish embryos exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) develop cardiac and vascular defects, including pericardial and yolk sac edema, heart looping defects and reduced peripheral blood flow. These effects are mediated by the zebrafish aryl hydrocarbon receptor 2 (AHR2). Although gene knockdown by morpholino technology is useful, and has provided considerable insight into the role of AHR2 in vivo, its efficacy is limited to the first few days of development. This work describes the construction and characterization of a dominant negative form of AHR2 (dnAHR2), and the establishment of transgenic zebrafish lines that express dnAHR2 in a ubiquitous manner. The expression of the dnAHR2 protein was verified by Western Blot analysis of protein lysates isolated from F1 offspring. In addition, dnAHR2 expressing fish showed reduced induction of AHR2 target genes and are protected against the endpoints of dioxin toxicity. A constitutively active form of AHR2 (caAHR2) has also been constructed, and transgenic lines expressing caAHR2 under the control of an edyzone inducible promoter are being established. The use of these modified AHR2s, driven by tissue specific promoters, will provide greater insight into tissue specific mechanisms of dioxin toxicity. (Supported by: NIEHS, NIH Grant F32 ES007015 and NIH RO1 ES012716)

ALDO-KETO REDUCTASE GENES IN ZEBRAFISH.


Orido-quinoines of PAH such as BaP-7,8-dione (BPQ) can participate in redox cycling leading to radical formation. BPQ is formed by the sequential action of CYP1A to form the 7,8-epoxide, epoxide hydrate to form BP-7,8-dihydrodiol, and dihydrodiol dehydrogenases (aldo-keto reductases or AKRs), to form the o-quinoine. Several mammalian AKRs catalyze the NADP-dependent oxidation of PAH trans-dihydrodiol to the o-quinoines, via the catechol, including human AKR1A1 and AKR1C (AKR1C1-AKR1C4) proteins. To address questions of oxidative damage to DNA induced by Ah receptor agonists during development, we have examined AHR1 family genes in zebrafish. Full-length coding sequences for the five zebrafish genes identified from the zebrafish genome were cloned and sequenced. The amino-acid sequences inferred for the full-length coding regions of the zebrafish sequences were up to 76% identical to mammalian AKR1s. Bayesian analysis of the multiple phylogenies with two sequences clustering with human AKR1A1s, and two sequences that clustered with the AKR1B1s. Exon-intron boundaries in the AKR1A-like genes were identical to each other and like those in known AKR1B1s. Likewise the three AKR1B-like genes had exon-intron boundaries that were similar to each other and like the exon-intron boundaries in mammalian AKR1B1s. However, one of these zebrafish genes had lower percent identity to the mammalian AKR1B1 suggesting it may represent a separate subfamily. The zebrafish AHR1A-like genes exhibited shared synergy with human AKR1A1. However, the zebrafish AKR1A-like genes have complicated syntenic relationships with the human AKR1Cs. The five genes exhibit very different patterns of expression during zebrafish development, and one of the AKR1B genes was expressed at high levels only during early development. These zebrafish AKR1s have also been heterologously expressed in E. coli, demonstrating the formation of functional proteins. (NIH R01ES015912)
epithelial (THLE) cell viability assay (LC50 at 72 h) and Zt toxicity (LOEC or LC100) was visually apparent; although there was not a one-to-one correlation based on concentration in the medium. These results illustrate the challenges of predicting compound uptake into ZL.

**378 COLIPA PROGRAM ON OPTIMIZATION OF EXISTING IN VITRO EYE IRRI TATION ASSAYS FOR ENTRY INTO FORMAL VALIDATION: TECHNOLOGY TRANSFER AND INTRA/INTER LABORATORY EVALUATION OF AN EPICULAR ASSAY FOR CHEMICALS.**

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The European Union will ban in vivo eye irritation tests on ingredients for cosmetic products after March 2009. COLIPA, (European Cosmetics Association) is actively working to bring in vitro eye irritation tests to formal validation with ECVAM. This poster details the Colipa program on technology transfer and reproducibility of MatTek Episcular™ assay as one of the two human reconstructed tissue assays. This Episcular protocol differs from previous time-to-toxicity protocols in that it uses a single exposure period for each chemical and a prediction model based on a cut-off in relative survival (≥ 60% – irritant I) (R36 and R41); >60% - non-classified (NC). Test substance exposure time is 50 minutes with a 2-hour post-exposure incubation for liquids and 90 minutes with an 18-hour post-incubation exposure for solids. After the post-exposure, tissue viability is determined by tetrazolium dye reduction (MTT). Combinations of 20 coded chemicals were tested in 7 laboratories. A standardized protocol and laboratory documentation were used by all laboratories. MatTek provided initial training and then each laboratory operated independently during the technology transfer study. Twenty liquids (11 NC9) by ECVAM plus 5 solids (3 NC2) were selected so that best exposure regimens could be assessed. Concurrent positive (methyl acetate) and negative (water) controls were tested in each trial. Chemical decoding occurred only after study completion. In all, 298 independent trials were performed and demonstrated ≥99.7% agreement in prediction (NC/II) across the laboratories. Coefficients of variation for the % survival of tissues across laboratories was generally modest (<16%) except where tissue survival values were low. Using these data, a formal submission was sent to ECVAM in support the protocol’s entry into a formal validation study.

**379 PROTECTIVE EFFECTS OF EXOGENOUS ANTIOXIDANTS ON LIPID PEROXIDATION AND ANTIOXIDANT ENZYMES IN MYCOTOXINS-INDUCED TOXICITY.**

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Mycotoxins are secondary metabolites of various moulds. During mycotoxins exposure in mammal’s cells there is a generation of reactive oxygen species (ROS) and mycotoxins can produce oxidative stress in exposed cells. Cells have different antioxidant mechanisms, the primary defense is offered by enzymatic and nonenzymatic antioxidants, which have been shown to scavenge free radicals and ROS. Antioxidants belonging to the second line of defense include glutathione (GSH), vitamin C and vitamin E. Mammal cells were individually exposed at different concentrations of mycotoxins (ranged from 1 to 50 μM). A second experiment was conducted to investigate the capacity of antioxidants (GSH, vitamin C, and vitamin E) to protect mammal cells of mycotoxins. After different time periods (ranged from 2 to 48 h), GSH content, the activity of antioxidant scavenger enzymes and generation of intracellular ROS by the sensitive fluorogenic probe 2,7-dichlorodihydrofluorescein diacetate (H2-DCFDA) were determined. Increases in lipid peroxides were observed in cells which grow up in media deficient of antioxidants and which were exposed to mycotoxins. Decrease in lipid peroxidation was observed in those assays which exposed mycotoxins together antioxidants. Significant decrease in GSH content and alternation of antioxidant enzymes were recorded in mammal cells exposed mycotoxins in deficient antioxidants media. The results obtained from use of different antioxidants and ROS scavengers suggested a role of mycotoxins in oxidative stress. The use of antioxidants provides protection against toxicity caused by mycotoxins in the mammal cells assayed. These results indicate ROS are formed from mycotoxins and its oxidant effects can be abrogated by glutathione and other exogenous antioxidants. This work was supported by the Science and Education Spanish Ministry (AGL-2007-61493).

**380 GATA2: A POTENTIAL CANIDATE GENE OF BLUE COHO SH TOXICITY IN JAPANESE MEDAKA.**

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Blue cohoosh (Caulophyllum thalictroides) (BC) is a perennial herb used by the Native American Indian women to induce labor and to treat other uterine complications. The safety and effectiveness of this herbal product has not yet been evaluated by the Food and drug administration of America (FDA), however, several reports indicated that BC by some unknown mechanisms is able to induce teratogenic effects in cardiovascular system in new born babies. For experimental verification of the teratogenic effects of BC we have used Japanese medaka (Oryzias latipes) embryo-larval development as our experimental model. Fertilized medaka embryos were exposed to methanolic extract of BC root (0-10 μg/ml) from 3-5 day post fertilization (dpf) and were used for biochemical and morphological analysis on 6 dpf, respectively. BC treated embryos demonstrated vascular abnormality in a dose-dependent manner; however, total cellular protein, RNA, and DNA contents of the embryos remained unaltered. Several transcription factor mRNAs such as emx2, en2, shh, wnt1, zic5 and ir3 which were expressed in the brain of medaka embryos during development were also unaltered after BC treatment. Craniofacial and tail cartilage development as observed on 10 dpf was found to be normal in BC-treated embryos. However, by applying subtractive Hybridization technique we observed that the transcription factor GATA2 was over expressed in BC-treated embryos. We therefore conclude that the teratogenic effect of BC in cardiovascular development of medaka was probably mediated through GATA2 signaling pathway (supported by COBRA P20RR021929).

**381 A CELL VIABILITY ASSAY FOR ESTIMATING IN VIVO TOLERANCE IN RODENTS.**

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The application of safety screening paradigms is expected to revolutionize the drug discovery process. However, with the exception of mechanistic assays for genetic toxicology and safety pharmacology screening, screening strategies to assess general safety are inadequate. In this regard, the value of in vitro cell viability assays for assessing in vivo chemical safety has long been debated with differing outcomes. A retrospective analysis of results from rat in vivo toleration studies (4-14 days, varying doses) conducted with diverse chemical matter that spans a broad array of therapeutic targets (protease inhibition, cGMP modulation, 3 GPCRs, and 3 kinase programs) shows that cell viability (using the SV-40 transformed human liver epithelial cell line), is generally predictive of overall safety outcomes using an equally weighted scoring system (0-4 component, 0-12 overall score). Scoring based on systemic toleration (mortality, body weight, food consumption, adverse clinical signs), clinical chemistry/hematology parameters (deviations from normal ranges), and organ pathology (necrosis or incidence and severity of histologic change). As an overall probability, compounds with LC50 values <10 μM were generally less tolerable (53%<4, 23% 4-8, 44%>8) than compounds with LC50 values 50-300 μM (67%<4, 21% 4-8, 13%>8, p < 0.004 by Fisher's Exact Test). Refinements predict overall toleration for individual compounds could be made when exposure was taken into consideration. Safety margins (LC50/AUCaverage) of <1 lead to higher adverse outcomes in rodent studies (0%<4, 29% 4-8, 71%>8) compared to safety margins >10 (61%<4, 27% 4-8, 12%>8). This work shows that overall toleration of new chemical matter in a short-term in vivo rat study can be predicted based on knowledge of its LC50 value and anticipated exposure values, reinforcing the old axiom that the dose makes the poison.

**382 DEVELOPING PREDICTIVE TOXICITY SIGNATURES USING IN VITRO DATA FROM THE EPA TOXICAST PROGRAM.**


A major focus in toxicology research is the development of in vitro methods to predict in vivo chemical toxicity. Numerous studies have evaluated the use of targeted biochemical cell-based and genomic assay approaches. Each of these techniques is potentially helpful, but provides only a partial view of the complex biology that leads to tissue, organ or whole organism toxic effects. Here we present the first results from the ToxCast program that combines multiple types of assays into “toxicity signatures” that are optimally predictive of particular in vivo toxicity endpoints.
A toxicity signature is in essence a function that takes as input the results of a set of assays run on a chemical, and produces a prediction of the toxicity of that chemical for a specific in vivo endpoint. We used the EPA ToxCast program Phase 1 data, which includes about 600 in vitro assays run on a total of 520 chemicals, the majority of which are pesticide active ingredients. The corresponding in vivo toxicity data is taken from the Toxicology Reference database (ToxRefDB), which is tabulated data from guideline animal studies for chronic, cancer, developmental and reproductive toxicity endpoints. We present a variety of machine learning approaches to mine this complex data set for toxicity signatures with both high sensitivity and specificity. These include linear discriminant analysis, support vector machines and neural networks. In addition to these automated approaches, we also present more hypothesis-driven, mechanism-based signatures including ones probing nuclear receptor-mediated liver toxicity. An important observation from these first analyses is that one often needs to include assays of multiple types, probing multiple mechanisms or pathways, to adequately predict in vivo toxicity across a wide range of chemicals. Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.

### 383 ALTERNATIVE PORCINE CORNEAL OCULAR REVERSIBILITY ASSAY CORRELATES WITH DRAIZE REVERSIBILITY DATA.


No established alternative ocular irritation assay can measure corneal tissue damage and reversibility. With the support of a Colgate-Palmolive Grant for Alternative Research, we have developed an alternative assay: Porcine CornealOpacity Reversibility Assay (PorCORA). PorCORA measures corneal damage and recovery for extended time periods using porcine corneas excised from hypotensive abattoir eyes. Test articles are dosed directly onto the corneal surface, and tissue damage and recovery are assessed by sodium fluorescein (NaFL) retention in the same corneas over time (21 days). We have confirmed NaFL retention results and corneal recovery in the PorCORA system via several approaches. Both fluorescence and reflective confocal microscopy confirm damage indicated by fluorescein retention in the cultured corneas. In addition, we have shown histological evidence that also correlates well with NaFL staining in the PorCORA assay. Overall, PorCORA is predictive and representative of corneal tissue damage and recovery. Here we report the results of a 32-reference chemical validation including chemicals from the following classes: acetates, acids, alcohols, alkyls, esters, hydrocarbons, inorganics, ketones, surfactants, and several solid compounds. PorCORA generates a mean (4 corneas per experimental condition) “days to clear” endpoint when corneas no longer retain NaFL stain (1-21 days). When compared to mean days to clear (corneal effects) from the ECETOC Eye Irritation Data Bank (historical Draize data), the correlation coefficient is r = 0.84, and r = 0.82 for the MMAS (ECETOC). To determine if the PorCORA system can distinguish between reversible and irreversible corneal damage, we considered corneas that retained NaFL at 21 days to be irreversible. PorCORA reversibility results (reversible vs. irreversible) correlated very well with ECETOC data, yielding 91% accuracy, 92% sensitivity, 89% specificity, 86% positive predictivity, and 94% negative predictivity for the 32 compounds tested.

### 384 PORFOCAL, FOR YOUR EYES ONLY!

M. R. Carathers, M. Piehl, R. C. Soda and D. R. Cerven, MB Research Laboratories, Spinnerstown, PA.

MB Research Laboratories has developed a procedure for the culturing of excised porcine corneas for a period of up to 21 days. Using these corneas, we have developed a method, PorFocal, to evaluate the effects of multiple doses of low-level irritants. Corneas are excised from enucleated porcine eyes from the abattoir, and can be cultured in a living state for up to 21 days. In order to detect and quantify low-level damage to corneal tissue, this assay was created to assess ocular irritation by measuring cell viability using a dead stain, Ethidium homodimer (EtH), and fluorescence confocal microscopy. For the PorFocal assay, eight cultured corneas (4 per tissue) were treated with the staining kit, a live cell stain. This assay has been further developed and a ratio of live to dead cells in each group could yield higher-sensitivity corneal irritation measurements.

### 385 IN VITRO MODELS FOR PREDICTION OF CHEMICAL METABOLISM AND TOXICITY.

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The use of cell lines for drug and chemical toxicity screening, although very attractive, has serious limitations because of the limited expression of cytochrome P450 isozymes. In addition, cell line toxicity assays give little easily measurable information about the toxic mechanism. In order to circumvent these issues we have generated a range of cell lines which have been engineered to report on different pathways of toxic insult. These include oxidative stress, DNA damage, NFkB signalling, apoptosis, heat shock response, etc. In addition, we have developed a number of adenoviral vectors which allow the expression of single or multiple human P450s in any cell line of interest. P450 expression was achieved using a novel approach using the foot-and-mouth disease virus peptide 2A sequence which allows the expression of multiple proteins of a single promoter system. Using this approach we have expressed CYP3A4, CYP2D6, CYP2A6 and CYP1A1 at levels at least equivalent to those normally found in human liver. In order to exemplify the utility of this model in cytotoxicity screening, we have taken a single cell line where the antioxidant responsive element (ARE), activated by Nrf2, is used to drive a luciferase reporter. We have then exposed these cells to either 7-ethoxyresorcinol or butylated hydroxyanisole and have demonstrated that ARE activity is only induced in cells which express cytochrome P450 enzymes CYP1A1 and CYP2A6. These data demonstrate the power of this experimental approach to give a more accurate assessment of toxicity associated with exposure to chemical agents. Acknowledgements are made to Millipore Inc. and NC3Rs in the funding of this work.

### 386 PREDICTIVE CYTOXICITY MEASUREMENTS BY MONITORING HEAT SHOCK PROTEINS, F38 AND THE CELL PROLIFERATION IN HIGH-CONTENT SCREENING CELL-BASED ASSAYS.


Heat shock proteins (HSPs) play crucial roles in maintaining cellular homeostasis and promoting cell survival by refolding or degrading damaged proteins in response to various cell stresses. There are five major HSP families including small HSPs, Hsp60 or chaperonins, Hsp70, Hsp90 and Hsp110. HSPs are involved in many cellular functions such as signaling, protein synthesis, folding and degradation, protein translocation and cell death. We hypothesized that multiplexed monitoring of HSPs in cells can be used for in vitro predictive cytotoxicity assessments. To test our hypothesis, we monitored multiple HSPs in cells to evaluate the cellular stress affected by toxic chemicals. We developed four quantitative cell-based imaging high-content screening (HCS) assays towards the following combinations of HSPs in cells: (1) Hsp27 and phosphorylated-Hsp27, (2) Hsp60 and Hsp90beta, (3) Hsp70 and Hsp90alpha, (4) FKBP52 (Hsp90). Cells were treated with different cytotoxic chemicals and then fluorescently labeled, imaged and quantitatively analyzed using a high-content analysis platform. We used cell number, nuclear size, p38 MAPK activation and Bcl/tn incorporation as known reference markers for cellular stress. Dose and time course responses of HSPs against different stressors show that many heat shock proteins are induced by different stressors at different times. Interestingly, these heat shock proteins respond at a lower dose in cells than in animal assay. Heat shock proteins in cells enables quantitative detection of cellular stress mediated by cytoxic agents in high content screening assays.

### 387 EFFECTS OF IN VITRO METABOLISM ON THE ENDOCRINE ACTIVITY OF SUBSTANCES IN THE YEAST-BASED REPORTER GENE ASSAY.


Text substances can be screened for potential endocrine activities using in vitro assays, e.g. yeast-based reporter gene assays (YAS) for androgenic and oestrogenic activities. The xenobiotic metabolic capacity of yeast cells, however, is not
well investigated. Using model compounds, we evaluated whether the addition of metabolically active rat liver in vitro systems increases the predictive value of the yeast assay. Testosterone (T) was almost completely metabolized by microsomes under the chosen incubation conditions. DME-DES (the non-estrogenic di-methyl ether of the estrogenic diethylstilbestrol) and DES-DP (DES-propionate) were also extensively metabolized. Incubates of T with active microsomes (Al) as well as heat-deactivated microsomes (HDC) were transferred into the YAS assay, and corresponding incubates of DES derivatives (DME-DES and DES-DP) were tested in the YES assay. In the YES assay, T and its HDC showed the well-established androgenic activity. The Al of T had a lower androgenic activity, demonstrating that the deactivation of the androgenic activity of T can be detected with this in vitro system. DME-DES and its HDC showed no estrogenic activity in the YES assay, whereas its AI showed an estrogenic response, demonstrating that the activation of the estrogenic activity of DME-DES can be detected with this in vitro system. Surprisingly, for DES-DP, the HDC as well as the AI showed direct estrogenic activity. Investigations of the metabolic capacity of the yeast cells itself revealed that their esterases are able to saponify DES-DP forming estrogenic DES. Thus, we demonstrated a successful integration of metabolic capacity in the YES/YAS in vitro assay, allowing for a more accurate assessment of the endocrine potential of test substances including their potential metabolite activation or deactivation.

**388 EVALUATION OF THE SKIN SENSITIZATION POTENTIAL OF HAIR-DYE, PARA-PHENYLENEDIAMINE BY NON-RADIOACTIVE MURINE LOCAL Lymph NODE ASSAY USING BROMODEOXYURIDINE WITH FLOW CYTOMETRY AND IMMUNOHISTOCHEMISTRY.**


Although para-phenylenediamine (PPD) has been widely used as hair-dye precursor throughout the world, a skin sensitization potential was suspected. In the current study, we investigated the skin sensitization potential of PPD using non-radioactive murine local lymph node assay (LLNA) with 5-bromo-2’-deoxyuridine(BrdU) using flow cytometry(LLNA:BrdU-FC) and immunohistochemistry(LLNA:BrdU-IHC) technique. Female Balb/c mice were applied topically with the increasing concentration of PPD, positive control DNCB or negative control, SLS on the dorsal of both ears for three consecutive days. To assess proliferating cells in lymph node, BrdU was injected (ip) to mice at 12hr and 24hr before sacrifice. After sacrifice, auricular lymph nodes were isolated and undergo lymphocyte preparation and tissue processing for immunohistochemistry. Proliferative response in draining lymph nodes was assessed by evaluating BrdU incorporation into cells using flow cytometry and immunohistochemistry. The weight of ear and lymph node in the mice treated with PPD, DNCB and SLS were also tested for the evaluation of irritation potential. As a result, PPD treatment significantly increased the stimulation index by LLNA:Brdu-FC and LLNA:Brdu-IHC in a dose-dependent manner and stimulation index of 3% PPD was more than three. Contact dermatitis reactions, consisting of increased ear weight and lymph node weight and the presence of inflammatory cells, were also observed by 3% PPD. These data were comparable to the previously reported EC3 value of PPD tested in LLNA assay using radioisotope. With this result, we could suggest that the new non-radioisotopic LLNA with flow cytometry and immunohistochemistry method can be a valid model for the evaluation of the skin sensitization potential.

**389 MECHANO-TOX IN VITRO MECHANISTIC TOXICITY SCREENING FOR DRUG DISCOVERY.**

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In a drug discovery setting, traditional in vitro screening, such as cytotoxicity assessment, is inadequate to identify all compounds that show toxicity in man. One of the reasons for this high false-negative rate is the failure of cells in culture to express all of the potential mechanisms for toxicity. We have put together a package (MECHANO-Tox) of mechanism-based in vitro models to address the toxicity of potential drugs on a mechanistic level. Phospholipidosis (PLD), the excessive accumulation of phospholipids in organelles within the cells, is a clinically relevant toxicological mechanism. More than 50 drugs, mainly cationic amphiphiles, are known to cause PLD. The PLD-Screen Phospholipidosis Assay, a cell-based in vitro screen, uses fluorescence to detect phospholipid accumulation. Tamoxifen causes lipid accumulation in treated patients. In the PLD-Screen, tamoxifen causes a 4-fold increase in cellular phospholipids at sub-lethal concentrations. Idiosyncratic toxicity is a major problem for drug screening. Proposed mechanisms for idiosyncratic toxicity include mitochondrial toxicity and the generation of reactive metabolites. The MITOXI-Screen Mitochondrial Toxicity assay facilitates the identification of mitochondrial toxicants. In this screen, cells are grown in conditions that render them sensitive (MITO-S) or resistant (MITO-R) to mitochondrial toxin. Treatment with Antimycin A causes complete growth inhibition of MITO-S cells at concentrations where no effect is observed on MITO-R culture. The RE-ACTI-Toxic assay detects affects of the estrogen diethylstilbestrol (DES) on the genotoxicities in vitro. Test compounds are subjected to metabolic activation in the presence of an electrophile. Adduct formation is monitored by LC/MS/MS. Tcyrine is known to form reactive intermediates that bind to CspC29 in vivo. The RE-ACTI-Tox Assay-S and DES-DP were from the reactive metabolite in vitro. Application of the MECHANO-Tox package will allow the earlier prediction of toxicity for classes of compound whose toxicity is difficult to identify through traditional studies.

**390 ASSESSMENT OF DRUG INDUCED CARDIOTOXICITY IN TRANSPARENT ZEBRAFISH.**


Cardiotoxicity is the leading cause of drug withdrawal and a major reason for clinical trial delays or failure. We and others have shown that pharmacologic response to well characterized cardiotoxins in zebrafish larvae is strikingly similar to responses in humans (Parng 2002; Langheinrich et al. 2003; Milan et al. 2003; Parng et al. 2003; Sefmera et al. 2003; Zhang et al. 2003; Parng et al. 2004a; Parng et al. 2005; Mcgrath et al. 2006; Mcgrath et al. 2008). Although zebrafish have only one atrium and one ventricle and beats per minute are faster than human (~150 vs ~70), drugs that elicit cardiomyopathy, arrhythmia, negative inotropic effects, or QT prolongation in man also cause bradycardia and slow circulation in zebrafish, drugs that rarely induce cardiotoxicity in normal clinical use did not cause significant cardiotoxicity in zebrafish. In a recent study, we assessed effects of short term exposure to 10 cardiotoxic drugs: astemizole, amiodarone, clozapine, clomipramine, erythromycin, haloperidol, lidocaine, quinidine, terfenadine, versapamil. Heart rate, rhythm, circulation and morphological endpoints were assessed visually. For heart rate and rhythm, 48 hour post fertilization zebrafish were exposed to compounds for 4 hours, and dose responses were generated. For morphological endpoints, zebrafish were exposed to compounds for 24 hours. Results for these 10 positive reference compounds were in good agreement with previously published results in zebrafish and there was 100% correlation with results in humans. The ability to assess morphology in the transparent animal without performing expensive and time consuming surgery and histology is a significant advantage. This convenient model can be used to profile compound safety and toxicity in a physiologically intact animal prior to assessment in mammals.

**391 IN VITRO MODEL TO PREDICT THE ORAL BIOAVAILABILITY OF AROMATIC AMINE HAIR DYES.**


Application via the oral route is a key requirement for the safety assessment of hair dyes. For a relevant estimation of the systemic exposure, consideration of a potential barrier for the uptake form the intestine is relevant. In pharmacology, CaCo-2 cell lines are widely used to predict the absorption rate of candidate drug compounds across the intestinal epithelial cell barrier in vitro. (Sambury et al., 2005). As an in vitro measure for the oral bioavailability we analysed the apparent permeability of test molecules across the intestinal epithelial cell barrier in vitro. The permeability characteristics (Papp of 50 mM after 60 min) of each individual molecule across the intestinal barrier was categorized by comparison to the reference substances Propranolol for high permeability (90 % absorption in humans) and ranitidine for low permeability (50 % absorption in humans). The obtained permeability category was compared to the percentage of bioavailability following oral dosage of the radiolabeled compound in a rat ADME study. For 12 out of 13 hair dyes, a high permeability was determined and for one molecule (aspirin) a low permeability was found. For all hair dyes tested, the results obtained in CaCo-2 cells correlated well with the in vivo findings. We conclude that CaCo-2 cells represent a promising in vitro model to predict the bioavailability after oral dosing and will contribute to further reduce animal testing.

**392 TESTING OF THE EPIOCULAR™ TISSUE MODEL FOLLOWING EXTENDED SHIPPING TIMES.**

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The EpiOcular tissue model has been commercially available since 1996 and is used extensively by companies to evaluate the ocular irritancy of new formulations and products. Functional quality control (QC) tests involve determination of the exposure time that reduces tissue viability to 50% (ET-50) following exposure to Triton P001
X-100 (0.3%). These QC tests show a high level of intra-lot reproducibility and long term inter-lot stability of the tissue model. Coefficients of variation (CV) in the standardized QC assay average <7% and the yearly average ET-50 has ranged from 22.0 – 27.5 minutes over the period 1996-2007. Recently, a new ocular irritancy protocol has been developed which builds on the domain of applicability to a broad variety of organic chemicals. The assay protocol was tested extensively in a European Cosmetic, Toiletry and Perfumery Association (Colipa) sponsored pre-validation study and will enter formal validation early in 2009. The current study investigated QC testing results following the extended times required for shipment of the tissue from the US to Japan. Over a 12-month period, 22 independent lots of EpiOcular tissue were shipped to Kurabo Industries (Osaka, Japan), an EpiOcular distributor. Standardized QC testing was performed 4-6 days later by Kurabo with the following results: ET-50 = 23.2 +/- 4.1 min (n = 22 tissue lots). All tissue lots tested within the QC acceptance range of 12.2 min < ET-50 < 37.5 min and compared favorably with the 1996 average ET = 24.9 +/- 6.3 minutes (upon which QC acceptance criteria are based). These results demonstrate successful shipment of EpiOcular and establish the feasibility of transfer of the in vitro ocular irritation assay to Japan and other venues requiring elongated shipping times.

**393 MODELING INDIVIDUAL VARIABILITY FROM IN VITRO DATA.**

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A major challenge in toxicology is identifying and quantifying the range and variability of human response. We have developed an in vitro model system combined with a multi-level approach to biostatistical modeling to investigate variability in human response. Twenty donors gave a blood sample six separate times over a period of at least six months. Peripheral blood mononuclear cells (PBMCs) were isolated and cultured for 48 hours in the presence of low levels (up to 200nM) inorganic mercury (Hg) and the immune stimulant lipopolysaccharide (LPS). Cells treated with LPS alone were used as a control in order to estimate baseline responsiveness. We measured cytokine production in the supernatants of the cell cultures treated with LPS alone were used as a control in order to estimate baseline responsiveness. These methods allowed us to define the range of cytokine production indicated that there is a significant and consistent dose-response increase in the production of TNFα with Hg treatment. We saw a considerable amount of variation in the baseline response to LPS (a 50-fold range, 61.5 pg/ml TNFα to 3465.4 pg/ml TNFα) and also in the subsequent response to Hg, both within and between people. Therefore, we chose to use this multi-level modeling approach with log-transformed data that allows for the quantification of both inter-individual and intra-individual variation using Markov chain Monte Carlo (MCMC) methods for analysis. "Individual-level" characteristics, such as sex and race, along with "visit-level" characteristics, such as monthly fish consumption and medication use, were tested in this model for their effects on cytokine production in response to Hg treatment. These methods allowed us to define the range of response to Hg among individuals. We have observed that while there is a considerable range in a person’s initial cytokine response to LPS, the slope of the dose-response curve to increasing Hg concentrations is highly consistent across all individuals tested.

**394 THE USE OF THE SKINETHIC HUMAN CORNEAL EPITHELIAL (HCE) MODEL TO PREDICT OCULAR IRRITANCY APPLIED TO A LARGE SET OF 435 INDUSTRIAL CHEMICALS: APPLICABILITY DOMAINS DEFINITION.**

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The 7th amendment of the EU Cosmetic Directive will lead to the ban of animal testing for cosmetic ingredients in March 2009. Thus alternative methods are urgently required in order to evaluate eye damage potential of chemicals. The HCE model from SkinEthic laboratories is a standardized epithelium reconstructed with immortalized human corneal epithelial cells. We have used the HCE model to evaluate in vitro eye irritation potential of chemicals. Because intrinsic cytotoxicity potential of chemicals is decisive factor affecting cornea after contact, we have used cell viability (MTT test) assessment as the main endpoint to classify chemicals. Adapted protocol assays were based on specific contact times and a specific post-treatment incubation period. The “long” hour + 16 hours HCE assay established on a set of 102 chemicals, was applied to an extended set of 435 industrial “real life” chemicals (EU classification: 367 Non classified, 49 R41 and 19 R36). The Prediction Model (PM) based on a 50% viability cut-off allowed the drawing up of 2 chemicals classes (irritants and non irritants). Inter and intra-batch reproducibility was assessed by using a spay control which evaluated the domain of applicability to a broad variety of organic chemicals. The assay protocol was tested extensively in a European Cosmetic, Toiletry and Perfumery Association (Colipa) sponsored pre-validation study and will enter formal validation early in 2009. The current study investigated QC testing results following the extended times required for shipment of the tissue from the US to Japan. Over a 12-month period, 22 independent lots of EpiOcular tissue were shipped to Kurabo Industries (Osaka, Japan), an EpiOcular distributor. Standardized QC testing was performed 4-6 days later by Kurabo with the following results: ET-50 = 23.2 +/- 4.1 min (n = 22 tissue lots). All tissue lots tested within the QC acceptance range of 12.2 min < ET-50 < 37.5 min and compared favorably with the 1996 average ET = 24.9 +/- 6.3 minutes (upon which QC acceptance criteria are based). These results demonstrate successful shipment of EpiOcular and establish the feasibility of transfer of the in vitro ocular irritation assay to Japan and other venues requiring elongated shipping times.

**395 EPIGENETIC CYTOXICITY OF METALS ON MOUSE EMBRYONIC STEM CELLS.**

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An ever-increasing comprehension of gene function leads to a realization that DNA sequences of targeted genes, albeit a very important hereditary indicator, are very much susceptible to a variety of modifications, triggered by exogenous elements. Such DNA alterations, along with modifications to associated proteins, interfere with gene expression by inducing errors in DNA packaging into chromatin and subsequently in replication and transcription. This study examines epigenetic cytoxicity of eight metals (As, Mn, Li, Hg, Cd, Ni, Pb, Cu) on pluripotent mouse embryonic stem (mES) cells (ES-D3, ATCC), derived from the cell mass of mouse blastocysts. During the first stage, the cells were seeded on collagen type-I coated 96 well plates, allowed to incubate for 24 hours, following one hour of exposure to corresponding metals. Cytotoxic effects were monitored using cell viability (MTT) assay. The data show the cells responded to the presence of metals by increasing proliferation, as compared to control, and directly proportional to increasing doses of the metals. In order to investigate a true epigenetic effect, a quantitative polymerase chain reaction (PCR) technique was performed. mES cells were seeded on 6 well plates, as above. At the end of the exposure period, cells were collected, pelleted and mRNA was extracted using Dynabeads® mRNA Direct™ Kit. The three template genes, Rad18, Top5a, and Ogg1 were chosen for their known role in DNA damage repair and genome stability. The PCR data reveals a decrease in expression levels of the three genes. Such a decrease in expression of DNA repair genes correlates with mutational events, thus suggesting that alteration of DNA repair mechanisms in the presence of acute metal exposure contribute to a variety of epigenetic events. (Supported in part by grants from Alternatives Research and Development Foundation, 35532-985; International Foundation for Ethical Research, 35262-985).

**396 A YEAST-BASED MODEL SYSTEM TO EVALUATE TOXICITY TO HUMAN STEROID HORMONE RECEPTOR SIGNALING.**

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Hsp90 protein complexes regulate key signaling pathways. Steroid hormone receptors (SHRs) are among the best characterized substrates for Hsp90 regulation and they serve as therapeutic targets in hormone-dependent cancers. Drugs that interact with Hsp90 have been developed as cancer therapeutic agents. However, drugs targeted to a SHR or Hsp90 chaperone-substrate complex may have toxic effects on other receptors or substrate proteins. We developed a model yeast system engineered to express human androgen, estrogen α, estrogen β, glucocorticoid, mineralocorticoid, or progesterone hormone receptors along with relevant reporter genes to explore this problem. Radiocil, an Hsp90 inhibitor, preferentially inhibited signaling by SHRs. We observed SHR signaling inhibition in yeast cells treated with radicicol concentrations (3nM-3μM) that were not generally toxic. Low doses of radicicol (<1 μM) did not decrease cell growth, but inhibited signaling by all six SHRs in a dose-dependent manner. IC50 values for signaling inhibition by radicicol were similar for all six SHRs (0.4 to 1.2 μM). Targeting one SHR in a cancer with this type of Hsp90 inhibitor may have off-target effects on other SHRs. This model system may be useful for assessing the toxicity of pharmaceutical agents and xenobiotics (e.g. endocrine disruptors) that exert effects on steroid hormone signaling pathways. Supported by NIH 5R33CA101622-03.)
A HIGH CONTENT SCREENING (HCS) APPROACH FOR IDENTIFYING ANTIBACTERIALS THAT IMPAIR MITOCHONDRIAL PROTEIN SYNTHESIS IN CELLS.

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Over 50% of antibacterials are protein synthesis inhibitors that target the bacterial ribosome as their primary mode of action. One of the challenges associated with the development of these compounds is mitochondrial toxicity since there is a structural similarity between bacterial ribosomes and the host’s mitochondrial ribosomes. Impairment of protein synthesis within mitochondria leads to depletion of mitochondrial DNA-encoded proteins. Hence, identifying drugs that impair protein synthesis in the off-target, the mitochondrion, is important in the drug discovery process. We describe here a high content screening (HCS) approach for identifying drugs that impair mtDNA-encoded protein synthesis in cells. HepG2 cells seeded in 96-well format were grown in a selection of drugs in a dose-response manner over a period of 5 days. The effect of each drug on mtDNA-encoded protein synthesis was assessed using an antibody against cytochrome c oxidase together with a fluorescently conjugated secondary antibody. Cytochrome c oxidase is a mitochondrial protein which has 3 subunits encoded by mtDNA and made by mitochondrial ribosomes. The effect of the drug on overall cytotoxicity was assessed with the nuclear stain, DAPI. Our results show that the assay is a high throughput method of identifying such drugs such as many antibacterials which impair mtDNA-encoded protein synthesis.

INTER-LABORATORY VALIDATION STUDY OF IN VITRO EYE IRRITATION TEST; SHORT TIME EXPOSURE (STE) TEST.

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Short Time Exposure (STE) test is an easy in vitro eye irritation test that assesses cytotoxicity in SIRC (rabbit corneal cell line) cells following a one 5 minute dose treatment. To assess reliability (including transferability and inter-lab reproducibility) and relevance of STE test, a validation study with 5 laboratories was conducted. The standard protocol of the STE test for this study was prepared and fixed before study began. At first, three standard substances (sodium lauryl sulfate, SLS; calcium thioglycollate, CT; and Tween 80, TW80) were evaluated to confirm reliability of the STE test. Three experiments of each substance were evaluated using 5%, 0.5% and 0.05% test material and saline as vehicle. The mean cell viability (CV) was then calculated. Both the CV and rank classification based on the prediction STE model were compared with their background data from the STE test developer. The CV of SLS and TW80 was less than 7% and more than 90% respectively, for all three doses in all laboratories. For CT 9%, 0.5% and 0.05% solutions showed less than 20% CV, about 70% CV, and more than 85% CV, respectively, in all laboratories. These CV values were similar with the developer’s background data. The rank classification of SLS, CT and TW80 were 3, 2 and 1 respectively, in all 5 laboratories. Also, these ranks were the same as developer background data. From these data, a good reliability of the STE test was obtained. To be completed by the end of 2008, we are further evaluating this assay’s reliability and relevance by testing in 5 labs for additionally 25 substances. This validation study is supported by JSAEE (Japanese Society for Alternative to Animal Experiments).

IN VITRO SKIN IRRITATION ASSESSMENT OF DYES AND COLORING CHEMICALS USING THE ECVAM VALIDATED EPISKIN ASSAY.


The in vitro reconstructed human epidermis EpiSkin has been scientifically validated in 2007 as a full replacement model for the Draize rabbit acute skin irritation test. The test is based on tissue viability assessment by using the MTT test and allows the discrimination between irritants (R38) and not classified (NC) chemicals. The MTT assay is a standard colorimetric method: Yellow MTT is reduced to pure formazan by epidermis living cells. Therefore, due to the reading method, any chemical able to color the tissue or induce indirect and non specific coloration absorbing closely to formazan can result in a possible viability overestimation. The purpose of this study was to assess the use of the validated EpiSkin protocol for the in vitro prediction of colored chemicals irritancy potential (e.g. hair dyes). Colored chemicals as dyes can be retained in the epidermis or difficult to wash-off and induce a possible residual staining. Consequently, after formazan extraction, non specific remaining color may modify final readings. In order to eliminate colorimetric interference, we introduced specific controls fitting to the validated protocol course. Control tissues followed the same treatment steps than treated ones, MTT step excepted. Stratum corneum were removed from all tissues before color extraction. These controls enabled the quantification of non specific optical density (NSOD) and the definition of acceptable limits. Therefore calculations take into account only the true OD related to mitochondrial activity of living cells. Histological analysis was conducted in order to document strong coloring chemicals results. R38 and NC dyes have been evaluated by using this strategy.

We showed that the EpiSkin assay is a suitable and reliable method for in vitro skin irritation prediction of chemicals likely to color tissues. The applicability domain of the EpiSkin skin irritation assay can therefore be extended to these chemical families.

QUANTITATIVE STRUCTURE–ACTIVITY RELATIONSHIP (QSAR) MODELS AS SUITABLE ALTERNATIVES FOR EXPERIMENTS WITH FISH.

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Quantitative-structure–activity relationship (QSAR) models are expected to play a crucial role in reducing the number of animals used for toxicity testing due to the adoption of the new European Union (EU) chemical control system called Regulation, Evaluation, and Authorization of Chemicals (REACH). In the present study, the potential of using in vitro toxicity data to predict the acute in vivo toxicity of chlorinated alkenes to fish was investigated. Chlorinated alkenes are widely used in the production of adhesives and lubricants, have large production volumes, and therefore have a large potential for environmental pollution. A fast, cost-effective method that minimizes animal use is therefore required to assess their toxicity. Acute cytotoxicity of a series of chlorinated alkenes to Chinese hamster ovary cells was observed at concentrations similar to those that have been shown previously to be toxic to fish. Strong correlations exist between the acute in vitro toxicity of the chlorinated alkenes and (i) hydrophobicity (modeled by the calculated log Kow (octanol–water partition coefficient); r2 = 0.88, r2 ext = 0.85 and r2 ext = 0.74) and (ii) in vivo acute toxicity to fish (r2 = 0.76). A QSAR model has been developed to predict in vivo acute toxicity to fish based on the in vitro data and even on in silico log Kow data only. The developed QSAR model is applicable to chlorinated alkenes with up to 10 carbon atoms, up to eight chlorine atoms, and log Kow values lying within the range from 1.71 to 5.70. Out of the 100 196 compounds on the EU market, the QSAR model covers 77 of them. These findings demonstrate that in vitro experiments and even in silico calculations can replace animal experiments and even in vivo experiments in the prediction of the acute toxicity of existing and new chlorinated alkenes that have similar chemical structures as the ones tested in the present study.
specificity. Microarray analysis of gene expression showed that chlorpromazine treatment with UVA irradiation caused changes in gene expression over time that were not observed without UVA irradiation. These genes include those for keratins, collagens and fibronectins. EPARS is a accurate and sensitive test for detecting photosensitive substances or doses representative of those that cause actual human skin reactions. Thus, EPARS is a highly predictive photosensitivity assay, with endpoints of inflammatory mediator and gene expression that allow for investigation into the mechanisms of photosensitivity in a wide variety of consumer products.

402 AN IMPROVED IN VITRO MODEL FOR SAFETY SCREENING OF DRUGS IN DEVELOPMENT: ACCURATE EVALUATION OF DRUG-MEDIATED CYP INHIBITION USING H-CASS MICROSOMES.

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Human liver microsomes are a well-characterized in vitro model for identifying cytochrome P450 (CYP) inhibition during drug development. Use of hepatic microsomes from human donors reduces concerns about species differences and also reduces the use of animals during preclinical development. However, the development of this model is limited by variations in enzyme activity among different microsomal preparations, variations among the isoforms within a single preparation, and also by overall low CYP isoform enzyme activity. In drug safety studies, low microsomal CYP enzyme activity can preclude accurate quantitation of CYP inhibition. Selective pooling of well-characterized microsomes can overcome these limitations. Using a proprietary algorithm, we created H-class microsomes that provide consistently higher activity across the panel of clinically relevant CYP isoforms, particularly those isoforms that are lower in activity. IC50 values of known inhibitors of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 were determined using isoform-selective substrates. The rate of CYP substrate metabolite formation was measured using HPLC-UV or LC/MS assays. Under these conditions, the average dynamic range of CYP enzyme activity increased by 90% in H-class microsomes relative to microsomes with lower activity, avoiding the occurrence of below quantifiable limit (BQL) data and allowing for accurate estimation of IC50 values for H-class microsomes. A highly important emerging tool for accurate CYP inhibition characterization during drug development.

403 A TRANSGENIC MODEL FOR ARSENIC METHYLATION AND ITS BIOLOGICAL EFFECTS IN DROPHILIA.

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When inorganic arsenic is ingested by humans there is metabolic conversion to methylated arsenical species, readily detectable in urine. Contrary to an earlier belief that methylated species represented metabolic detoxification products, it is now accepted that certain methylated arsenicals are more toxic than their inorganic progenitors, particularly where cells are exposed chronically to low levels. The genotoxicity of such methylated species, however, caused, seems to be much greater than that of inorganic arsenic, and is likely to be relevant to the carcinogenic potential exhibited by long term arsenic exposure. We wished to explore this potential in a highly genetically manipulable background in order to shed light on the critical mechanisms and pathways involved in arsenic-mediated carcinogenesis. Others had shown that Drosophila exposed directly to methylated arsenicals exhibit a clearly analogous genotoxic response, but fail to do so when fed inorganic arsenite owing to apparent absence of the relevant methylation pathway. We circumvented this by introducing the human gene for the enzyme arsenic(III) methyltransferase (AS3MT), under easily manipulated regulatory control, into Drosophila. We have confirmed that transgenic flies can be induced to express an antigenically cross-reactive form of the human enzyme, and that its expression appears to affect neither the normal developmental cycle nor fertility of the transgenic lines. Nor is there any obvious differential toxic effect (e.g., enhanced mortality) when arsenite is fed to transgenic lines either uninduced or induced for expression of AS3MT. Nonetheless, when larval extracts from such lines were analyzed for arsenic speciation via HPLC-ICP-MS it was found that both mono- and dimethylated arsenical species were present in large amounts, but only when AS3MT was induced. Thus the model can now be used to explore the nature and extent of genotoxic outcomes in many informative genetic backgrounds, as well as the effects of different polymeric variants of AS3MT found in exposed human populations.

404 ENVIRONMENTAL MUTAGENESIS AND CYTOCHROME P450 ACTIVITIES IN THE NEMATODE CAENORHABDITIS ELEGANS.

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The nematode Caenorhabditis elegans is emerging as an important alternative model in toxicology. It offers unique advantages over conventional models, including ease of maintenance, short life cycle, genetic manipulability, well-developed bioinformatics, and high-throughput capability. A major concern about using the C. elegans model in toxicology, however, is a lack of understanding about its xenobiotic metabolism capabilities. There is also limited information on how C. elegans responds to known human mutagens. The current study addresses these questions using two different approaches: (1) testing toxicity and DNA damage induced by known environmental mutagens that require metabolic activation, in strains of C. elegans carrying mutations in DNA repair genes; and (2) characterizing the catalytic activities of C. elegans microsomes using standard CYP substrates. Aflatoxin B1 (AFB1) exposure was found to significantly inhibit (P < 0.05) the growth and reproduction of C. elegans, and have a greater effect on repair-deficient than repair-proficient strains. Benzo[a]pyrene (BaP) was no more toxic to repair-deficient than repair-proficient animals, however. These results suggest that C. elegans metabolizes AFB1, but not BaP, to DNA-reactive forms. Furthermore, C. elegans microsomes were found to have a lower cytochrome C reductase activity compared to rainbow trout microsome (6.8 vs 29.6 nmol/min mg, respectively) and show no 7-ethoxycoumarin-O-deethylase (EROD) activity at a detection limit of 2 pmol/min mg. Further experiments are being carried out to identify any DNA damage caused by AFB1 and BaP exposure as well as the presence of any higher eukaryote CYP-like activities in C. elegans.
Isolated trout livers were perfused using methods designed to preserve tissue viability. EPA/ORD/NHEERL/Mid-Continent Ecology Division, Duluth, MN.

3.7 to 4.8). The effect of fu on CLh was studied by manipulating the concentration of bovine serum albumin in the perfusion buffer using an experimental design wherein each liver acted as its own control. Free chemical concentrations after perfusion were measured by trans-epithelial electrical resistance (TEER) and sodium fluorescein (NaFl) leakage, and inflammatory cytokine release (interleukin (IL)-1α, IL-1β, IL-6, and IL-8) were examined. When compared to untreated controls, two irritating test articles, benzalkonium chloride and nonoxynol 9, reduced tissue viability to <10% and TEER to <60% and increased NaFl leakage by 11-24% and IL-1α and IL-1β release by >100%. Four other non-irritating materials had minimal effects on these parameters. Assay reproducibility was confirmed by testing the chemicals using three different production lots and by using tissues derived from cells of three different donors; coefficients of variation were <12%. In conclusion, decreases in TEER and cytokine release appear to be useful endpoints for preclinical toxicity screening of chemicals, formulations, and medical devices and should be investigated further as an in vitro alternative for the current RVI assay.

Isolated trout livers were perfused using methods designed to preserve tissue viability and function. Liver performance was evaluated by measuring O2 consumption, vascular resistance, K+ leakage, glucose flux, lactate flux, alanine aminotransferase leakage, and metabolic clearance of 7-ethoxycoumarin (CLh,7-EC) changed in nearly direct proportion to changes in the free concentrations of 7-EC. Clearance rates corrected for declining metabolic activity equation was subsequently developed to correct measured levels of clearance for decreasing metabolic activity over time. To illustrate the value of this preparation, experiments were conducted to examine the effects of protein binding on hepatic clearance of 7-EC. Clearance rates corrected for declining metabolic activity (CLh,7-EC,CORR) changed in nearly direct proportion to changes in the free concentrations of 7-EC, effluent to the liver, as predicted by theoretical models of liver function. Additional studies were performed to characterize the concentration-dependence of 7-EC metabolism. Metabolic activity increased linearly with the total concentration of 7-EC in the effluent to the liver in constant levels of CLh,7-EC,CORR values for four livers averaged 12.0 ± 5.0 mL/h/g-liver (mean ± SD, n = 60 individual determinations) and were in good agreement with an estimate of hepatic clearance obtained by extrapolating published in vitro data from isolated trout hepatocytes. The extended viability of isolated trout livers achieved in this study creates new opportunities for research on hepatic function in fish. This abstract does not necessarily reflect U.S. EPA policy.

METABOLISM OF 7-ETHOXYCOUMARIN BY ISOLATED PERFUSED RAINBOW TROUT LIVERS.

The venous equilibrium model is widely used to describe hepatic clearance (CLh) this model predicts that CLh will approximately equal the product of intrinsic clearance (Clint) and efferent to the liver, as predicted by theoretical models of liver function. Additional studies were performed to characterize the concentration-dependence of 7-EC metabolism. Metabolic activity increased linearly with the total concentration of 7-EC in the effluent to the liver in constant levels of CLh,7-EC,CORR values for four livers averaged 12.0 ± 5.0 mL/h/g-liver (mean ± SD, n = 60 individual determinations) and were in good agreement with an estimate of hepatic clearance obtained by extrapolating published in vitro data from isolated trout hepatocytes. The extended viability of isolated trout livers achieved in this study creates new opportunities for research on hepatic function in fish. This abstract does not necessarily reflect U.S. EPA policy.

AN EVALUATION OF THE VENOUS EQUILIBRIUM MODEL FOR HEPATIC CLEARANCE USING ISOLATED PERFUSED RAINBOW TROUT LIVERS.
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410 REDUCTIVE METABOLISM OF N-(1-(3,4-METHYLENEDIOXYPHENYL) PROPAN-2-YL)-N-METHYLDIAMINONITROMETHANE IN HUMAN HEPATOCYTES.
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3,4-Methylenedioxymethamphetamine (MDMA) is a designer drug with a central nervous system stimulant and hallucinogenic action, and controlled all over the world. Metabolism of MDMA has been studied well and Cytochrome (CYP) 2D6 and 2C19 are responsible for the metabolism. MDMA is oxidized to MDA and MDA is further metabolized to MDEA (N-desmethylmethamphetamine (MDMA) is reduced into MDEA). The metabolism of MDMA has been studied well and Cytochrome (CYP) 2D6 and 2C19 are responsible for the metabolism. MDMA is oxidized to MDA and MDA is further metabolized to MDEA. The metabolism of MDMA has been studied well and Cytochrome (CYP) 2D6 and 2C19 are responsible for the metabolism. MDMA is oxidized to MDA and MDA is further metabolized to MDEA.
various coatings specific to the intended use. The core composition of QDs commonly consists of Cd and Se, and less commonly, Hg, Te, In and As. While metals utilized for this core composition can be toxic, the wide variety of QD preparations makes it difficult to predict cellular response in vitro or in vivo. In order to gain a better understanding of their disposition within the liver, human hepatocellular carcinoma (HepG2) cells were exposed to poly(maleic anhydride-alt-1-tetradecene), tri-n-octylphosphine oxide (PMAT-TOPO) co-polymer CdSe-based QDs at varying concentrations (5-100 nM). QD uptake into HepG2 cells was evaluated at 6 and 24 hours using both confocal and traditional fluorescent microscopy. HepG2 cells sequestered QDs from culture media in a time- and dose-dependent manner. QD uptake was pronounced by 3 hours of exposure. Cell viability (as measured by LDH release) was not altered. Our preliminary results suggest that this particular class of stable QDs has no adverse effects on HepG2 cells under the conditions tested. Currently, we are conducting similar experiments with human primary hepatocytes. Primary cells reflect pharmacokinetic aspects of liver more closely than HepG2 cells, including absorption, distribution, metabolism, and excretion of xenobiotics. In contrast to HepG2 cells, the availability of primary cells is limited. Therefore, we are using HepG2 cells to optimize experimental parameters which we will then test with primary hepatocytes. This approach optimizes the use of primary cells and also assesses the suitability of HepG2 cells as an in vitro model for nanoparticle toxicity studies as compared to primary hepatocytes. This work was supported by NIEHS Grants R01ES016189.

**412 MITOCHONDRIAL TOXICITY OF USNIC ACID: MECHANISM OF ACTION CONTRIBUTES TO ITS IDIOSYNCRATIC HEPATOTOXICITY.**


The lichen metabolite usnic acid has been promoted as a dietary supplement for weight loss, although serious cases of hepatotoxicity have been associated with its use. Even under conditions accumulating that mitochondrial impairment contributes to the etiology of drug-induced hepatotoxicity. Accordingly, we examined effects of usnic acid (UA) on mitochondrial function using isolated organelles, transformed HepG2 cells, and primary rat and human hepatocytes. In freshly isolated rat hepatocytes, usnic acid rapidly depleted ATP and was cytotoxic in a dose and time dependent manner. In isolated rat liver mitochondria, usnic acid profoundly uncoupled respiration and exacerbated Ca2+-induced permeability transition. At higher concentrations it inhibited immunocaptured mitochondrial Complex-V (ATPase).

In intact HepG2 cells, UA decreased mitochondrial membrane potential, depleted reduced glutathione, resulting in cell injury. The observed mitochondrial uncoupling would consume calories and dissipate the energy as heat, which would facilitate weight loss. However, such mitochondrial impairment yields cytotoxic oxidative and bioenergetic stress that induce irreversible mitochondrial failure, and hence the hepatotoxicity associated with usnic acid.

**413 EFFECT OF ROTENONE AND CYCLOSPORIN A ON HUMAN AND RAT LIVER SLICES.**


Liver slices have emerged as a model of toxicity that bridge between simpler in vitro cell culture models and fully integrated in vivo models of toxicity. The present study was undertaken to examine the toxicity of rotenone (ROTE), a mitochondriald poison, and cyclosporine A (CSA), an inhibitor of the bile salt export pump (BSEP). Liver slices from both rat and human donors were cultured for 6, 24 and 88 hours in the presence of either DMSO (0.1%), rotenone or CSA at 0.1, 0.3, 1.0, 3.0, 10, 30, and 50 (CSA) or 100 μM (ROTE). Biochemical endpoints including ATP, LDH, K+ were measured at each time point. In addition, mRNA was extracted for each slice of each group and transcript profiles obtained using either rat or human microarrays (Affymetrix). Finally selected slices were analyzed histologically to determine the extent of toxicity and to correlate findings with the biochemical and genomic data. Rotenone exhibited toxicity in liver slices as early as 6h with ATP being the most sensitive endpoint. ATP was diminished by >50% at 6h by 10 μM ROTE. Liver slices were resistant to CSA up to 50 μM with little biochemical evidence of toxicity. For both compounds, the gene expression results could be categorized into early transient changes, early persistent changes, and late changes. In addition, molecular evidence could be delineated consistent with the proposed toxicologic activity of each compound. The comparison of rat and human data shows congruence as well as differences in how liver slices from different species respond to these compounds.

**414 OPTIMIZING CULTURE CONDITION FOR IMPROVED PREDICTION OF HEPATOTOXICITY OF HIGH PROTEIN BINDING COMPounds.**


In vitro toxicity tests are generally performed under conditions with relatively low protein concentrations. Theoretically, non-protein bound fraction of drugs are pharmacologically and toxicologically active in vivo. Therefore, the concentrations having effects in such test systems can not be directly compared with in vivo plasma concentrations of high protein binding drugs. We investigated the value of in vitro predictive hepatotoxicity tests performed in the presence of human serum albumin and human alpha-1 acidic glycoprotein. Cytotoxicity test against human hepatocytes, mitochondrial membrane potential in hepatocellular carcinoma HepG2 cells, and steatosis assay (fat accumulation) in immortalized human hepatocyte FA-N4 cells were performed using reference hepatotoxic and non-hepatotoxic drugs. Therapeutic index, the ratio of the toxic concentration to the peak plasma concentration at effective dose, was employed to assess the value of these tests. As a result, hepatotoxic drugs tended to have narrow therapeutic indexes as compared with non-hepatotoxic drugs in all three tests. Toxic effects of drugs with high protein binding (>90%) were generally impaired by adding plasma proteins to the cell culture systems. However, the degree of improvement of toxic effects was not correlated well with plasma protein binding ratio of these drugs. That is, some drugs showed marked decrease in toxic effect but the others showed only marginal decrease nevertheless comparable high protein binding levels. Consequently, it is more valuable to actually perform the in vitro toxicity tests with physiological protein concentrations than to speculate from the plasma protein binding ratio of the drug and the results of in vitro toxicity tests performed with low protein concentrations.

**415 XENOBIOTIC METABOLIZING ENZYME (XME) EXPRESSION IN AGING HUMANS.**


In the presence of foreign compounds, metabolic homeostasis of the organism is maintained by the liver's ability to detoxify and eliminate these xenobiotics. This is accomplished, in part, by the expression of XMEs, which metabolize xenobiotics and determine whether exposure will result in toxicity. Our previous microarray studies demonstrate that age alters the expression of XMEs in rats and mice. To determine if aged humans also exhibit changes in XME expression, we examined gene expression profiles using Affymetrix Human U133 Plus 2.0 arrays in liver samples from young (21-45 years) and old (60+ years) men and women. Five to seven livers per age group were profiled. Differentially expressed genes (DEG) were determined using a one-way ANOVA (p<0.05) followed by a Benjamini-Hochberg FDR multiple testing correction (q<0.05) using Rosetta Resolver®. Principal component analysis showed a clear age-dependent separation in expression profiles between young and old hepatic transcripts in males and females. We identified 370 genes that were altered between young and old men and 1163 genes that were altered between young and old women. Top canonical pathways were identified using Ingenuity® Pathway Analysis. Protein ubiquitination pathway is affected in both older men and women. We found that age caused minimal numbers of changes in the gene expression of XMEs (8 in males and 53 in females between young and old, respectively). Most of these changes were in the expression of Phase III genes (transporters). The expression of solute carriers increased with age in men, and the majority decreased with age in women. In addition, we identified 93 or 49 gene expression differences between men and women in the young or old groups, respectively, some of which were XMEs. These studies indicate that the livers from aging humans exhibit a number of changes in XMEs that may lead to differences in the metabolism of xenobiotics. (This abstract does not necessarily reflect US EPA policy).

**416 THE FETAL/NEONATAL MOUSE LIVER EXHIBITS TRANSCRIPTIONAL FEATURES OF THE ADULT PANCREAS.**


Metabolic homeostasis of the organism is maintained by the liver’s ability to detoxify and eliminate xenobiotics through the expression of xenobiotic metabolism enzymes (XME). The fetus and neonate have been hypothesized to exhibit in-
creased sensitivity to xenobiotic toxicity. This project was designed to examine differentiated expression genes (DEG) in early life stages in the livers from male C57BL/6 and C3HHeJ mice. Gene expression profiles were generated using Affymetrix Mouse 430 2.0 arrays for early life stages (gestation day (GD) 19, postnatal day (PD) 7, PD32) and compared to young adults (PD67). Principal component analysis (PCA) showed a clear age-dependent separation in expression profiles between GD19, PD7, and PD67 hepatic transcripts. Differentially expressed genes (DEG) were determined using a one-way ANOVA (p<0.05) by Rosetta Resolver®. A Benjamin-Hochberg FDR multiple testing correction (p<0.05), and a fold-change cutoff of 1.5. Gene expression changes were the most abundant at the early time points in male C57BL/6 (10.068 and 5.989 DEG for GD19 and PD7 respectively). We found 139, 93, and 12 XMEs significantly altered in the GD19, PD7, and PD32 when compared to PD67, respectively. qRT-PCR confirmed altered expression for selected genes. Because the fetal liver is a site of hematopoiesis, we hypothesized that some of the differences in gene expression between GD19 and PD67 were due to the presence of hematopoietic progenitor cells. We compared the liver profiles with a mouse tissue microarray dataset which included adult tissues involved in hematopoiesis. Suprisingly, PCA and hierarchical clustering showed embryonic liver clustering with embryonic and adult pancreas and separate from adult liver and hematopoietic tissues. These results indicate that there are extensive gene expression differences between early (fetal, neonatal) and adult liver and that the fetal/neonatal liver exhibits transcriptional similarities with the pancreas. (This abstract does not necessarily reflect US EPA policy).

**417 GSH MODULATION, A STRESSED LIVER MODEL TO EVALUATE DRUG-INDUCED HUMAN LIVER INJURY.**


GSH levels are modulated in human liver slices to provide insight into whether drug induced hepatotoxicity is enhanced by a poor liver GSH status. Liver slice GSH levels were modulated in several ways: 1) depletion of GSH with BSO (L-buthionine-S-sulfoximine) which inhibits the synthesis of GSH, 2) reduction of GSH by APAP (acetaminophen) at a concentration associated with hepatotoxicity, 3) reduction of GSH by DEM (diethyl maleate) which is a thiol conjugating agent, 4) reduction of GSH by BSO, DEM or APAP were not further reduced by μM MMI. An increase in liver slice AST and ALT leakage was enhanced by co-treatment with RH being the most sensitive to this effect; most likely due to their low basal GSH levels. In contrast, heps with high basal levels of GSH were not as sensitive to this effect. However, there was still a clear response to the HTX in all species except for PD32, where RH was the most sensitive. We conclude that this assay is a robust platform for discovery of RH with the development of a screening assay in discovery with RH and a development assay with DH, HH, MKH in later stage drug development.

**419 DEVELOPMENT OF IN VITRO TOXICOGENETIC MODELS FOR HEPATOTOXICITY.**

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Numerous studies support the fact that a genetically diverse mouse population may be useful as an animal model to understand and predict toxicity in humans. We hypothesized that cultures of hepatocytes obtained from inbred and mixed mouse strains can produce data indicative of inter-individual differences in in vivo responses to hepato-toxins. In order to test this hypothesis and establish whether high-throughput in vitro studies using cultured hepatocytes from genetically distinct mouse strains are feasible, we aimed to standardize cell isolation and culture conditions and determine whether near-physiological maintenance of the cells isolated from different mouse inbred strains can be achieved. Hepatocytes were isolated from 15 strains of mice (AJ, B6C3F1, BALB/c, C3H/HeJ, C57BL/6J, CAST/EJ, DBA/2J, FVB/NJ, BALB/cByJ, AKR/J, MRL/MpJ, NOD/LtJ, NZW/LacJ, PWD/Ph) and WSB/EiJ) and cultured for up to 7 days in traditional 2D culture. The cells have been assessed for viability and functionality on a daily basis by measuring production of lactate, pyruvate, and urea, as well as leakage of lactate dehydrogenase as a measure of membrane integrity. We also employed calcine and ethidium fluorescence staining to assess cell viability at 1, 3, 5 and 7 days of culture. Our data shows that high yield (48 to 87 million hepatocytes/mouse) and viability (86 to 98%) can be achieved across a panel of strains. Furthermore, we conclude that cell function of hepatocytes isolated from different strains and cultured under standardized conditions is comparable and cells remain viable and metabolically active as indexed by lactate, pyruvate and urea production. These experiments open new opportunities for high-throughput and low-cost in vitro assays that may be used for studies of toxicity in a genetically diverse population. (Supported, in part, by grants from NIHES R01-ES051241 and US EPA RD-833825)

**420 PRECISION-ORGAN SLICE TECHNOLOGY TO EVALUATE LIVER TOXICITY OF PERFLUORINATED COMPOUNDS (PFCs).**

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PFCs are a class of widespread environmental contaminants that are resistant to hydrolysis and persist in the environment. Once absorbed they are poorly eliminated from the body and have been shown to be hepatotoxic and cause developmental, reproductive, immunological and endocrine effects in rodents and non-human primates. To evaluate chain length and functional group dependent effects of PFCs directly without the influence from absorption or elimination, an in vitro liver slice model system was proposed. The objective of this study was to assess the use of liver slices to evaluate and compare the toxicity from exposure to a single PFC (initially assessed using perfluorooctanoate, PFOA) vs. mixtures of PFCs. Precision-cut rat liver slices were prepared with a Brendel/Vitron tissue slicer and incubated in media for up to 48 hours in a high oxygen environment. Liver slices were exposed to 0.1% DMSO (vehicle) or PFOA (400 μM; serum concentration in rats associated with toxicity) for 6, 12, 24 and 48 hours. Viability of liver slices was assessed by measuring ATP and marker enzyme activities to assess cytotoxicity (AST, ALT, LDH, SDH) and evaluated H&E-stained slices histopathologically. In general, ATP levels were lower in slices exposed to PFOA compared to vehicle exposed slices at 12 hours of exposure. After 12 hours, all marker enzyme activity measured in PFOA exposed liver slices were at or below 50% of values measured in vehicle exposed slices and approached 0% by 48 hours. These results correlated with changes observed histopathologically in which ~30% of the cells in slices exposed to PFOA for 12 hours appeared viable. Slices exposed to the vehicle were found to be ~30% viable at 48 hours, while no viable cells were observed at either 24 or 48 hours in

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421 CROSS-FUNCTIONAL DRUG CLASS COMPARISON OF THE TRANSCRIPTOMES OF IDIOSYNCRATIC HEPATOTOXICANTS IN PRIMARY HUMAN HEPATOCYTES: ASSOCIATION WITH KNOWN DEATH MEDIATORS.

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Idiosyncratic drug reactions (IDRs) continue to confound development of new drug candidates, and these unanticipated toxicities pose a substantial risk to the public health and to the pharmaceutical industry in general. These adverse reactions are not predictable using standard pre-clinical strategies and therefore are unrecognized until the very late stages of clinical evaluation. This type of toxicity is not limited to any one class of drugs, but has been manifested in a wide spectrum of pharmacologic classes including quinolone antibiotics, thiazolidinediones (TZDs), and in neurological therapies. Uncovering common hepatotoxic mechanisms that link distinct classes of compounds would add great value to the fundamental understanding of late-stage adverse reactions. To compare potential similarities in mechanisms of hepatotoxicity, primary human hepatocytes were exposed to 3 well-known drugs associated with hepatic IDRs, including trovafloxacin (quinolone class), tigloylzone (TZD class), and nefazodone (anti-depressant) for 24h. Levofloxacin and rosiglitazone, devoid of hepatic IDRs, were also evaluated as negative controls. Subsequently, these cell systems were analyzed for transcriptomic changes using Affymetrix human genome microarrays. Each of the three test items invoked a dose-dependent increase in the total number of gene expression changes. With potential toxicological implications, the IDR-invoking compounds modulated over 300 transcripts in common and apart from the negative comparators. Further interrogation of the roles of these gene products revealed an association with known death mediators, such as TGF-β, GADD45β, PARP, MitoEaf1, and IRF-1. In addition, critical genes controlling cell cycle were consistently dysregulated by the IDR associated agents, including CCNL1, BCL3, MDM2, and IGFBP. Thus, evaluation of overlapping transcriptomic changes, in the context of cross pharmaceutical class IDRs, may provide insights into common mechanisms of toxicity.

422 MULTIPARAMETRIC HEPATOTOXICITY SCREENING IN HEPG2 CELLS WITH COMPARISON IN PRIMARY HEPATOCYTES.

Cell Biology, MDS Pharmaceutical Services, Bethel, WA. Sponsor: J. Britaux.

Drug-induced hepatotoxicity remains a major cause for failures during drug development. Hepatic apoptosis induction, phospholipidosis and neutral lipid accumulation detection late in the drug development pipeline has contributed to these setbacks. Earlier identification of potential liabilities can result in recommendations to minimize these risks during lead optimization as well as understand their relevance in provoking clinical hepatotoxicity. Development of a protocol in vitro multiplexed high content screening platforms have enabled more comprehensive predictive early toxicity assessments previously only attainable in more complex in vivo models. In this study we used multiplexed high content screening with automated fluorescence microscopy and image analysis in a 384-well microtiter plate format to identify and quantify apoptosis induction, phospholipidosis and neutral lipid accumulation potential of sixteen compounds in HepG2 cells and compared findings obtained using this approach with primary hepatocytes. Selected compounds included acetaminophen, amitriptyline, aminotriazole, ceftriaxone, chlorpromazine, isoproterenol, tigloylzone, rosiglitazone, valproic acid, propranolol, aminodarone, cyclosporine A, mehtotrate, staurosporine, toloxtenon and terfenadene. High concordance was found with the reported toxicity profiles for the tested compounds. Comparative responses in primary hepatocytes were attained using a novel data analysis approach. Our high-throughput in vitro multiplexed hepatotoxicity screen offers comprehensive predictive information allowing pre-selection of drug scaffold design with longer-term risk considerations and can provide evaluations in normal primary hepatocytes.

423 ASSAY VALIDATION OF HEPATOTOXICITY IN qHTS USING HUMAN PLATEABLE CRYOPRESERVED HEPATOCYTES.

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The liver is the key organ for the biotransformation of exogenous and endogenous compounds to aid in their clearance from the body. In many cases, the metabolism of these compounds is critical to protect the body from toxic levels, but in some cases, the metabolites of parental compounds may incur hepatotoxicity. Taking the metabolism of compounds into account is essential not only to drug development, drug safety and environmental health issues, but also to evaluate the risks associated with injury to the liver. The ability to screen compounds to assess hepatotoxicity is crucial, but has been traditionally limited to in vivo testing for accurate evaluation, which is not desirable for ethical and efficiency reasons. In vitro testing, which reduces animal use, has become a viable option with the advent of specific markers and sensitive reagents. The development of quantitative high throughput screening (qHTS) allows one to test an enormous number of potential toxins in research and commercial laboratories, like primary human hepatocytes. For proof of concept, we have developed methods to utilize plateable cryopreserved human hepatocytes (PCHH) in a 1536-well format in order to screen known toxic compounds. PCHH were plated at two different cell concentrations in collage-coated 1536-well plates. Test compounds, dosed over a four-log range, were added four hours after plating and incubated for an additional 40 hours. Intracellular ATP content was measured to determine IC50 for 12 compounds, including donoxubicin, taminofen, staurosporine, and phenylmercuric acetate. The signal-to-background ratio assay window is above 100-fold, which indicates that the 1536-well plate format is robust and can be utilized for assessing hepatotoxicity using human primary hepatocytes in a qHTS platform. The wide range of toxicos identified from the qHTS approach illustrates the utility of this platform. Additionally, qHTS can be employed to further explore the susceptibility of hepatocytes to various mechanisms of toxicity using appropriate cellular death markers.

424 MULTIPLEX CYTOTOXICITY ASSAY FOR PRIMARY HEPATOCYTES.

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A multiplex cytotoxicity assay for primary human (freshly isolated and cryopreserved) and rat hepatocytes (freshly isolated) in 96-well format was developed to help identify modes of toxicity. Broadly, cytotoxicity can be classified as apoptosis or necrosis. Cell necrosis is generally viewed as cell swelling and leakage of cellular components due to loss of membrane integrity, while apoptosis is viewed as cell shrinkage with activation of catalytic enzymes (ex., caspases). Understanding the mode of toxicity could be valuable in guiding structure-activity relationships during drug discovery. The multiplex cytotoxicity assay detects apoptosis and necrosis in a single assay, using lactate dehydrogenase (LDH) leakage, caspase 3/7 activation, and mitochondrial function (resazurin dye reduction) as endpoints. The performance of individual assays (ex., ranges of assay linearity and cell seeding densities) was examined and optimized under multiplexed conditions using compounds with known modes of toxicity, Staurosporine, an apoptosis inducer, activated caspase 3/7 without causing LDH leakage and perhexiline, a necrotic toxicant, caused LDH leakage without caspase activation. Ibutrofen, potassium dichromate and quinine caused changes in both caspase activity and LDH leakage, and all compounds decreased cell viability as measured by mitochondrial function (dye reduction). The concentration-response curves, however, were different for each endpoint for every compound, suggesting a limitation of a single-endpoint assay to detect cytotoxicity adequately. Within LDH endpoint there was difference between fresh and cryopreserved hepatocytes further highlighting the limitations of single-endpoint assay. With three cytotoxicity endpoints from a single treatment, the multiplex assay offers the unique advantages of 1) better detection of cytotoxicity that could be missed using a single endpoint, 2) insight into the mode of toxicity such as apoptosis or necrosis, 3) cost and time-effective operation, and 4) better data quality by using same treatment conditions.

425 PROTECTION BY THE PROLYL HYDOXYLASE INHIBITOR, EDHB, AGAINST CELL KILLING AFTER CHEMICAL HYPOXIA IN CULTURED RAT HEPATOCYTES.

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BACKGROUND: KCN and iodoacetic acid (IAA) simulate the ATP depletion and reductive stress of anoxia by inhibiting respiration and glycolysis, respectively. Our AIM was to study the effects of ethyl 3,4-dihydroxybenzoate (EDHB), a prolyl hydroxylase inhibitor and HIF-1α stabilizing agent, on killing of cultured rat hepatocytes during such chemical hypoxia. METHODS: Rat hepatocytes cultured overnight in 24-well plates were exposed to 2.5 mM KCN plus 200 μM IAA in the presence of 0-1000 μM EDHB, and cell viability was monitored by propidium iodide fluorescence. RESULTS: In the absence of KCN+IAA, EDHB up to 200 μM
had no effect on viability, but higher concentrations caused toxicity. In the presence of KCN+IAA, EDHB decreased cell killing from ~80% to ~25% after 2 h. However, higher concentrations caused toxicity. In the presence of KCN+IAA, EDHB decreased cell killing from ~80% to ~25% after 2 h.

**828 REACTIVE OXYGEN SPECIES RELATED MECHANISMS PARTICIPATE IN THE SENSITIVITY OF M-CSF-PRIMED RAT KUPFFER CELLS TO LPS CHALLENGE.**

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Kupffer cells (KCs), tissue macrophages in the liver, represent about 20% of the hepatic nonparenchymal cells. Reactive oxygen species (ROS) modulate the activity of many signal transduction pathways. Little is known about the mechanisms by which triggering (mobilizing) factors (M-CSF) priming modulates the sensitivity of KCs to lipopolysaccharide (LPS). Here, we hypothesized that NADPH oxidase-dependent ROS contributes to LPS-induced activation of MAPK family members and NF-kB in M-CSF primed rat primary KCs in vitro. A novel system was established to isolate and culture high purity KCs from rats involving enzymatic liver perfusion, elutriation centrifugation, and cell sorting with ED2 (KC marker). M-CSF (10ng/mL) in culture medium promoted relative increased number of KCs which survived for over 4 weeks in vitro. M-CSF priming also enhanced a mature phenotype and morphology, and the ability to phagocytize FITC-latex beads. Although not detectable in KCs without LPS treatment, TNEs and IL-6 markedly increased after exposure to LPS, with peak levels (>100-fold) seen at day 7 by ELISA. In addition, M-CSF priming significantly enhanced LPS-induced ROS production (2-fold), and CD14 expression (2.5-fold). Further, M-CSF priming markedly enhanced LPS-stimulated activation of MAPK extracellular receptor activator kinase 1/2 (ERK1/2), p38 and c-jun-N-terminal kinase (JNK), as well as activation of NF-kB with decreased cytoplasmic I-kBα expression, a critical step in NF-kB activation. Pretreatment with diphenyleneiodonium, an inhibitor of NADPH oxidase, reduced LPS-induced increases in ERK1/2, JNK and NF-kB, and decreased I-kB. Taken together, sensitization of M-CSF primed KCs appears to be mediated through enhanced signaling through TLR4/CD14, which increases NADPH oxidase-derived ROS and subsequent MAPK and NF-kB in KC. Therefore, blocking ROS-dependent signaling may be a promising strategy to prevent untoward liver effects elicited by LPS-mediated KC sensitization. (Supported in part by NIH CA100908)

**829 MALE-PREDOMINANT EXPRESSION OF HEPATIC TRANSPORTER OATP1A1 EXPLAINS THE GENDER DIFFERENCE IN PLASMA LEVELS OF UNCONJUGATED BILE ACIDS IN MICE.**

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After developing a LC-MS/MS method for quantification of bile acids in plasma, liver, and bile, we noted that the plasma concentrations of unconjugated bile acids (alpha-muricholic acid, beta-muricholic acid, cholic acid, and deoxycholic acid) were lower in male than in female mice. To identify the underlying mechanism for this gender difference in circulating unconjugated bile acids, mRNA levels of hepatic genes involved in bile acid biosynthesis, conjugation, and transport were quantified in adult male and female mice. No gender difference was observed in the mRNA expression of the rate-limiting bile acid synthetic enzymes, namely the bile acid conjugation enzymes (Fap5 and Mbbaat), or the canicular bile salt export pump (Bsep). However, male mice exhibited slightly lower expression of sodium taurocholate co-transporting polypeptide (Ntcp), but much higher expression of organic anion transporting polypeptide 1a1 (Oatp1a1). To determine whether male-predominantly expressed Oatp1a1 accounts for the lower plasma levels of unconjugated bile acids in male mice, plasma concentrations of unconjugated bile acids were quantified in wild-type and Oatp1a1-null mice. Oatp1a1-null mice were subjected to a diet supplemented with 0.3% deoxycholic acid (DCA). After treatment, plasma DCA concentration in Oatp1a1-null male mice was about 40-fold higher than in wild-type male mice. In conclusion, this study demonstrates that male mice have lower plasma concentrations of unconjugated bile acids than female mice, and that this is due to higher expression of Oatp1a1, which transports unconjugated bile acids from plasma into liver. (Supported by NIH ES059716, ES057079, ES031714, ES09649, and RR021940).

**840 ALCOHOL CIRRHOSIS UPRREGULATES MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN TRANSPORTER EXPRESSION IN HUMAN LIVER.**

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Transporters are an integral component for liver detoxication of drugs and environmental chemicals. Previous studies in rodents have demonstrated that drug- and disease-induced liver injury alters transporter expression. However, limited data...
exist regarding regulation of transporter expression with various types of liver injury in humans. Alcohol-induced liver disease represents a major type of liver injury that is present in the American population with approximately 13,000 deaths per year because of alcohol-induced liver cirrhosis. Because there is a sizable population that presents with liver cirrhosis, it is important to know whether cirrhosis alters transporter expression for two main reasons. First, it is important to understand pharmacokinetics due to alterations in transporter expression. Second, it is important to understand how the liver adapts to different types of injury and the molecular mechanism by which adaptation occurs. Therefore, we examined the expression of major drug transporters in livers from normal non-cirrhotic and cirrhotic humans.

Total RNA was isolated and mRNA expression was quantified using the Branco DNA Signal Amplification Assay. Cirrhosis significantly increased the mRNA expression of Multidrug Resistance-Associated Protein (MRP) 2 and 4, and tended to increase Organic Anion Transporting Polypeptide 1B1 (OATP1B1). Cirrhosis increased MRP2-5 protein expression in liver. Because MRP2-4 are coordinately increased with genes regulated by the NRF2-KEAP1 pathway, we hypothesized that NRF2 is activated during cirrhosis. Cirrhosis increased NRF2 levels in liver nuclear fractions and increased the mRNA and protein expression of NADPH:Quinone Oxidoreductase 1 (NQO1), a gene well characterized to be upregulated through this pathway. In summary, in humans, MRP2 is appreciably increased during cirrhosis, which is associated with activation of NRF2-KEAP1 pathway.

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431 AROMATIC HYDROXYLATION IS A MAJOR NOVEL PATHWAY IN THE METABOLISM OF THE MYCOTOXIN ZEALENALONE.

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We have recently reported that zearalenone (ZEN) undergoes extensive oxidative metabolism with rat liver microsomes, resulting in the formation of eight mono-hydroxylated metabolites; however, the exact chemical structures of these novel oxidative ZEN metabolites were not disclosed (Pfeiffer et al., Mol Nutr Food Res 2007, 51, 861-877). It was the aim of the present study to reveal the oxidative metabolism of ZEN in human liver microsomes (HLM). We report here that HLM convert ZEN to four major metabolites through monohydroxylation at the aromatic ring, and two minor metabolites monohydroxylated at the aliphatic moiety. The aromatic hydroxylation of ZEN is supported by the following findings: (i) loss of one deuterium in each of the four major, but not of the minor, metabolites when ZEN specifically labeled with deuterium at both aromatic positions was incubated with HLM, (ii) the four major metabolites were substrates of the enzyme catechol-O-methyltransferase, and (iii) two of the four major metabolites were identical in LC-MS and GC-MS analysis with reference compounds synthesized from ZEN through nitration to two isomeric mononitro-ZENs, followed by reduction to the respective amines and subsequent oxidation to quinone imines, hydrolysis to quinones, and reduction to catechols. The structures of the synthetic compounds were established by 1H and 13C NMR spectroscopy. The other two aromatic hydroxylation products were identified as the respective zearalenols. Evidence for the aromatic hydroxylation of ZEN under in vivo-like conditions was obtained when ZEN was incubated with precision-cut rat liver slices: analysis by LC-MS revealed the presence of two glucuronides and one methyl ether of one of the aromatic hydroxy-ZENs. In cod, this study has clearly shown that ZEN is prone to aromatic hydroxylation. Further investigations are now warranted to demonstrate this novel pathway in ZEN metabolism in vivo and to assess its toxicological relevance. Supported by “Food and Health” of KIT.

432 METABOLISM COMPARATIVE CYTOTOXICITY ASSAY (MCDA) AND CYTOTOXIC METABOLIC PATHWAY IDENTIFICATION ASSAY (CMPA) WITH CRYOPRESERVED HUMAN HEPATOCYTES FOR THE EVALUATION OF METABOLISM-BASED CYTOTOXICITY IN VITRO: PROOF-OF-CONCEPT STUDY WITH AFLATOXIN B1.

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The Metabolism Comparative Cytotoxicity Assay (MCDA) and the Cytotoxic Metabolic Pathway Identification Assay (CMPA) have been developed to allow a systematic investigation of the role of P450 metabolism in xenobiotic toxicity. The MCDA was designed for the initial identification of the role of metabolism in cytotoxicity by comparing the cytotoxic potential of a toxicant in metabolically competent (cryopreserved human hepatocytes) and metabolically incompetent (CHO) cells, in the presence and absence of an irreversible, non-specific P450 inhibitor, 1-aminoazobenzotriazole (ABT). The CMPA was designed to identify specific P450 isoforms involved in metabolic activation via cytotoxicity evaluation in the presence and absence of isoform-selective P450 inhibitors. Results of a proof-of-concept study with the MCDA and CMPA with a known hepatotoxicant, aflatoxin B1 (AFB1), are reported. In the MCDA, AFB1 was found to have significantly higher cytotoxicity in human hepatocytes than CHO cells, therefore confirming its requirement for biotransformation to be toxic. AFB1 was found to effectively attenuate AFB1 P450 metabolism, leading to decreased metabolic activation. AFB1 had no effects of AFB1 cytotoxicity in CHO cells. In the CMPA, AFB1 cytotoxicity was found to be attenuated by ketoconazole and diethylthiocarbamate, but not by furafylline, quinidine, and sulfaphenazole. The results suggest that the isoforms inhibited by ketoconazole (mainly CYP3A4) and diethylthiocarbamate (mainly CYP2A6 and CYP2E1), but not the isoforms inhibited by furafylline (mainly CYP1A2), sulfaphenazole (mainly CYP2C9) and quinidine (mainly CYP2D6) are involved in the metabolic activation of AFB1. This proof-of-concept study suggests that MCDA and CMPA with cryopreserved human hepatocytes are potentially useful for the evaluation of the relationship between human xenobiotic metabolism and toxicity.

433 MITOCHONDRIA AS A SITE OF METABOLISM OF DICHLOROACETATE.

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Dichloroacetate (DCA) is a common contaminant of municipal drinking water and is a therapeutic agent for the treatment of some metabolic disorders. By inhibiting mitochondrial pyruvate dehydrogenase kinase, DCA stimulates the activity of the pyruvate dehydrogenase complex, and thus reduces the production of lactate and other pyruvate precursors. Pharmacokinetic studies in humans and rodents showed that DCA treatment results in a prolongation of its elimination half-life, probably through inhibition of glutathione transferase zeta 1 (GSTz1, also known as maleylacetate transferase isomerase), the only enzyme so far known to catalyze the conversion of DCA to glyoxylate. Like other GSTs, GST1 is present in the cytosol, and in vitro studies of DCA biotransformation have investigated the properties of the cytosolic enzyme. Since DCA is known to be taken up into and concentrated in mitochondria, its principal site of action, it is of interest to explore the role, if any, of mitochondria in the biotransformation of DCA. Initially, suspensions of washed mitochondria from human or rat liver were incubated with 14C-DCA in the presence or absence of ATP, Coenzyme A, glutathione, or mixtures of these cofactors. Over a 30 min incubation period, significant biotransformation was observed only when glutathione was present. The products were identified as glyoxylate, glycine, CO2, and oxalate. Hepatic mitochondria from rats treated with DCA for 8 weeks at 500 mg/kg did not convert 14C-DCA to metabolites. Expression of GSTz protein was detected in human and rat liver mitochondria by Western Blotting against a rabbit antibody to human GSTz1. Studies of sub-mitochondrial fractions showed that GSTz was present in the soluble, but not the membrane compartments of mitochondria. We conclude that hepatic mitochondria are able to metabolize DCA as evidenced by the presence of GSTz1 and in vitro studies of DCA biotransformation. In order to investigate the properties of the cytosolic enzyme, mitochondrial fractions were isolated from human liver mitochondria and incubated with 14C-DCA at different concentrations. The products of DCA biotransformation were identified by radioactive thin layer chromatography. The data show that DCA is metabolized by a mitochondrial enzyme, possibly involving GSTz isozymes. Supported in part by the US Public Health Service, ES 07355 and ES 014617.

434 IN VITRO METABOLIC STABILITY OF CHEMICALS IN RAINBOW TROUT (ONCORHYNCHUS MYKISS) COMPARED TO HUMAN CRYOHEPATOcyTES.


Assessment of bioaccumulation of chemicals that may be found in the environment is becoming increasingly important. REACH legislation is in effect in European countries with the aim of improving protection of human health and the environment. Other environmental and government organizations in the world, including the US, are joining this effort. Bioconcentration factor (BCF) assessment has been based on in vivo modeling. In vivo testing requires large numbers of test organisms and is time and cost intensive. Incorporation of in vitro methodologies into BCF assessment and an extrapolation method has been developed (Cowan-Elberty et al., 2007). Phase I and Phase II drug metabolizing enzymes mediate the biotransformation of xenobiotics in mammalian and some aquatic species (e.g., fish, crustaceans and molluscs). The present study was designed to utilize cryophotocytes from rainbow trout, and assess metabolic stability of three chemicals (nonylphenol, methoxychlor and ethinylestradiol). The metabolism of these chemicals was compared to their metab-
olim in human cryohepatocytes. One year old rainbow trout (male and female) were euthanized, livers flushed through the hepatic vein. Hepatocytes were isolated by collagenase perfusion and cryopreserved. Liquid nitrogen. Human livers were perfused and hepatocytes isolated and cryopreserved. Test chemical incubations were performed at pH 7.8 and 12 °C (trout) and pH 7.4 and 37°C (human) to reflect physiological conditions of each species. Disappearance of parent chemical was measured by LC-MS/MS or GC-MS. Differences were observed in the metabolism of each chemical and between species. These results demonstrate the possible use of rainbow trout cryopreserved hepatocytes as an in vitro model system to predict bioaccumulation of environmental chemicals.

345 COBALT CHLORIDE, A LEAKAGE PRODUCT OF COBALT NANOPIRATES, IS TOXIC TO RAT LIVER MITOCHONDRIA: A CARBON 13 NMR STUDY.

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It is known that cobalt chloride (CoCl2) may be released from cobalt nanoparticles but its cellular toxicity remains poorly characterized. The objective of the present study was to test if CoCl2 interferes with the metabolism of the liver. Given the importance of lactate as a physiological substrate of the liver, we have conducted a study in vitro to characterize the effect of CoCl2 on hepatic lactate metabolism. For this, we used cryopreserved liver slices from 48-h-fasted Wistar rats that were incubated in oxygenated Krebs-Henseleit buffer containing either unlabeled lactate or variously labeled Krebs-Henseleit buffer. The labeling patterns of the glycolytic intermediates of CoCl2 treated liver slices were compared to control liver slices. It is concluded that, in rat liver cells, CoCl2 is toxic to the mitochondria leading to a fall of cellular ATP levels that results in a strong inhibition of lactate removal and lactate gluconeogenesis as well as of uric acid and ketogenesis.

346 BILIARY EPITHELIAL CELL CULTURE PREDICTS TOXICITY BY 1-ARYLXOPYRONAN-2-OL IN THE RAT.

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Exposure of cells lining the biliary tract to high concentrations of drugs or metabolites due to biliary clearance renders the cells susceptible to cyotoxic cell damage. Although the incidence of drug-induced biliary toxicity is low, the often progressive nature of toxicity and uncertain translation across species warrants minimizing as a potential liability during drug discovery. The aim of these studies was: (1) develop and characterize a primary culture of rat biliary epithelial cells (REC), (2) evaluate its utility to predict biliary toxicity detected in rats treated with an ary ether of naphtalene, and (3) interrogate the system with other known biliary toxicants. A rat BEC cell line that expresses cholangiocyte markers gamma-glutamyl transpeptidase and cytokeratin 7 was established. UGT1A1, 1A2, 1A6, and GSTY2C mRNA were detected in the cells at 16, 53, 43, and 77% of levels in liver, respectively. CYP1A2 and CYP3A1 mRNA levels were only detected at 0.1% of liver levels. The cells retained expression of several transporters in culture, specifically, Abca1, Abcg1, Mdr2, Mdr3, Mrp1 and Mrp2, that were expressed at 8, 16, 29, 53, 57, and 97% of levels found in ileum, respectively. The cells were used to test whether hyperplasia and degeneration of small bile ducts caused by 1-arylxypropan-2-ol in the rat was due to direct cellular injury. The parent molecule and its hydroxycid metabolite, 3-aryloxy-2-hydroxypropanonic acid, were not toxic, but the diol metabolite 3-aryloxypropane-1,2-diol decreased cell viability by 65% (IC50 = 10μM) implicating this metabolite as the potential proximal biliary toxicant. Interestingly, the hydroxycid metabolite selectively increased bile cell proliferation in vitro suggesting a differential role of the metabolites in the hyperplasia and biliary cell loss detected with the parent molecules in rats. This system was also sensitive to treatment with the biliary toxicants chlorpromazine, tobramycin, ticlopidine, and propafenone.

347 MEASUREMENT OF LIVER ALANINE AMINOTRANSFERASE (ALT1 & ALT2) ACTIVITY BY D-CYCLOSERINE INHIBITION.

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ALT activity is routinely used as an indicator of hepatocellular damage, but ALT is also an important enzyme involved in gluconeogenesis and amino acid metabolism. The cloning and expression of human and rat ALT1 and ALT2 proteins has allowed the development of immunoassay methods to measure ALT isoforms (ALTiso) and mass and there is some indication that isoenzyme analyses may differentiate between hepatocellular injury versus metabolic adaptation. As an alternative approach, we used recombinant ALT1 and ALT2 proteins to develop an assay to measure ALTiso activity based on their differential inhibition by D-Cycloserine. At optimal D-Cycloserine concentrations, there is a 35% to 40% difference in inhibition between the recombinant ALTiso isoforms. Human and rat recombinant ALT1 and ALT2 enzymes are similarly differentially inhibited. A preliminary reference range (n=30) of ALT1 and ALT2 activities in fasted normal rat liver homogenates is 134 to 381 and 26 to 118 μU/mg of protein, respectively. Intra-assay (n=3-6 replicates) and inter-assay precision (n=3, 4 replicates over span of 3days) for ALT1 was 5.9% and 3.5% and for ALT2 was 8.7% and 10.2%, respectively. ALTiso activity in working dilutions of the liver homogenate prepared in the 125mM Tris buffer, pH 9 with 150mM NaCl, 25% glycerol and Roche Complete EDTA-free Protease Inhibitor cocktail was stable for 3 days at 4°C. The stock liver homogenate was stable for 30 days at 4°C and for 3 freeze-thaw cycles, but lost approximately 25% of the ALT2 isoactivity when stored for 3 days at 4°C. The measurement of ALTiso activity by D-Cycloserine inhibition provides an alternate quantitative method. The method should be adaptable to laboratory automation and potentially applicable for use with both human and rat samples. Current efforts are to utilize this assay for characterization of changes in liver ALT activity in animals treated with xenobiotics and to optimize a serum ALT isoenzyme assay using this method.

348 IN SITU BIOMONITORING USING CAGED JUVENILE CHINOOK SALMON (Oncorhynchus tshawytscha) AT A RIVER SUPERFUND SITE IN THE PACIFIC NORTHWEST.

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Contaminated sediments represent a potential threat to human and ecological health. Due to the complexity of these materials, there is a great deal of uncertainty associated with risk characterization of sediments. A series of in situ studies were conducted at a Superfund site in the Pacific Northwest. River sediment at the site is contaminated with polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs). Current studies are aimed at measuring biomarkers in caged juvenile Chinook salmon (Oncorhynchus tshawytscha). Based on existing data, four stations were selected for water and sediment sampling. Salmon fingerlings were also caged and exposed at each sampling station for 7-8 days. Following sacrifice, fish livers were analyzed for body burden of PAHs and PCBs. Mean levels of contaminants present in sediment samples collected each July from 2004 through 2007 ranged from 1394 to 3037 ng/dry g for total PAHs and from 16 to 1904 ng/dry g for total PCBs. Mean total PAHs in surface water collected ranged from 231 to 1541 ng/L and PCBs were only detected during one sampling event. Analysis of fish tissue detected total PAH levels as high as 251 ng/g wet weight, while PCB levels were below detection. DNA adduct analysis was performed by 32P-postlabeling of salmon gill tissue, which was collected during the 2004, 2006, and 2007 sampling events. DNA adduct levels were significantly higher in the exposed salmon versus controls at selected stations in 2004 and 2006, however no significant difference was present during the 2007 sampling event when exposure levels were lower than other years. Results confirm that tissue concentrations provide a sensitive measure of contaminants in the water column. However, DNA adduct analysis is useful as a marker of genetic damage when high exposure levels of contaminants are present. This research was supported by NIEHS Superfund Basic Research Program Grant No. P42ES09397.
metabolites which has the potential to intercalate into DNA. The first aim of this study is the monitoring of BaP and BbF from soils of the Cacharillas marshalland and tissues of Ochrochomis mossambicus (tilapia) that inhabit within the marsh. The Cacharillas marshalland is surrounded by multiple industries and a thermoelectric plant that are potential sources of environmental contaminants including PAHs and PCBs. The fact that it continues to be used for recreational activities presents a potential source of exposure to carcinogens to surrounding communities. Soils (sludge) and tilapias were obtained from potential contaminated areas in the marshland. The optimized and applied methodology for extraction and isolation of PAHs included: Ultra sonic and microwave extraction techniques for soils and fish respectively, followed by column filtration (silica, alumina and sodium sulfate). For optimization of the method control sludges and tilapias were spiked with BaP, BbF and the internal standard (decabromophenyle). Analysis of the samples and identification of the monitored PAHs were performed by HPLC analysis. Preliminary results indicate the presence of PAHs on tilapias and soil samples at the parts per million (ppm) levels. Furthermore, evidence of bioaccumulation was suggested since the concentration level detected on tilapias were higher than in soil samples. Mass characterization of these carcinogens is being performed by Electrospray ionization massspectrometric techniques. Similar concentrations of BaP and BbF found on sediments or fish will be used to evaluate their potential cytotoxic effects on human normal cells. This study presents evidence of the presence of environmental carcinogens on the Cacharillas marshland.

**TRANSLATING IN VITRO ESTROGENIC ASSAY RESULTS TO ECOLOGICAL RISK ASSESSMENT.**

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In vitro assay results are increasingly used to evaluate endocrine activity of a wide range of environmental pollutants. However, translating in vitro assay results into the existing risk assessment framework is challenging since regulatory guidance on this issue is currently lacking. The United States Environmental Protection Agency (US EPA)’s Endocrine Disruptor Screening Program (EDSP) plans to screen and test potential endocrine disrupting chemicals, starting in 2009, and will rely largely on the results of in vitro assays in its initial screening stage (Tier I). It is yet to be determined how these screening results will be translated into ‘risk’, communicated to the public, and potentially used for regulation. We developed an in vitro fish estrogen receptor (ER) competitive binding assay to quantitatively assess estrogenicity of several known active pharmaceutical ingredients (APIs) e.g., progesterone, gestodene, and medrogestone. We subcloned zebrafish ERα and incubated this receptor with one of the APIs along with a positive control, the relative potency obtained from the in vitro assay was used to derive a pre-dicted no (adverse) effect concentration (PNEC) for each API, based on their relative binding affinities to ERα and the literature-reported PNEC for ethinylestradiol (EE2). These results were used to provide risk-based guidance with existing risk assessment procedures. We propose that, along with the results from ‘traditional’ toxicity testing, this approach can be used to evaluate the ecological risk of estrogenic chemicals. These methods also provide an approach as to how future screening results of US EPA EDSP could be interpreted.

**THE CYTOCHROME P450 (CYP) GENE SUPERFAMILY IN DAPHNIA PULEX.**

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Cytochrome P450s (CYPs) are essential eukaryotic enzymes for making endoergicous structural, signaling, and defensive molecules. A subset of CYPs have evolved as critical components of the detoxification systems of vertebrates and invertebrates. Data mining and manual curation of the Daphnia pulex genome identified 79 functional CYP genes, and three CYP pseudogenes. These CYPs belong to 4 clans, 15 families, and 19 subfamilies. The CYP 2, 3, 4, and mitochondrial clans are the same four clans found in other sequenced protostome genomes. Phylogenetic comparison of D. pulex CYPs to insects, vertebrates and sea anemone (Nematostella vecten-sis) CYPs using MrBayes show that the CYP1 clan, and to a lesser degree, the CYP4 clan has expanded in D. pulex, whereas the CYP3 clan has expanded in insects. However, the expansion of the D. pulex CYP2 clan is not as great as the expansion observed in deuterostomes and the nematode C. elegans. Mapping of CYP tandem repeat regions demonstrated the unusual expansion of the CYP370 family of the CYP2 clan. The CYP370s are similar to the CYP15s and CYP303s in insects that have always been solo genes but in Daphnia they make up about 20% of all the CYP genes. Lastly, our phylogenetic comparisons provide new insights into the potential origins of otherwise mysterious CYPs such as CYP46 and CYP19 (aromatase). Overall, the cladoceran, D. pulex has a wide range of CYPs with the same clans as insects and nematodes, but with distinct changes in the structure of each clan.

**TEMPORAL EXPRESSION OF FOUR ESTROGEN-RESPONSIVE PLASMA PROTEINS IN ADULT SHEEPSHEAD MINNOW EXPOSED TO STRONG AND WEAK ESTROGEN AGONISTS.**

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Protein profiling via high throughput mass spectrometry can be used for detection of biomarkers that can be applied diagnostically to screen chemicals for endocrine modifying activity. In previous studies using sheephead minnows (Cyprinodon variegatus, SHM), mass spectral analysis detected four peptides (2950.5, 2972.5, 3003.4, 3025.5 m/z) in the plasma of estrogen-treated male fish and untreated gravid females compared to unexposed males. In this study, accumulation and clearance of the four protein biomarker was determined in SHM during and after cessation of exposure to either the native ligand 17β-estradiol (E2) or the weak estrogen agonist, bisphenol-A (BPA). Fish were continuously exposed to either 0.3 μg E2/L, 0.5 μg BPA/L or a triethanol glycyl solvent control for 7 days using a flow-through dosing system followed by placement in untreated seawater for the remainder of the 20 day experiment. Fish were sampled on days 1, 3, 5, and 7 of exposure followed by sampling at discrete intervals for up to 13 days post-exposure. At each interval, plasma from 5 fish was analyzed for presence of the four peptide biomarkers by MALDI-TOF-MS. Exposure to E2 and BPA caused significant increases in the four protein biomarkers during the exposure period. Average levels of expression ranged from 30 fold (2972 m/z) to approximately 80 fold over control values in the 2950 m/z protein biomarker. After cessation of chemical exposure, mean biomarker expression levels of the E2 treated fish remained relatively constant for 5 days followed by gradual plasma clearance reaching solvent control values by day 20. In contrast, BPA treated fish demonstrated significantly elevated expression (p=0.05) of the four biomarker proteins through day 20 with the 2950 and 3025 m/z markers remaining 56 and 29 fold higher, respectively, than solvent control values. Results of this study indicate time dependent clearance of the estrogen-responsive proteins from the plasma which may be detectable for several weeks after initial exposure to an estrogenic compound.

**EVALUATION OF THE EFFICIENCY OF THE TREATMENT BY PHOTOCHEMICAL TREATMENT BY PHOTOELECTROCATALYSIS AND CONVENTIONAL CHLORINATION IN THE REMOVAL OF THE Azo Dyes from Aqueous Samples Using the Salmonella Mutagenicity Assay.**

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Introduction: The literature shows that the conventional treatment by activated sludge employed by the dyeing industry has not effectively removed the azo dyes from effluents as well as the mutagenicity. Objectives: To compare the use of the photocatalyticalysis with conventional chlorination as an alternative method for the treatment of aqueous samples that contain mutagenic azo dyes, using CI Disperse Red 1 (CAS n. 2872-52-8), CI Disperse Orange 1 (CAS n. 2581-69-3) and CI Disperse Red 15 (CAS n. 3180-81-2). Methods: Initially, we evaluated the mutagenicity of the original dyes with the strains TA98, YG1041 and YG1042. In order to preliminarily evaluate the efficiency of the degradation product, we used the strain TA98. Salmonella mutagenicity assay was performed using the pre-incubation protocol, in the presence and absence of S9. The photocatalytic oxidation experiments were performed in a photoelectrochemical reactor equipped with water refrigeration using an ultra-thermostatic bath and Ti/TIO2 thin film electrodes. Conventional chlorination was performed with chlorine gas. Results: The original studied dyes tested positive for the strains TA98 and YG1041, and for both, we observed a decrease of the effect in the presence of S9. Apparently photocatalyticalysis completely removed the mutagenicity of the dyes and seems to be more efficient than chlorination for the treatment of mutagenic dyes and this result was published in Electrochimica Acta (in press). Conclusion: Probably photoca-
MOLECULAR CLONING OF AKR1A GENE AND ITS COMPARATIVE EXPRESSION WITH CYPIA IN RESPONSE TO BENZO(A)PYRENE TREATMENT IN TILAPIA (OREOCROMIS NILOTICUS).


Azo(pyrene) (BaP) is a polycyclic aromatic hydrocarbon widely distributed in aquatic ecosystems. This compound requires bioactivation by cytochrome P450 1A (CYP1A) and aldo-keto reductase 1A (AKR1A) enzymes to carry out its toxic effects such as DNA damage, reactive oxygen species generation, among others. BaP is also able to regulate the expression of CYP1A and AKR1A genes through different pathways. AKR1A gene expression has not been studied in Tilapia (Oreochromis niloticus), a tropical fish of great ecological and economical importance. The aim of this study was to compare the BaP inductive effects on AKR1A and CYP1A gene expression in Tilapia's liver by measuring their mRNA levels. We performed a time-response curve using 20 mg/kg BaP given intraperitoneally and livers were collected at 3, 6, 12, 18, 24, 72 and 120 h after treatment. Heparin cDNA was obtained from tilapias and analyzed by Quantitative Real-Time PCR assay. Previously, AKR1A cDNA was cloned, sequenced and compared with those AKR1A cDNA reported sequences in the GeneBank DNA database. AKR1A from Tilapia has a 324 pb open reading frame encoding a 108 amino acid protein with a theoretical molecular weight of 12 kDa. The AKR1A amino acid sequence is closely related to zebrafish (80% identity). In liver, CYP1A mRNA expression was constitutively higher than of AKR1A mRNA. On the other hand, BaP exposure remarkably induced both genes but CYP1A gene expression is evidently higher than that of AKR1A. In addition, mRNA CYP1A expression peaked at 6 h whereas AKR1A did so at 24 h. Thus, AKR1A is present in tilapia's liver and is BaP inducible; the time peak shown by CYP1A and AKR1A suggests that the induction mechanism by which these genes are modulated might be somewhat different. Efforts should be done to investigate the pathways differences for the induction of AKR1A and CYP1A mRNAs.

THE CYTOCHROME P450 EFFECT ON THE CLEAVAGE OF THE CHROMOPHORE GROUP OF THE MUTAGENIC DYES DISPERSE RED 1, DISPERSE ORANGE 1 AND DISPERSE RED 13 AND THE RELATION BETWEEN THE GUANOSINE REACTION AND AZO BOND FOR THEIR MUTAGENICITY.

F. M. Chequer, M. V. Zanoni, and D. P. Oliveira.

A lot of dyes are used as food additives and the azo dyes are the most used. Although Sudan dyes are nonauthorized they have been illegally used to enhance or to maintain the appearance of food products. According to the literature Sudan I is mutagenic for different systems, but few information was found about the toxicity of Sudan III that has chemical structure similar to Sudan I. In this work, we investigated the ability of Sudan III as well as its reduced metabolites with guanosine, a DNA nucleotide. Guanosine (at 1,4x10^-4 mol.L^-1) was added to Sudan III (5,5x10^-5 mol.L^-1) and the reactions were monitored using a diode array spectrophotometer. Using a spectroeletrochemical method Sudan III at 5,x10^-5 mol.L^-1 was reduced for 40 minutes (25oC) at an oxidative potential -1.5 V, on the absence of oxygen. The mutagenicity of Sudan III was preliminary evaluated with Salmonella/microsome assay, using pre-incubation protocol and TA98 and TA100 strains, in the presence and absence of S9, using the maximal dose of 1mg/plate. The Sudan III reacts with guanosine, generating a stable product with spectral absorbance different from the original dye. After the reduction step, we observed that the Sudan III red color changed to purple indicating that the azo bond was modified and this radical also interacts to guanosine. At the tested conditions, Sudan III tested negative for the mutagenic activity. Our results clearly demonstrated that the azo dyes are toxic/mutagenic, as well as their breakdown products. The objectives of this work were: (1) To evaluate of the mutagenicity of Disperse Red 1 (CAS: 2872-52-8), Disperse Orange 1 (CAS: 2581-69-3) and Disperse Red 13 (CAS: 3180-81-2) using the micronucleus assay (MN); (2) To investigate if the cytochrome P450 is able to break the azo bond; (3) To determine if the mutagenic activity could be related to the link of chromophore group with the guanosine. The cytochrome-block micronucleus assay was used to evaluate the mutagenicity of the dyes in human lymphocytes and HepG2 cells. The action of Cyt P450 on the azo bond was monitored by the decrease of the spectral absorbance of each dye after incubation with S9 for 45 minutes at 37oC. The guanosine was added to different concentrations of each dye and the reaction was also monitored by spectrophotometer. The three dyes showed dose dependent increase in the frequency of MN, for both lymphocytes and HepG2. The incubation of the dyes with S9 clearly reduced the color, indicating cleavage of azo bond. The reaction of guanosine with the dyes lightly reduced the spectral absorbance of the azo bond, suggesting that the bond of this nucleoside with chromophore group is weak. We concluded that the dyes Disperse Red 13, Disperse Red 1 and Disperse Orange 1 are mutagenic. The chemical studies showed that the azo bond was cleaved after oxidation by S9, and similar results should be reproduced in in vivo situations. Therefore, further studies are necessary in order to elucidate the chemical structure of compounds formed after the cleavage of azo bond by Cytochrome P450 isoforms. The mutagenic effect of the dyes does not seem to be related with the binding of guanosine with the chromophore group.
A GC-MS method was developed for four suspect bioaccumulative chemicals (including several isomers): Sanjinol, Coniferan, Precyclemone B, and Vernaldehyde with log Kow values of 4.3, 4.9, 5.2, and 5.9 respectively. The GC-MS method allowed for parent quantification, characterization of fragments from profiling experiments and separation of potential metabolites. Samples were incubated at various points (extending to 60 or 120 minutes) at two different concentrations of S9 protein (0.2 and 1 mg/ml) and three chemical concentrations. Incubations included enzymatic cofactors NADPH, UDPG and PAPS. Zero-time incubations, heat-treated, untreated control, solvent alone, and no S9 served as controls to distinguish between enzymatic metabolism and nonspecific chemical degradation. All four chemicals were rapidly metabolized (range 28% to 98%) by trout S9 fractions after one hour. This turnover was due to enzymatic action as confirmed by the control test groups. These results support the previously unknown metabolism of these compounds that which decreases their estimated BCF values. This type of data will be central for the future modeling of aquatic bioaccumulation.

**449 THE EFFECT OF 2, 4-DINITROPHENOL ON ZEBRAFISH SWIM PERFORMANCE.**

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Examination of fish swim performance is a potentially environmentally relevant, non-lethal endpoint for examining fish survivability. Zebrfish (Danio rerio) served as the model to investigate the acute affect on swim performance of a toxicant that acts as an oxidative uncoupler. Fish (n=10 per group) were aqueously exposed in a static medium to 0.6, and 12 mg/L 2,4-dinitrophenol for 24 hours. Critical swimming speed (Ucrit, a measure of swimming speed and endurance) was determined using a Loligo Systems mini swim tunnel in clean water. Fish were recorded at various intervals of Ucrit with a high speed camera. The acquired images were used for swim motion analysis. Following Ucrit analysis, fish were euthanized and their whole body triglyceride levels were determined as a marker of energy stores. No fish died during the control exposure, while mortalities in the 6 and 12 mg/L exposure groups were 20 and 30 percent, respectively. Ucrit was significantly decreased (p<0.05 in Tukey’s test after 1-way ANOVA) in fish exposed to 12 mg/L, but not 6 mg/L 2,4-dinitrophenol. However, the oxidative uncoupler did not have a significant effect on swim speed, total distance, or tail length. In conclusion, this study shows that acute exposure to a mitochondrial uncoupler significantly affects critical swimming speed of zebrafish, while mortality in the 12 mg/L is not significant.

**450 DNA SEQUENCE CHARACTERIZATION OF GOWANUS CANAL SOIL BACTERIA WITH POTENTIAL USE IN BIOREMEDIATION.**

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Bioremediation is a process that uses either naturally occurring or deliberately introduced microorganisms to break down environmental pollutants to return the natural environment altered by contaminants to its original condition. Our group is interested in soil microbiota from a particularly polluted area of Brooklyn New York: the Gowanus Canal (GC). The GC was a hub for Brooklyn’s maritime and commercial shipping activity. The industrial sector around the canal grew substantially over time to include stones and coal yards, manufactured gas plants, tanneries, factories for paint, chemical plants, and sulfur producers, all of which emitted substantial water and airborne pollutants. With six million annual tons of cargo produced and trafficked through the waterway, the GC, in the beginning of the 20th century, ranked among the nation’s busiest and most polluted canal. In this study we aimed to isolate and characterize naturally-occurring GC soil bacteria with bioremediation potential. Soil samples from GC were diluted, plated on starch agar and fed to larval European corn borer. The combined activity of the lepidopteran-active traits was assessed using lyophilized leaf incorporated into an artificial diet to assess potential interaction among the lepidopteran-active traits. To assess the potential for interaction among the rootworm-active traits, the rootworm-active proteins were performed to evaluate root feeding damage after infestation with western corn rootworm. Treatments included a control, the single event products and the combined trait product. The combined activity of the rootworm-active proteins was consistent with dose additivity, and there was no interaction between the rootworm and lepidopteran-active traits. To assess potential interaction among the lepidopteran-active proteins, and between the rootworm- and lepidopteran-active proteins, bioassays were performed to evaluate root feeding damage after infestation with western corn rootworm. Treatments included a control, the single event products and the combined trait product. The combined activity of the rootworm-active proteins was consistent with dose additivity, and there was no interaction between the rootworm- and lepidopteran-active proteins. In conclusion, the combined activity of the rootworm-active proteins was consistent with dose additivity, and there was no interaction between the rootworm and lepidopteran-active traits. To assess potential interaction among these Bt proteins, demonstrating a lack of interaction allows studies completed for the individual products to be used to inform the safety of the combined trait product. The potential for interaction among the rootworm-active, the lepidopteran-active, and between the rootworm-active and the lepidopteran-active proteins was evaluated with a whole plant bioassay assay and bioassays with corn leaf tissue. To assess the potential for interaction among the rootworm-active Bt proteins and between the rootworm and lepidopteran-active proteins, bioassays assays were performed to evaluate root feeding damage after infestation with western corn rootworm. Treatments included a control, the single event products and the combined trait product. The combined activity of the rootworm-active proteins was consistent with dose additivity, and there was no interaction between the rootworm- and lepidopteran-active proteins.

Since in last years has decreased oyster production, it is important to identify bio-
chemical biomarkers to infer about the health status of these organisms by exposure to pollutants. Within the biochemical biomarkers are those for oxidative stress. The thiobarbituric acid reactive species (TBARS) and total levels of glutathione (total GSH) show information about the oxidative damage and the antioxidant defense (no enzymatic) of organism, respectively. The aim of this study was to obtain information about the REDOX balance in Crassostrea spp. Oysters were sampled at different sites within Boca Camichin regarding to TBARS and total GSH levels. These results suggest that the organisms are expected to be chemical specific (such as metabolism and cellular injury rate造成的 variation among the models for the three chemicals arise in a few parameters expected to be chemical specific (such as metabolism and cellular injury rate constants). The BBDR model was used to quantitatively indentify limits of cellular in-jury and proliferation that would result in a significant increase in tumor incidence.

Results of the generalized BBDR model simulations provide evidence to inform the use of cytotoxicity and cellular proliferation as key events within this MOA for these chemicals. This BBDR model or subsequent versions in this iterative process may prove useful in cellular and cellular proliferation can be considered a cancer MOA for chemicals that produce liver tumors when their chemical specific parameters are available. (This abstract does not reflect USEPA policy.)

**454** BIOLOGICALLY-BASED MODELING OF MODULATION OF MAP KINASE SIGNALING PATHWAY BY LF IN TOXIGENIC PHASE OF ANTHRAX INFECTION


Anthrax (Bacillus anthracis) owes its virulence to the production of the multicomponent anthrax toxin which mediates the toxigenic (terminal) stage of infection. One toxin component, lethal factor (LF), cleaves most isoforms of mitogen-activated protein kinase kinases (MAPKK) of the organism. This interferes with a major signaling pathway linking the activation of membrane receptors, such as TLR4, to the transcription of several genes, including those encoding pro-inflammatory cytokines, as well as the p38 MAPK dependent expression of pro-survival genes. We have developed an initial modeling framework involving interaction of LF with host cells (macrophages). Cleaving MAPKK by LF leads to a decline (to zero) in the signaling output that has a characteristic half-time that can be correlated with the net potency of the toxin, as exemplified by reductions in macrophage viability in vitro in a number of studies. LF gains access to the host cell cytosol after it is secreted by germinating spores (early infection) or after it is endocytosed during toxemia (later, systemic infection). In either case, translocation of LF from the endosome to the cytosol is mediated by pores formed by another secreted toxin component, protective antigen (PA). A kinetic model has been developed that simulates the accumulation of LF in the macrophage cytosol over time. Our model is applied to published in vitro/in vivo dose-response and time-course datasets showing the differential susceptibility of various macrophages to LF/PA and/or bacterial exposures, often together with MAPKK cleavage data. This model, in combination with a proliferation model that simulates LF dosimetry and a description of the cellular response to signal reduction (alterations in gene expression leading to changes in cytokine production, apoptosis etc.), provides a model framework that predicts the time courses of the physiological effects of anthrax lethal toxin in biologically varied individuals.

**455** DEVELOPMENT OF A QUANTITATIVE MODEL INCORPORATING KEY EVENTS IN A HEPATOIC MODE OF ACTION TO PREDICT TUMOR INCIDENCE


Biologically-Based Dose Response (BBDR) modeling of environmental pollutants can be utilized to inform the mode of action (MOA) by which compounds elicit adverse health effects. Chemicals that produce tumors are typically described as either genotoxic or non-genotoxic. One commonly proposed MOA for non-genotoxic carcinogens is characterized by the key events of cytotoxicity and regenerative proliferation. The increased division rate associated with such proliferation causes an increase in the probability of mutations, which can result in tumor formation. In this presentation, three carcinogens which are thought to induce tumors in mice through a cytotoxic mode of action (chloroform, carbon tetrachloride, and dimethyl formamide) are quantitatively compared using a generalized BBDR quantitative model for tumor incidences. For each compound, a physiologically based pharmacokinetic (PBPK) model is developed and linked to a pharmacodynamic model of cytoreduction and cellular proliferation. The rate of proliferation is then linked to a clonal growth model which predicts tumor incidences. Comparisons of the BBDR simulations and parameterizations suggested that the only significant variation among the models for the three chemicals arise in a few parameters expected to be chemical specific (such as metabolism and cellular injury rate constants). The BBDR model was used to quantitatively indentify limits of cellular in-jury and proliferation that would result in a significant increase in tumor incidence.

Cancer development involves an escape from apoptosis and oncogenic-senescence after the carcinogen exposure. These can occur simultaneously in a multicellular organism and reflects in Weibull’s time-to-tumor model. Different anti-oncogenic (of p53, p27, Arf) effects are multiplicative to bring about cancer development in our calculations. In contrast, radiation induced adenocarcinoma of thyroid resulted from genetic damages and specific fusion of anti-oncogenes in proximity. These anti-oncogenes control cell division (Ret) and differentiation (Pas8). By reviewing genetics literature of thyroid multinodular follicular carcinomas, we propose the following thyroid carcinogenic mechanism: Exposure to ionization radiation leads to DNA double-strand breaks in those ‘‘chromosomal-territories’’ of actively transcribing endocrine gland such as the thyroid. Most likely this is accompanied by chromosomal translocation to neighboring loci of a different chromosome. These include the recombination of anti-oncogenes in proximity controlling cell division (Ret) and cell differentiation (Pas8) of a progenitor thyroid cell. This loss-of-heterozygosity leads to apoptosis/senesce escape. Unlike many cancer cells, the re-sulted cancerous cells somehow retain the differentiated thyroid functions due to the proximity of anti-oncogenes controlling cell division and differentiation. In addition, gene conversion occurs in neighboring cells instead of translocation. Thus it can lead to the appearance of multinodular follicular carcinoma in the thyroid with differences in the DNA sequence as identified in a single individual. The chances of different mutation at the same loci via mistakes in the DNA replication can also occur but in a magnitude six-order-of-magnitude lower.

Disclaimer: The opinions and conclusions in this abstract are only those of the authors and do not reflect the institutions they represent.

**456** CHROMOSOMAL TRANSLOCATION IN THYROID CARCINOGENESIS


Cancer development involves an escape from apoptosis and oncogenic-senescence after the carcinogen exposure. These can occur simultaneously in a multicellular organism and reflects in Weibull’s time-to-tumor model. Different anti-oncogenic (of p53, p27, Arf) effects are multiplicative to bring about cancer development in our calculations. In contrast, radiation induced adenocarcinoma of thyroid resulted from genetic damages and specific fusion of anti-oncogenes in proximity. These anti-oncogenes control cell division (Ret) and differentiation (Pas8). By reviewing genetics literature of thyroid multinodular follicular carcinomas, we propose the following thyroid carcinogenic mechanism: Exposure to ionization radiation leads to DNA double-strand breaks in those ‘‘chromosomal-territories’’ of actively transcribing endocrine gland such as the thyroid. Most likely this is accompanied by chromosomal translocation to neighboring loci of a different chromosome. These include the recombination of anti-oncogenes in proximity controlling cell division (Ret) and cell differentiation (Pas8) of a progenitor thyroid cell. This loss-of-heterozygosity leads to apoptosis/senesce escape. Unlike many cancer cells, the re-sulted cancerous cells somehow retain the differentiated thyroid functions due to the proximity of anti-oncogenes controlling cell division and differentiation. In addition, gene conversion occurs in neighboring cells instead of translocation. Thus it can lead to the appearance of multinodular follicular carcinoma in the thyroid with differences in the DNA sequence as identified in a single individual. The chances of different mutation at the same loci via mistakes in the DNA replication can also occur but in a magnitude six-order-of-magnitude lower.

Disclaimer: The opinions and conclusions in this abstract are only those of the authors and do not reflect the institutions they represent.
A morphometry model of the rabbit lung for particle deposition modeling.

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A rabbit lung model was developed based on information available in the published literature. The airways of the lung were described with a symmetric geometry, similar to what has been used for typical path deposition models. Although the rabbit lung is known to be more asymmetric or monopodial than this idealized model, it is expected that, on average, regional deposition (e.g., tracheobronchial or pulmonary) will be adequately characterized by this model. The lung airways are grouped by branching generation, which is the number of bifurcations down from the trachea to the airway. The airways are described by the average number, length, diameter, branching angle, and gravity angle for the airways in each branching generation. The geometry of the tracheobronchial (TB) region (generations 1-14) was defined by integrating the data primarily from Kliment (1974), Ramirez et al. (2001), and Schlesinger and McFadden (1981), and Rodriguez et al. (1987) data were used to define the pulmonary (PUL) region. The morphometry data were used in a typical path deposition model based on the work of Yeh and Schum (1980), and Wojcicki (1988). Deposition via diffusion, sedimentation, and inertial impaction was modeled for TB and PUL deposition. An empirical function of the diameter and airflow rate was fitted to Raabe (1975) data and used for extrathoracic (ET) deposition. The model predictions were in good agreement with the data. Monte Carlo analysis of deposition with variability distributions for breathing frequency, tidal volume, and breath pause time shows significant variation in deposition. It is expected that, on average, regional deposition (e.g., tracheobronchial or pulmonary) will be adequately characterized by this model. The lung airways are grouped by branching generation, which is the number of bifurcations down from the trachea to the airway. The airways are described by the average number, length, diameter, branching angle, and gravity angle for the airways in each branching generation. The geometry of the tracheobronchial (TB) region (generations 1-14) was defined by integrating the data primarily from Kliment (1974), Ramirez et al. (2001), and Schlesinger and McFadden (1981), and Rodriguez et al. (1987) data were used to define the pulmonary (PUL) region. The morphometry data were used in a typical path deposition model based on the work of Yeh and Schum (1980), and Wojcicki (1988). Deposition via diffusion, sedimentation, and inertial impaction was modeled for TB and PUL deposition. An empirical function of the diameter and airflow rate was fitted to Raabe (1975) data and used for extrathoracic (ET) deposition. The model predictions were in good agreement with the data. Monte Carlo analysis of deposition with variability distributions for breathing frequency, tidal volume, and breath pause time shows significant variation in deposition, though there is less variability for 1 mm particle predictions. Predictions of deposition in the human lung are significantly higher than that of rabbit lungs.

An integrated QSAR-PBPK model for simulating inhalation and dermal kinetics of volatile organic chemicals (VOCs) in humans.

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Quantitative-structure-activity relationship (QSAR) approach is potentially useful for estimating chemical-specific input parameters for developing physiologically-based pharmacokinetic (PBPK) models. QSARs for partition coefficients, metabolic parameters and absorption rates can be used to fit the rapid development of PBPK models for simulating the pharmacokinetics and internal dose of chemicals. The objective of this study was to develop an integrated QSAR-PBPK model for simulating the pharmacokinetics of VOCs in humans exposed by inhalation and/or dermal routes. For this purpose, available experimental data on human blood-air (Pc), fat-air (Pfa), octanol-air (Poa) and water-air (Pwa) for 35 hydrocarbons (alkanes, alkylenes, aromatic hydrocarbons...) were analyzed. The resulting QSARs, along with tissue composition data, permitted the calculation of the muscle:air, liver:air, fat:air, skin:air, and Poa for VOCs that could be described using the following fragments: CH3, CH2, CH, C, C-C, H, benzene ring and H in benzene ring structure. The predicted partition coefficients were within a factor of 0.77–1.15 of the experimental values. The skin permeability coefficients (Kp) were also calculated on the basis of log Pow and molecular weight, whereas the CLint for humans was obtained by fractional body weight scaling of QSAR-derived CLint in rats. The QSAR algorithms were then incorporated along with the equations constituting PBPK model, within Microsoft EXCEL spreadsheet. The integrated QSAR-PBPK model adequately simulated the pharmacokinetics of m-xylene in humans on the basis of molecular structure both for inhalation (33 ppm, 7 h) and dermal (1 mmol/L, 20 min) exposures. The integrated QSAR-PBPK modeling approach developed in this study would be potentially useful as a tool for predict- ing in vivo kinetics and bioaccumulation of chemicals in humans under data-poor situations.

PBPK/BD model of Francisella tularensis in rhesus monkeys.

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Although physiological modeling of chemicals in animals and humans is now being employed as a regulatory tool for risk assessment, the same technology for microbial agents is in very early stages of development. The need for bio kinetic/dynamic models for microbial agents is in very early stages of development. The need for biokinetic/dynamic models for microbial agents is great, particularly for biohazard agents of concern for potential intentional releases. We are developing a physiologically based biokinetic/biodynamic (PBPK/BD) model for inhalation infection of Francisella tularensis, the agent causing tularemic disease, in rhesus monkeys. The model was adapted from a prototype model for deposition and clearance of Bacillus anthracis spores and includes pulmonary deposition of particles, bacteria-phagocyte interactions, and transport of intra- and extracellular bacteria through the lung and thoracic lymph nodes. The model was optimized for predicting kinetics of disease progression for the highly virulent SCHU-S4 strain of F. tularensis. The literature for F. tularensis is equivocal regarding the requirement for bacterial uptake into phagocytes to proceed growth in non-human primates. Therefore, we used the model to explore the effects of alter native growth scenarios on simulated kinetics of infection. By allowing for growth of bacteria following escape from phagocytes, the model is able to replicate observed kinetics of bacterial growth in lung and thoracic lymph nodes of Rhesus monkeys. This work illustrates the utility of modeling and simulation for testing mechanistic hypotheses about microbial pathogenesis that could be validated experimentally.

A Physiologically Based Biokinetic and Biodynamic (PBPK-BD) Model of Systemic Bacillus Anthracis Toxins.

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A multispecies physiologically based biokinetic and biodynamic (PBPK-BD) model of inhalation anthrax is being developed. The goal is to quantitatively describe the mechanisms of Bacillus anthracis (BA) spore lung deposition and clearance, BA spore germination and growth, subsequent release of anthrax toxins, and role of toxin systemic kinetics and dynamic response in experimental outcomes in order to perform cross-species extrapolation of disease response. A key element for simulating the whole animal anthrax disease response is to mechanistically and quantitatively describe the biokinetics of protective antigen (PA) as it is released from vegetative BA, circulates via the blood stream to organs then attaches to cell membranes via PA receptors, capillary morphogenesis protein (CMG2) or tumor endothelial marker (TEM8). The system model has been developed and initially parameterized according to PA receptor density and kinetic data. Relative density values for PA receptors were derived from published studies in which mRNA expression levels of CMG2 and TEM8 were measured in tissues of mice, while binding affinities of PA to its receptors were derived from published binding stoichiometry studies. The model predicted the half-life of PA in blood, which was validated by published data on serum PA in mice following IV dosing with PA, and serum PA in rats following IV injection of lethal toxin (PA + lethal factor). This systemic model also makes good predictions of circulating PA levels in the serum of New Zealand White rabbits with inhalation anthrax. Further, we can predict organ-specific pathology with the inclusion of PA receptor density in our model. Together with our previously developed BA inhalation and proliferation models, the systemic model enables the simulation of circulating toxin levels in animals with inhalation anthrax and the associated organ pathology. This work was funded in part by the Defense Threat Reduction Agency as project BA06TAS022.

Use of a PBPK model to determine the potential effects of albumin and PON1 esterase activity on chlorpyrifos pharmacodynamics.

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Paraoxonase (PON1) is found in the liver and blood where it hydrolyses the active oxon metabolites of a number of organophosphorus (OP) insecticides, including chlorpyrifos. Human developmental differences and polymorphisms in PON1 activities have led to concerns about OP exposures in potentially sensitive populations. The role of albumin esterase in the hydrolysis of chlorpyrifos-oxon has been demonstrated in vitro. Albumin esterase does not show the same developmental differences or polymorphisms that have been seen for PON1, as blood levels of this protein are fairly constant through life stages and across species. A validated PBPK/BD model for chlorpyrifos was used to determine the potential role of PON1 esterase activity differences and albumin esterase activities on chlorpyrifos-oxon detoxification. Humans are potentially exposed to chlorpyrifos as residues on food, so oral exposures of 3 doses/day were simulated. Brain esterase shows -10% inhibition in a healthy adult with blood oxon of -0.5 mmol/L, equivalent to a daily exposure of -1.6 mg chlorpyrifos/kg/day. Young children are reported to have -1/3 the PON1 activity of adults. In an individual with 1/3 PON1 activity, a modeled 10% brain esterase inhibition would occur at -0.35 mg chlorpyrifos/kg/day. In a theoretical situation where only albumin esterases are present in the complete absence of PON1 activity, the PBPK model predicts 10% brain esterase inhibition at
will include additional validation from targeted studies, validation using in vivo biomarker data for rodents as input. Future developments of the framework, we found good agreement between the output of simulations and measurements. This open source software package has features for both forward dosimetry, in which a calibrated model is used to predict biomarker data from known exposures, and reverse dosimetry, in which the model is used to reconstruct absorbed dose and exposure from collected biomarker data. To this end, the framework contains tools for Monte Carlo and Bayesian analyses, statistical post-processing and visualization of results, and a graphical user interface. As a first step toward a general OP mixture dosimetry model, we have implemented a physiologically-based pharmacokinetic (PBPK/PD) model for a mixture of chlorpyrifos [O,O-diethyl O-(3,5,6-trichloro-2-pyridyl)phosphorothioate] (CP) and diazinon [O,O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl)phosphorothioate] (DZ). Using this model within the framework, we have found good agreement between the output of simulations and published results for CP and DZ exposure biomarkers in rodents. We also found good agreement between reconstructed and experimental dosing scenarios using in vivo biomarker data for rodents as input. Future developments of the framework will include additional validation from targeted in vivo studies, validation using human exposure data, and dose reconstruction using biomarker levels from human epidemiological databases. This project is supported by EPA STAR Grant R833451.

**465 USING QSAR TO PREDICT PBPK/PD MODEL PARAMETERS FOR ORGANOPHOSPHORUS CHEMICALS.**

C. D. Ruark, C. Hack, P. J. Robinson and J. M. Gearhart, HJF, Wright-Patterson AFB, OH.

In vitro rates of phosphorylation, dealkylation, and regeneration by acetylcholinesterase, butyrylcholinesterase, carboxylesterase, and other esterases are important pharmacodynamic parameters, while metabolic activation and clearance are important pharmacokinetic parameters of organophosphorus (OP) compounds and their metabolites. These parameters are key for accurate characterization of physiologically-based pharmacokinetic/pharmacodynamic predictions in animals and humans but are not currently available for many OP compounds. This data gap can be filled through the methodology of quantitative structure-activity relationship (QSR), and the results can be applied to assess the health effects and variability in human populations with OP exposure. The relationships presented here between structure and cholinesterase activity of OP compounds were modeled using ACD/8.0 and Codessa 2.51 (Semichem, Inc.). In order to describe the structure of these OP compounds, regression techniques were applied to over 500 analyzed topological, geometric, constitutional, thermodynamic, electrostatic, and quantum mechanical descriptors to obtain statistically verified equations for the QSR models. The results of an AChE bimolecular rate constant regression, with a total of 679 structures, show that an R² of 0.71 can be achieved with 40 descriptors. The best one-parameter correlation was found to be the average nucleophilic reactive index for a carbon atom with an R²<12. The AChE data set was found to be normally distributed, with rate constants ranging from log₁₀(1.638) to log₁₀(10.450) M⁻¹ s⁻¹ with a mean of log₁₀(4.406). Finally, the assessment of the applicability domain was described through the leave-one-out principle yielding a Q² = 0.67. With the development of a QSAR toolbox complementing PBPK/PD modeling of OP chemical warfare agents, better assessment of these chemical threats can be made to assist in medical response management and planning in the future. This project received support from the Defense Threat Reduction Agency - Joint Science and Technology Office, Basic and Supporting Sciences Division.

**466 ACCOUNTING FOR STEREO-ISOMERS AND IONIZATION IN A PBPK/PD MODEL OF VX EXPOSURE.**

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Historically, chemical warfare nerve agent (CWNW) exposure threats were thought of as primarily lethal exposure level effects restricted to the combat theater, yet civilian population exposures are now of concern as well as evidenced by terrorist attacks with sarin in Japan in 1994 and 1995. In order to better describe the complex kinetics and dynamic effects of VX, an existing physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) model for VX (based on previously developed PBPK/PD models for diisopropylfluorophosphate and sarin (GB)) was extended to account for stereoisomers and the ionization of VX. This extended model simulates exposure via intravenous, subcutaneous, or dermal exposure in the guinea pig or Gottingen minipig. It consists of 3DIB models: one for each of the stereoisomers of VX and one for cholinesterase (AChE, BuChE, and CE) inhibition by VX. The model also accounts for differences in diffusion, urinary clearance, binding to red blood cells (RBCs) and plasma, and cholinesterase inhibition, regeneration, and aging due to ionization of VX by calculating instantaneous concentrations of ionized and non-ionized chemical using the Henderson-Hasselbalch equation and tissue-specific pH values. Simulated endpoints included fluoride ion regenerated VX G-analog in RBCs and plasma, plasma and tissue VX concentrations, and inhibition on acetylcholinesterase (Jakubowski et al., 2003). Species differences in response, due to differing pharmacokinetic profiles of VX, were addressed. The blood concentration and urinary excretion of VX were used as a confirmation of the low in vivo metabolism of VX relative to other CWNWs, like GB and soman. This extended model, validated with multiple data types in different species, provided the basis for predicting data from human IV and oral exposure presented in a peer-reviewed publication (Sidell and Groff, 1974). (This project received support from the Defense Threat Reduction Agency - Joint Science and Technology Office, Basic and Supporting Sciences Division.)
467 ASSESSING THE INFLUENCE OF DIETARY MANGANESE VARIABILITY AND INHALED EXPOSURE BY PHARMACOKINETIC MODELING.

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Manganese (Mn) is an essential dietary element for various important biological functions. Symptoms of central nervous system toxicity are known to occur at high inhalation concentrations of exposures. Although abundant in food and drinking water, health risk concerns are generally aimed at the potential exposures in some populations to Mn airborne particles from industrial ore and alloy production, commercial use, and car emissions. The objective of the present study is to assess the daily dietary contribution of Mn on the tissue accumulation of Mn from inhalation exposure. With a recently developed physiologically-based pharmacokinetic (PBPK) model for adult rodents and nonhuman primates, variability of steady-state blood and brain Mn levels in a simulated adult human population was determined using Monte Carlo techniques. Population model simulations of steady-state tissue concentrations were generated with population dietary Mn intake distribution data in North America (mean: 2.43 mg/day [0.07 – 6.2 mg/day], standard deviations: 1.8 mg/day). A 4-fold difference in blood and brain Mn concentrations was determined between the 5th and 95th percentile values based on dietary only input. Brain and blood Mn concentrations increased by 2 times at high dietary input and an inhaled exposure of 0.05 mg Mn/m3 (the current U.S. EPA RfC). 2-fold difference 5th and 95th percentile blood and brain Mn concentrations from an inhaled input at 0.5 mg Mn/m3 suggests an important contribution of a variable population diet. Since Mn tissue levels are regulated to prevent toxicity over a definite range of exposures, determining the changes above baseline tissue Mn levels from inhalation is crucial for a dosimetry-based risk assessment. Evaluating the variability of dietary Mn intake enables the identification of a possible point of departure tissue Mn reference concentration for the health risk assessment of Mn.

468 UNDERSTANDING Mn KINETICS IN RATS DURING GESTATION AND LACTATION USING PBPK MODELING.

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A PBPK model describing Mn kinetics in rats during the perinatal period was developed to improve our understanding of inhalation exposure conditions that could lead to increased central nervous system Mn concentrations in developing rats or humans. The model includes changes in physiology of the rat during gestation and lactation and describes Mn behaviors in the body with saturable tissue binding, homeostatic control of uptake and excretion, and tissue-specific increases in Mn from inhalation. A series of model modifications were made for the pregnant, lactating dam, fetus, and pups compared to adults, including: efficient, but saturable, maternal-fetal transfer of Mn, tissue binding capacities in the developing fetus and pup, increased Mn absorption in the lactating dam to maintain maternal homeostasis in the face of Mn loss through milk, more efficient gastrointestinal tract absorption, and low basal, but inducible, biliary excretion in pups. The ability of the pup to enhance biliary excretion when challenged with excess Mn was also required for simulating inhalation exposure during the early postnatal period. In general, these changes were consistent with the biology of essential metals during development. The resulting model simulations match a variety of published studies on maternal Mn homeostasis during gestation and lactation, milk Mn levels, placental levels, gestation fetal tissue levels, and changing patterns of neonatal tissue Mn levels for both normal dietary intake and with Mn inhalation. The successful description of Mn concentrations across these lifestages suggests that the present model can help to understand the relationship of Mn tissue concentrations in target organs across different developmental stages and their potential risks. This PBPK model will be useful in assessing whether infants and children should be regarded as susceptible populations for Mn inhalation.

469 DEVELOPMENT OF A PBPK MODEL FOR HEXAVALENT CHROMIUM IN RATS AND MICE TO ESTIMATE EXPOSURE TO ORAL MUCOSA AND SMALL INTESTINE EPITHELIUM.

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In a recent NTP bioassay, hexavalent chromium (CrVI), administered as sodium dichromate in drinking water, induced tumors in the intestine of mice and the oral mucosa of rats. The mode of action for CrVI carcinogenicity is thought to be associated with its reduction to trivalent chromium (CrIII) in the epithelial tissues of the portal of entry. The purpose of this study was to extend the PBPK model of oral CrVI exposure in rats developed by O’Flaherty et al. (2001), in order to simulate the exposure of the target tissues in both rats and mice. The O’Flaherty rat model was extrapolated to the mouse using known physiological parameter values and allometric scaling of chromium kinetic parameters. An oral mucosa compartment was added to the rat model and compartments for the stomach, small intestine and large intestine were added to the mouse description. The model was then used to assess uptake and tissue distribution of total chromium in the NTP kinetic studies (i.e., exposure in drinking water for 21 days followed by sacrifice 48 h after removing the dosing solution from the animals). An apparent non-linear, dose-dependent uptake of chromium from drinking water in these studies could be adequately described using Michaelis-Menten kinetics. The model was also able to reproduce data from other studies of chromium kinetics in rats and mice from the literature. After incorporating magnetic resonance imaging data on the rate of reduction of CrVI to CrIII in the intestinal lumen and tissues, the model will be used to predict target tissue exposure to CrVI and CrIII under the experimental conditions of the NTP bioassays, as well as at lower concentrations, in support of the design of an experimental study to evaluate the potential for a dose-dependent transition in the mode of action for CrVI carcinogenicity.

470 HARMONIZATION OF CYCLIC SILOXANES PBPK MODEL STRUCTURES.

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Cyclic siloxanes, D4 and D5, are low molecular weight siloxanes which are mainly used as intermediates in the production of silicone polymers. D4 and D5 can also be found in various consumer products. The general population may be exposed to low levels of these siloxanes through dermal, inhalation and oral routes while workplace exposures mainly occur by inhalation. There are currently six published PBPK models describing aspects of the pharmacokinetics of D4 and D5 for various exposure routes in rats and human. Each model addressed the unique kinetic properties of D4 and D5 (highly lipophilic yet with low blood/air partition coefficients) and provided a reasonable description of the available time-course data. However, these models also had specific characteristics, differing among the suite of models, that accommodated the kinetic differences among the different exposure routes and the differing breadth of specific data sets. In this work, we have developed a suite-of internally consistent, multi-compartment PBPK models to describe the variety of datasets across species, routes, and compounds. Our approach was to use inhalation and dermal exposure data to develop a `base’ model that was then used to evaluate results from oral administration. The time-course behavior of D4 in plasma after oral gavage is very different from the inhalation and dermal kinetics. The base model underestimated the inhalation rate and plasma concentration in the postexposure period after oral exposure. To account for this route-specific kinetics, a process whereby the orally administered compound(s) are assumed to enter the systemic circulation as a micro-emulsion that distributes to the various lipid pools and the liver, and becomes further distributed to the body via efficient venous blood was incorporated into the base model. Although this approach improved model fits to the oral uptake data, it is unclear whether the differences between the base model and oral uptake model are intrinsic to oral administration or an artifact of high bolus doses in lipophilic vehicles.

471 ADDRESSING PUBLIC EXPOSURES TO PRIORITY SOLVENTS USING HUMAN PBPK MODELS.

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Determining public health outcomes from exposure to environmental chemicals is a challenging and controversial research area. ATSDR is mandated by Congress to identify significant human exposure levels, develop methods to determine such exposures, and design strategies to mitigate them. Translational research is underway to develop human physiologically based pharmacokinetic (PBPK) models for priority environmental contaminants. This research focuses on development of a generic PBPK model structure for simulation of three routes of exposure (oral ingestion, dermal absorption, inhalation) to eight priority VOCs (acetone, perchloroethylene, chloroform, trichloroethylene, benzene, carbon tetrachloride, methylene chloride,
and vinyl chloride). Coded using Berkeley Madonna simulation software, the model consists of blood, rapidly and slowly perfused tissue, fat, liver, kidney, and skin compartments. Previously published human PBPK models were slightly modified to conform to our model structure, while chemical specific parameters for each VOC were retained. To evaluate model fidelity, predictions of our generic model were compared with published model predictions as well as published kinetic data. Good agreement was generally obtained for all VOCs. PBPK models allow evaluation of multiple exposure pathways and describe the impact of physiological state (e.g. enzyme induction, exercise), disease state (e.g. obesity, asthma, diabetes), reproductive state, life stage and life style on dosimetry and public health. This work is supported by a contract with Eastern Research Group (Contract no. 0133.19.011/1 for the CHCL3 model) and Cooperative Agreement U50US000778 (for additional VOCs).

**472 DEVELOPMENT OF A RAT PBPK MODEL FOR A PROMINENT N-ALKANE, TETRADECANE, FOUND IN AVIATION FUELS JP-8 AND S-8.**

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The US Air Force is transitioning from petroleum-based Jet Propellant-8 (JP-8) to a 50:50 blend of JP-8 and synthetic jet fuel (S-8) derived from a Fischer-Tropsch synthetic process using natural gas or coal. The goal of this project is to develop a PBPK hydrocarbon mixture model. In the present study we report on the development of a PBPK model for inhaled aerosolized tetradecane (C14). Rates were exposed only to 4 hr 89 mg/m3 of aerosolized C14 with 84% representing aerosol droplets size 4μm MMAD. C14 was also monitored in fuel studies in which male F344 rats were exposed via nose-only inhalation to aerosolized fuel concentrations of 900, 1060, 200 mg/m3 for JP-8, S-8, 56:50 blend. In these exposures C14 was present at 3.4 mg/m3 (JP-8), 11.7 mg/m3 (S-8), and 1.0 mg/m3 (Blend) representing approximately 1% of each fuel droplet. Exposures to a “designer mixture” of eight prominent fuel hydrocarbons were also conducted with C14 present at 62 and 137 mg/m3, comprising 14% of each droplet. Droplet size range for these studies was 1-3μm. The C14 PBPK model compartments consisted of the liver, fat, brain, richly and slowly perfused tissues. The model was configured for gas exchange of the vapor phase of C14 and for deposition and systemic uptake of aerosol droplets in the upper respiratory tract and deep lung. Fractional deposition of droplets was predicted using the Multiple-Path Particulate Dosimetry (MPPD2) model. Interesting features of this C14 PBPK model include rapid uptake and slow elimination from fat, interconversion into tissues, bidirectional behavior affecting flow limited, and rapid uptake of aerosol droplets. For C14 only exposure, the model adequately simulates tissue kinetic behavior compared to in vivo time course data points. In the presence of fuel, simulations also tracked well with tissue loading and clearance in the rat, responding to MPPD2 model predicted regional deposition. This is the largest n-alkane known to be used in a PBPK model. (AFOSR Grant #: FA9550-07-1-0132)

**473 DEVELOPMENT OF A MULTIRoute HUMAN PBPK MODEL FOR BROMODICHLOROMETHANE (BDCM).**

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BDCM is an animal carcinogen and developmental toxicant. Due to its presence as a disinfection byproduct in finished drinking water, BDCM may pose a risk for exposure via ingestion, inhalation or dermal exposure. Utilizing a unique data set wherein human subjects were exposed to BDCM by both the dermal and oral routes (Leavens et al., 2007), we have developed a refined human multi-route PBPK model. Compartmental models in the skin are liver, gut, fat, brain, kidney, and slowly and rapidly perfused tissues; tissue transport is blood-flow limited and metabolism occurs in the liver by a single pathway. Chemical-specific parameters for tissue solubility and metabolism as well as initial organ volumes and blood flow rates were obtained from the literature; subject-specific values were available for height, weight and fat volume. The preliminary model adequately predicted individual blood tissue time course data following dermal exposure, whereas the same model poorly predicted blood tissue time course data following oral exposure. Sensitivity analysis (SA) allowed identification of parameters that were uniquely sensitive for dermal compared to oral exposure and identification of parameters most appropriate to estimate from the available human data. Based on SA, parameters governing oral absorption and metabolism were critical for the oral route, whereas for the dermal route skin permeability was the single most influential parameter. Model-based analysis suggests the need for a second pathway for metabolic clearance (a mechanism not included in other PBPK models for BDCM). Preliminary in vitro studies support this conclusion. These results point to the critical role for model-based analysis in both the design and interpretation of data from human studies. (This abstract does not necessarily reflect Agency policy).

**474 CONTRIBUTION OF TOXICOKINETIC MODELING FOR THE STUDY OF THE IMPACT OF PHYSICAL EXERTION ON THE ASSESSMENT OF OCCUPATIONAL EXPOSURE TO ACETONE (ACE).**

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This study aims at assessing the impact of workload on urinary levels of ACE and its potential influence in the previous determination of the corresponding biological exposure indices (BEI). A physiologically based pharmacokinetic (PBPK) model was developed and validated in order to simulate weekly occupational exposure (8h/day, 5 days) to ACE at the threshold limit value (TLV) according to the American Conference of Governmental Industrial Hygienists (500 ppm). Simulations were then conducted under workload levels expressed as metabolism in both the design and interpretation of data from human studies. (This abstract does not necessarily reflect Agency policy).

**475 PBPK MODELING AS A TOOL FOR PREDICTING THE WOMEN LIFETIME EXPOSURE OF PBDE: FROM INFANTS TO ELDERLY.**

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Polybrominated diphenyl ethers (PBDE) are brominated flame retardants. Because PBDEs migrate to humans, and because children, infants and fetuses are thought to be more sensitive particularly for non-cancer effects there is a need to understand the tissue distribution. The objective of this project was to use a PBPK model to characterize the influence of age-specific physiological parameters on the body burden during lifetime exposure to PBDEs, including gestational status. A PBPK model has been developed to describe the ADME for BDE-47 in women during their lifetime including lactational and transfer from mother to fetus. This model contained 6 compartments: brain, liver, fat, rest of the body, placenta, and fetus and are connected through systemic circulation. Model parameters were obtained from the literature. Schecter et al (2007) reported that blood concentration in women range from 1.92 and 175.2 ng BDE47/g lipid corresponding to 48% of total BPDEs. Simulations were performed to predict the exposure that will result in equivalent tissue concentrations for a woman who is 25 years old. The major factor influencing the tissue concentration is the elimination such as extraction coefficient (E). For example, considering a low blood concentration range of 3.13 ng/g lipid in blood at 25 year old woman this woman need to be exposed to 0.00122 ug of BDE-47/kg of body weight/day for a E=0.0000) or at 54 ug of BDE-47/kg of body weight/day (for E=0.005). Similarly, for the higher concentration of 75.2 ng of BDE-47/g lipid in blood at 25 year old this women will be exposed to 0.11 ug of BDE-47/kg of body weight/day for a E=0.0000) or 85.5 ug of BDE-47/kg of body weight/day (for E=0.005). Development of lifetime PBPK model provides a powerful tool for risk assessment. During lifetime, women go through different critical stages thus, understanding the relationship between these critical stages and dose metric parameters are important determinants in understanding the latency of disease, the clinical signs of which may appear many years after the exposure.
PHYSIOLOGICAL MODEL BASED EVALUATION OF DIFFERENTIAL GASTROINTESTINAL ABSORPTION PATTERNS OF TCDD IN RAT AND HUMANS: IMPLICATIONS IN HUMAN HEALTH RISK ASSESSMENT.

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Human health risk assessments for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) involve extrapolation of data from animals to humans and/or data directly measured in humans. Physiologically based pharmacokinetic model (PBPK) is a tool that has been used in TCDD human health risk assessment. Exposure of TCDD in animals and humans results from oral absorption. In almost all PBPK modeling activities published to date, absorption of TCDD is described with data including volumes, transit times, lengths, and radii. Absorption is modeled via a system of linear and non-linear rate equations that consider 6 compound states, 18 compartments and 3 states of eroded material. The results show that an oral administration of 1mg per day in the fasted and fed rats resulted in approximately 50% difference in the amount absorbed with higher absorption observed in the fasted rat. Similarly, an oral administration of 1mg per day in the fasted and fed human adults resulted in approximately 250% difference in the amount absorbed with higher absorption observed in the fasted humans. In addition, the absorption of TCDD is in both rats and humans occurred predominantly in the duodenum and jejunum. The overall amount absorbed in fasted rats and humans is approximately the same as the respective amounts absorbed in the respective GI compartments. However, the total amount absorbed in fasted rats is approximately 3X times higher than the limited absorbed in fed humans and the same ratio applies to the respective GI compartments. The implications of the differential absorption in the human health risk assessment estimates are analyzed.

USE OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL TO PREDICT TISSUE DISPOSITION IN PIGS FED MELAMINE.

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The recent detection of melamine in milk products and the previous detection of melamine in food and products has resulted in occult 50% difference in the amount absorbed with higher absorption observed in the fasted and fed rats. Similarly, an oral administration of 1mg per day in the fasted and fed human adults resulted in approximately 250% difference in the amount absorbed with higher absorption observed in the fasted humans. In addition, the absorption of TCDD is in both rats and humans occurred predominantly in the duodenum and jejunum. The total amount absorbed in fasted rats and humans is approximately the same as the respective amounts absorbed in the respective GI compartments. However, the total amount absorbed in fasted rats is approximately 3X times higher than the limited absorbed in fed humans and the same ratio applies to the respective GI compartments. The implications of the differential absorption in the human health risk assessment estimates are analyzed.

PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING OF BROMODICHLOROMETHANE IN HUMAN.

E. Demchuk and H. Hansen. Computational Toxicology Laboratory, Agency for Toxic Substances and Disease Registry, Atlanta, GA. Sponsor: B. Fowler.

Bromodichloromethane (BDCM) is a major brominated trihalomethane found in public water supplies after chlorine disinfection. The sources of water contamination with bromine include the Superfund National Priority List (NPL) sites. The ATSDR Sequoia/Hazard database suggests that approximately 15% of NPL sites are bromine contaminated. The common routes of exposure to BDCM include inhalation of near-saturated air during showering and bathing, concomitant long-term contamination of air circulation systems in living areas, transdermal uptake (especially associated with swimming pools and hot tubs), and oral exposure with drinking water. Despite widespread exposures, the health effects of BDCM in humans are not fully understood, and safe exposure levels for routes other than oral have not been established. The present study describes a new physiologically-based pharmacokinetic (PBPK) model specifically designed and parameterized to assess BDCM exposures in humans. The flow-limited PBPK model includes lung, skin, liver, kidney, fat, slowly and rapidly perfused tisue compartments linked by the systemic circulation. The model was validated using actual human exposure data. Additional realism to the model was conveyed by appropriate consideration of differences in human physiology introduced by changing exercise load. It was found that the transdermal route may contribute significantly under the whole-body exposure conditions. The transdermal route may become dominant in well-evacuated environments. It was found that contribution of transdermal exposure for competitive swimmers could be up to 3-4 times greater than from inhalation exposures.

AGE- AND GENDER-STRUCTURED DISTRIBUTIONS FOR PHYSIOLOGICAL PARAMETERS: ASSESSMENT OF HUMAN VARIABILITY IN THE EXPOSURE-INTERNAL DOSE RELATIONSHIP FOR DICHLOROMETHANE (DCM).

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The U.S. EPA is revising its IRIS assessment for DCM, a high production volume chemical that has been used in products such as paint strippers, in manufacturing drugs, film coatings, electronics, and polyurethane foam, and in other processes. DCM exposure causes a variety of effects in multiple organs of rats and mice. David et al. (Regul Toxicol Pharmacol, 2006, 45:55-65) developed a PBPK model to describe DCM dosimetry among humans. Markov-Chain Monte Carlo analysis was used to estimate distributions for metabolic parameters but then a full-population distribution for the polymorphism in glutathione S-transferase was used. The body weight (BW) distribution ranged 7-135 kg, which includes individuals from several months old to obese adults. But when we set physiological parameters for a 1- or 70-year-old person based on Clewell et al. (Toxicol Sci, 2004, 79:381-93), the predicted internal doses fell well outside the distribution otherwise predicted. Thus, the physiological parameter distributions used by David et al. do not appear to fully characterize human variability. We then developed distribution functions for key physiological parameters, including BW, in the U.S. population based on census data and other published analyses. Age and/or gender could either be specified or randomly chosen from a full population distribution (gender ratio depends on age). Then age- and gender-specific distributions were defined for BW, alveolar ventilation, cardiac output, and fat and liver fractions of BW. Using these revised distributions, for example, increased the ratio of the 99th percentile mean liver-specific GST-1 mediated metabolism for the general population from 0.87 to 3.52.6. The mean value for this metric increased from 3.6 to 6.4x10^6 mg/L liver/day. If model predictions are to replace default methods (and uncertainty factors, the model should fully describe the population variability. The analysis, results, and opinions presented are those of the authors and do not necessarily represent EPA policy or decisions.

EVALUATING PBPK MODELING TECHNIQUES USING CALIBRATION DESCRIPTORS.


PBPK models have demonstrated their usefulness in both the human and rodent contexts and are increasingly used in risk assessment. One of their applications is the prediction of quantities not directly observed, such as tissue concentrations over time and the area under such concentration curves (AUC). Published models rarely have confidence intervals for estimated parameters or model predictions. The utility of these confidence intervals depends on their reliability, but that may be affected by model selection. To investigate these issues, we focus on the transport of compound into tissue; the biologically-relevant diffusion-limited compartments or simplified flow-limited compartments. We constructed a generic eleven compartment PBPK model with diffusion-limited liver and adipose compartments and used a protocol typical for the National Toxicology Program toxicokinetic studies to generate test data. One thousand test data sets were created for each of several compound with varying diffusion characteristics. A series of three models were evaluated for the compounds, including diffusion-limited transport for both liver and adipose, for liver but not adipose, and flow-limited transport for both liver and adipose. Utilizing simplex-based optimization routines, the simulated data sets are
used to estimate parameter sets associated with all three PBPK models. Model selection statistics are computed and evaluated. Confidence regions for both parameter estimates and the AUC for tissue compartments are found and their coverage evaluated. The results suggest that including biologically-relevant diffusion-limited compartments in PBPK models, even for compounds which are essentially flow-limited, may yield more reliable confidence intervals for model predictions despite requiring the estimation of additional parameters.

481 MITOCHONDRIAL FUNCTION AND OXIDATIVE STRESS IN DIABETIC NEPHROPATHY IN STREPTOTOTOZIN-TREATED RATS.


Male S-D rats were given streptozotocin (STZ: 60 mg/kg, ip) to induce diabetes. We investigated cellular and mitochondrial glutathione (mtGSH) status and energetics in renal cortical mitochondria and primary cultures of proximal tubular (PT) cells from STZ-treated rats at 30 and 90 days post-STZ (with age-matched control rats). Diabetic nephropathy (DN) typically develops at 45-60 days post-STZ. We tested the hypothesis that diabetes, and ultimately DN, are characterized by enhanced oxidative stress that leads to mitochondrial dysfunction and that, while there may be an initial up-regulation of mtGSH transport, these carriers are down-regulated as rats progress to a state of DN. Histopathological changes were observed even at 30 days and were more extensive at 90 days. Primary cultures of PT cells from 30-day STZ rats exhibited greater morphological injury than those from control rats after exposure to thiols oxidants (tetr-butyl hydroperoxide, mexitil, vinyl ke tone) or a mitochondrial toxin (antimycin A). Although mitochondrial State 3 respiration with succinate as substrate was significantly higher in renal mitochondria from STZ rats (respiratory control ratio ~ 4.7 in STZ vs. 3.4 in control), PT cells from control rats exhibited higher mitochondrial membrane potential (JC-1 fluorescence) than PT cells from STZ rats. Levels of reactive oxygen species (2,7- dichlorofluorescin fluorescence) were significantly higher in PT cells from STZ rats as well, consistent with an enhanced oxidative stress. Although rates of mitochondrial GSH transport were only modestly different between control and STZ rats, mRNA expression of the two mitochondrial GSH carriers (the dicarboxylate carrier [Slc25a10] and the 2-oxoglutarate carrier [Slc25a11]) was significantly enhanced in renal cortex from STZ rats. Diabetic nephropathy (DN) typically develops at 45-60 days post-STZ. We investigated cellular and mitochondrial glutathione (mtGSH) status and energetics in renal cortical mitochondria and primary cultures of proximal tubular (PT) cells from STZ rats as well, consistent with an enhanced oxidative stress. Although rates of mitochondrial GSH transport were only modestly different between control and STZ rats, mRNA expression of the two mitochondrial GSH carriers (the dicarboxylate carrier [Slc25a10] and the 2-oxoglutarate carrier [Slc25a11]) was significantly higher in STZ rat kidney at 30 days, consistent with an initial up-regulation of mitochondrial GSH transport. These results demonstrate that diabetes causes enhanced oxidative stress and mitochondrial dysfunction and that there are early and modest compensatory changes in mitochondrial GSH transport.

482 MULTIDRUG RESISTANCE PROTEIN 2 MEDIATES TRANSPORT OF N-ACETYL-S-(1, 2-DICHLOROVINYL)-L-CYSTEINE.

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N-acetyl-S-(1,2-dichlorovinyl)-L-cysteine (Ac-DCVC) is a metabolite of a common industrial contaminant and potent nephrotoxicant trichloroethylene (TCE). Ac-DCVC may be deacetylated in the renal proximal tubule by aminoacylase 3 (AA3, also significantly inhibited the ATP-mediated transport of glutathione by Mrp2. The transport of Ac-DCVC by Mrp2 was confirmed using IC-MS whereas no transport of DCVC was detected. The results suggested that Mrp2 is involved in the renal excretion of Ac-DCVC. Given the much lower toxicity of Ac-DCVC to mice compared to DCVC in renal proximal tubules. Using immunohistochemistry and immunoelectron microscopy, we localized Mrp2 to the apical membrane of mouse S1 nephron segment where it co-localized with AA3. Mouse Mrp2 was then expressed in the mouse proximal tubule mPCT cell line. Microsomal membranes were isolated from these cells and used to make inside-out vesicles. The ability of Mrp2 to transport Ac-DCVC into the vesicles was first evaluated in an assay, which measured the effect of competing substrates on transport of Ac-DCVC. While exposure to 10 μM Ac-DCVC significantly inhibited the transport rate of glutathione whereas DCVC had no effect. The inhibiting effect of Ac-DCVC was higher than the typical substrate of Mrp2, estradiol 17-β-D-glucuronide. Several other mercapturic acids, good substrates of AA3, also significantly inhibited the ATP-mediated transport of glutathione by Mrp2. The transport of Ac-DCVC by Mrp2 was confirmed using LC-MS whereas no transport of DCVC was detected. The results suggested that Mrp2 is involved in the renal excretion of Ac-DCVC. Given the much lower toxicity of Ac-DCVC to mice compared to DCVC in renal proximal tubules, it could be suggested that the typical localization of AA3 may protect from the Ac-DCVC-mediated toxicity in S1 tubule via prevention of cyttoplasmic deacetylation of Ac-DCVC. In addition, Mrp2 and AA3 may form a transport metabolon mediating the coordinated transport and deacetylation of Ac-DCVC. (NIH ES12935)

483 IMPAIREDrenal FUNCTION AND GLUCOSE METABOLISM IN CALCINEURIN A ALPHA KNOCKOUT MICE.

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Calcineurin, a serine/threonine protein phosphatase was originally discovered as a target of the immunosuppressant drug cyclosporine and since then calcineurin inhibitors have been successfully employed to prevent rejection of transplanted organs in humans. However, long term use of cyclosporine results in adverse drug reactions including nephrotoxicity and post-transplantation diabetes mellitus. Availability of catalytic subunit isoform specific calcineurin knockout mice has led to important advances in our understanding of cyclosporine-induced toxicity. Previously, immunological defects in calcineurin A beta knockout mice have been reported. Due to the early lethality of the calcineurin A alpha knockout mice, evaluation of role of calcineurin A alpha had been difficult. Recently, we rescued these mice from early lethality and mice were evaluated for important organ function tests. Calcineurin A alpha knockout mice show significant increases in the blood urea nitrogen (BUN) and decreases in glomerular filtration rate (GFR) with inflammatory and fibrotic changes upon histopathological examination of the kidneys which are similar to those observed in transplant patients with cyclosporine-induced nephrotoxicity. These findings are also supported by increased expression of TGFbeta and fibronectin by alpha knockout cells. These mice also showed elevated blood glucose levels with decreased tolerance to intra-peritoneal glucose administration which could be similar to cyclosporine-induced post-transplantation diabetes mellitus, however further evaluation is required. Taken together these results show that the pathophysiology of calcineurin A alpha knockout mice is similar to calcineurin inhibitor toxicity and that these mice could be a useful model in studying the mechanisms of calcineurin inhibitor toxicity.

484 2-BROMOETHANAMINE (BEA) INDUCES PAPILLARY NECROSIS BY IMPAIRING THE FUNCTION OF TONEBP UNDER HYPERTONIC STRESS IN KIDNEY CELLS.

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Sponsor: G. Franklin.

Renal papillary necrosis (RPN) may occur as a consequence of long-term abuse of analgesics including nonsteroidal antiinflammatory drugs (NSAIDs). While this is probably mediated by direct toxicity of the drug, the molecular mechanisms whereby NSAIDs induce papillary necrosis are not well understood. A major limitation to the study of RPN pathogenesis is the required chronic exposure before clinical detection of injury. In the present study, 2-bromoethanamine (BEA), a well-known papillotoxic drug, was employed to simulate rapid NSAID toxicity using inner medullary conducting duct (IMCD3) cells. While exposure to 10 μM BEA had no effect on cellular viability under isotonic conditions, when cells were subjected to a sublethal hypertonic stress (550 mOsm/kg H2O) a 50% loss in cell viability was determined in the first 24 hours and nearly complete cell death was determined after 48 hours. This data suggest that BEA exerts cytotoxicity only under hypertonic conditions. Since ToneBP (Tonicity Enhancer Binding Protein) is a transcription factor that is critical to cell survival in hypertonic conditions, we undertook experiments to examine the effect of BEA on ToneBP expression. Exposure of cells to 10 μM BEA resulted in a substantial reduction in ToneBP protein expression after 24 hours. Furthermore, hypertonicity induced expression of ToneBP target genes including aldose reductase, betaine/GABA transporter and Hsp70 was markedly blunted in BEA treated cells. In addition, in BEA treated IMCD3 cells, ToneBP was not translocated to the nucleus under acute hypertonic stress for proper transcription of target genes. Finally, we found a substantial decrease in ToneBP expression in medullary kidney tissues from mice injected with BEA (150 mg/kg). Our data therefore supports the notion that ToneBP is a potential target for BEA and possibly other nephrotoxins leading to the process of papillary necrosis.

485 POTENTIAL TOXICITY OF GLUCOSAMINE MEDITATED THROUGH TRANSFORMING GROWTH FACTOR β (TGFβ).

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POTENTIAL TOXICITY OF GLUCOSAMINE MEDITATED THROUGH TRANSFORMING GROWTH FACTOR β (TGFβ).

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Glucosamine, a well-known antiinflammatory agent, is a potential over-the-counter dietary supplement for the treatment of joint pain. The potential toxicity of glucosamine has been less extensively studied. The objective of this study was to examine the potential toxic effects of glucosamine and compare these effects to a well-known nephrotoxic agent, acetylsalicylic acid (ASA). We undertook experiments to examine the effect of glucosamine on TGFβ expression. Exposure of cells to 10 μM glucosamine resulted in a substantial reduction in TGFβ protein expression after 24 hours. Furthermore, hypertonicity induced expression of TGFβ target genes including aldose reductase, betaine/GABA transporter and Hsp70 was markedly blunted in BEA treated cells. In addition, in BEA treated IMCD3 cells, ToneBP was not translocated to the nucleus under acute hypertonic stress for proper transcription of target genes. Finally, we found a substantial decrease in ToneBP expression in medullary kidney tissues from mice injected with BEA (150 mg/kg). Our data therefore supports the notion that ToneBP is a potential target for BEA and possibly other nephrotoxins leading to the process of papillary necrosis.
Osteoarthritis, a degenerative joint disorder, is the most common form of arthritis affecting at least 20 million Americans, a number that is expected to double over the next two decades. While it is estimated that up to 5 million Americans have consumed the dietary supplement (H. Kennedy: Clin. Ther. 27:1847-1858, 2005), glucosamine as a treatment for joint pain or as a prophylactic for healthy joints, is efficacious, safety and mode of action remain controversial. In this study, five concentrations of glucosamine (15, 30, 120, 300 and 600 mg/Kg/day, oral gavage), separate and in combination with chondroitin sulfate, were given to lean Zucker rats for 6 weeks. While the treatment did not significantly alter serum leptin, uric acid, uric acid/creatinine, glucose, insulin, uric acid/creatinine, or triglycerides, TGF-β mRNA expression in kidney tissue was decreased. A two-fold increase in TGF-β mRNA expression in kidney from rats sacrificed at 4 hours after the last glucosamine (600 mg/Kg/day) treatment was also detected. TGF-β is known to stimulate extracellular matrix formation in response to collagenase. While this extracellular matrix can be beneficial to damaged cartilage in arthritic joints, it can produce pathogenic sclerotic conditions in kidney (S. Chen: Seminars in Nephrology, 23:532-543, 2003). Research was supported in part by IAG 224-93-001 between the NCTR/FA and NIEHS/NTTP.

848 POTENTIAL ROLE OF OXIDATIVE STRESS IN RESVERATROL PROTECTION OF CISPLATIN IN VITRO RENAL TOXICITY.

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Nephrotoxicity is a major adverse effect associated with cisplatin, a cancer chemotherapeutic agent. The development of interventions to delay or prevent the cisplatin associated renal toxicity would be of direct clinical relevance. Resveratrol (RES) is a naturally occurring phytochemical reported to possess some anticancer and antioxidant properties. This study tested the hypothesis that RES reduces the oxidative stress associated with cisplatin. Male Fisher 344 rats (200-250 g) were anesthetized with isoflurane and the kidneys were isolated, decapitated and rinsed for 3 min at ice cold Krebs buffer. Renal cortical slices were prepared and 50-100 mg of tissue was pre-incubated with ethanol (VEH) or 30 μg/mL resveratrol (RES, final concentration) for 30 min at 37°C under an oxygen atmosphere. The tissue was then incubated for 120 min in 3 ml oxygenated Krebs containing 0, 75, or 150 μM cisplatin in an oxygen atmosphere and constant shaker at 37°C. Loss of membrane integrity was evaluated as leakage of lactate dehydrogenase (LDH) following the 120 min incubation. Total renal glutathione, glutathione peroxidase activity, lipid peroxidation and protein carbonyls were measured as markers of oxidative stress. Lipid peroxidation was increased (p<0.05) by 75 and 150 μg/ml cisplatin relative to VEH values. A 30 min pre-incubation with RES prevented cisplatin-mediated increase in lipid peroxidation and protein carbonyls. RES pre-incubation and cisplatin did not alter glutathione peroxidase activity. RES also had no effect on cisplatin-mediated decline in total renal glutathione levels. These findings support the hypothesis that RES pre-incubation diminishes cisplatin in vitro renal toxicity and oxidative stress. (Supported by NIH Grant 5P20RR016477 to the West Virginia IDeA Network for Biomedical Research Excellence).

489 ANALYSIS OF RENAL BIOMARKERS IN RAT URINE SAMPLES FROZEN FOR 44 MONTHS FROM A STUDY ON PUROMYCIN AMINONUCLEOSIDE (PAN).

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Introduction- Following the FDA/EMEA suggestions on new renal biomarkers based on the C-Path Predictive Safety Testing Consortium (PSTC) submission, we have quantified the proposed renal biomarkers in samples of urine collected from control and PAN-treated rats. The samples had been retained frozen (-20°C) for 44 months. Methods- Wistar and Sprague Dawley (SD) rats (3 males/group) received a single IV injection on day 0 of either saline or PAN at 100 mg/kg. A third group was given 75 mg/kg IV on days 0, 1 and 2. Urine was collected pre-treatment; serum and urine were collected on days 3 and 10 prior to autopsy and analyzed for standard parameters. Kidneys were processed and stained with either hematoxylin and eosin or Desmin and examined histologically. RBM’s multiplex quantitative immunoassays developed for the PSTC work were used for quantifying the levels of β2 microglobulin, calbindin, clusterin, cystatin-C, kidney injury marker 1 (KIM-1) and 8 other markers. Results- Histologically, SD rats showed a similar slight mesangial thickening in the renal glomeruli with both treatment regimes. Wistar rats, however, showed a slightly greater change, particularly in the rats given a single dose – as clearly demonstrated by Desmin staining. Creatinin, total protein and N-acetyl-D-glucosaminidase recorded on Day 10, generally correlated with the histopathology. The frozen samples analyzed by RBM proved usable and several markers showed marked rises on Day 10 in all treated groups, with KIM-1, Clusterin and β2-Microglobulin being the most sensitive. Individual variation masked any such effects on Day 5. Conclusions- A panel of multiple biomarker assays were sensitive detectors of Puromycin-induced renal damage in urine stored for 44 months at -20°C. An elevation of several markers was evident on Day 10 (KIM-1, Clusterin, β2-Microglobulin and Neutrophil gelatinase-associated lipocalin in particular).

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1250 mg/L females compared to controls. The kidney was a target organ for beta-observed in the 625 and 1250 mg/L male groups, but were also observed in the 312 to 625 mg/L. Corresponding increases in renal observed in the kidneys of male rats exposed to 1250 mg/L and a few males exposed related effects on survival, clinical observations, organ weights, or gross lesions. both sexes of the 1250 mg/L group was observed within the first few weeks of the study was overcome as the study progressed, suggesting an initial aversion to the palatability of this concentration of beta-picoline. In rats, there were no treatment-related effects on survival, clinical observations, organ weights, or gross lesions. There were significant decreases in terminal body weights and mean water consumption of both sexes of the 625 and 1250 mg/L groups. Hyaline droplets were observed in the kidneys of male rats exposed to 1250 mg/L and a few males exposed to 625 mg/L. Corresponding increases in renal α2u-globulin concentrations were observed in the 625 and 1250 mg/L male groups, but were also observed in the 312 mg/L males. There was also an increase in the severity of nephropathy in males in the 625 and 1250 mg/L groups and an increase in the incidence in the 625 and 1250 mg/L females compared to controls. The kidney was a target organ for beta-picoline toxicity in rats. The observed lesions in this study are consistent with α2u-globulin-associated nephropathy in male rats.

N-cadherin expression dictates acute injury in response to mercuric chloride and simulated ischemia.

Acute kidney injury often occurs on a background of chronic kidney disease. We have established an aging model of CKD in our laboratory that recapitulates the increased sensitivity to acute kidney injury seen in the elderly. However, the mechanisms underlying the increased susceptibility of the aging kidney to injury remain poorly defined. As previous studies from our laboratory have demonstrated that the N-cadherin/β-catenin complex is a target of acute nephrotic and ischemic injury in the kidney, we examined expression of N-cadherin in the aging kidney. Aging is associated with increased injury. The results of this study suggest that the loss of N-cadherin/β-catenin expression increases the susceptibility of the aging kidney to injury, but not confluence of L and LN cells, a cadherin/catenin-deficient cell line and L cells stably expressing N-cadherin and β-catenin, respectively, were challenged with mercuric chloride (10 μM) or a simulated ischemic insult (mineral oil overlay) for 2-6 hr in serum-free media. Cell viability was assessed using the neutral red viability assay. Interestingly, a significant loss of viability was seen at all timepoints in L but not LN cells, 20% of control in L cells; 100% of control in LN cells at 6 hr). A similar result was seen when the cells were challenged with a simulated ischemia: at 6 hr LN cell viability was 60% of control while L cell viability was 20% of control. The data suggest that the loss of N-cadherin and β-catenin could account for the increased susceptibility of the aging kidney to injury as the lack of N-cadherin/β-catenin complex is associated with increased injury.

Pharmacologically-induced mitochondrial biogenesis aids in recovery of function of oxidant-injured renal cells.

Decreased mitochondrial function is associated with diseases affecting metabolic homeostasis and acute organ injuries, such as acute kidney injury (AKI). A primary consequence of impaired mitochondria in the pathophysiology of AKI is depletion of ATP; which can limit the ability of injured cells to recover and restore organ function. Thus, there is a need for pharmacological agent to enhance mitochondrial biogenesis and restore cellular functions. The compound SRT1720 is a potent activator of the NAD-dependent protein deacetylase SIRT1, an enzyme known to activate PGC-1α, the master regulator of mitochondrial biogenesis. We hypothesized that SRT1720 will induce mitochondrial biogenesis in renal proximal tubular cells (RPTC) and restore mitochondrial functions following an acute injury. RPTC treated with increasing concentrations of SRT1720 or diltren were examined for changes in mitochondrial proteins, oxygen consumption, and ATP. RPTC were then exposed to the model oxidant tert-butyl hydroperoxide (TBHP, 400μM), and injured cells were examined for changes in morphology and density, and ATP levels. In a reconstituent SIR1 activity assay, SRT1720 enhanced SIR1 activity 2-fold at 0.1μM and 8-fold at 30μM compared to controls. RPTC treated with 1 to 10μM SRT1720 exhibited concentration-dependent increases in nuclear-encoded ATPase β and mitochondrial encoded NDI protein, observed in both 625 and 1250 mg/L groups. At 3μM SRT1720, basal and FCCP-uncoupled respiration and ATP levels increased approximately 50% over controls. TBHP induced RPTC death and loss at 24hrs. The remaining sublethally injured cells exhibited morphological changes and 50% decrease in ATP levels. In contrast, sublethally injured RPTC treated with SRT1720 exhibited control levels of ATP. Data from these studies indicate that SRT1720 enhances SIR1 activity, induces mitochondrial biogenesis, and increases mitochondrial function within 24hrs in RPTC. Furthermore, SRT1720 restored ATP levels in oxidant-injured cells, suggesting that it may be useful in the treatment of AKI.

Effect of urinary constituents on the differential sensitivity to ethylene glycol-induced renal toxicity in Wistar and F344 rats.

Ethylene glycol (EG) is nephrotoxic because it metabolizes to oxalic acid, which precipitates with calcium to form calcium oxalate monohydrate (COM), accumulates in the kidney and produces necrotic damage. The EG metabolic pathway in humans and rats is considered to be similar. Previous studies have shown that rat cells are more sensitive than human cells to oxalate damage, although Wistar and F344 cells react the same. Wistar rats accumulate more oxalate in the kidney and are more sensitive to kidney toxicity than the F344 rat, but the mechanism for the strain difference is not known. We hypothesized that the supersaturation of calcium oxalate (CaOx SS) in the urine of Wistar rats is higher than that of F344 rats, thereby allowing for greater COM crystal deposition in the Wistar rat. Age-matched male Wistar and F344 rats were treated with 0.75% EG in drinking water for 24hr. Urine was collected at 0, 2, 4, 6, 8 and 24 hr and analyzed for key electrolytes that were used to calculate the ion-activity product as an index of the CaOx SS. Our data supported the differential sensitivity to renal toxicity from EG between the two rat strains (Wistar > F344). CaOx SS did not differ between the two rat strains before EG treatment. After treatment, plasma oxalate level and urine oxalate excretion were markedly greater in Wistar rats than in F344 rats, while urine calcium was slightly decreased in Wistars. These results led to a higher CaOx SS in urine of EG treated Wistar rats, which could explain the enhanced accumulation of oxalate in the kidney. The mechanism for the greater urinary oxalate excretion in the Wistar may relate to differences in renal oxalate transporters. Supported by the American Chemistry Council.

Metabolites of diethylene glycol (DEG) are responsible for the renal toxicity associated with acute DEG poisoning.

Diethylene glycol (DEG) is a chemical intermediate, industrial solvent and antifreeze agent. Ingestion of DEG, primarily through misuse of adulterated pharmaceuticals, has been linked with a large number of cases of acute kidney failure. DEG targets the kidneys, liver and central nervous system, but the mechanisms of toxicity and whether DEG or its metabolites are responsible for the toxicity remain unknown. The purpose of this study was to correlate the kinetics of DEG and its metabolites with the development of toxicity to determine the responsible toxic agent. Adult male Wistar rats were placed in one of four treatment groups including: water, low dose DEG (2 g/kg), high dose DEG (10 g/kg), or high dose DEG + 4-methylpyrazole (4-MP; an inhibitor of the presumed DEG metabolic pathway). Compared to controls, rats treated with high dose DEG had significantly decreased blood bicarbonate levels, urine pH and blood pH, with increased blood urea nitrogen (BUN), serum creatinine and serum AST. These parameters indicated that metabolic acidosis began at about 24 h post DEG treatment. Metabolic acidosis occurred to a small extent in the low dose DEG group, but the animals recovered by 24 h. No signs of any toxicity were observed in the DEG + 4-MP treated group throughout the 24 hr incubation time. DEG was completely eliminated from the plasma by 24 h, except in rats experiencing the most severe toxic syndromes. Ethidium homodimer renal perfusion revealed that the DEG-induced proximal tubular necrosis was blocked by treatment with 4-MP. These results demonstrate for the first time that a metabolite(s) of DEG, rather than DEG itself, is responsible for its toxicity, and that 4-MP
may be a useful antidote in treating DEG poisoning by blocking its metabolism. This project is supported by research agreement with American Chemistry

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The beta-lactam antibiotic cephaloridine, which has been withdrawn from the market, is used as a model compound in nephrotoxicity studies. In isolated rabbit renal

commercial MPS passed the agar diffusion assay regardless of whether the solution was

Biocompatibility testing of medical devices usually requires an assessment of cytotoxicity. Assay selection and protocol design often depend on a specific testing standard rather than on the specific form and function of the medical device. The chemical and/or physical characteristics of some medical devices may predispose them to false positive results in certain cytotoxicity assays. A direct comparison of toxicological studies, for instance, recommending fewer sampling time points and (i) reduces respiration. In the present study, we have re-examined this problem using isolated rabbit renal proximal tubules. For these, tubules were incubated for 4 hours in oxygenated Krebs-Henseleit buffer containing either 1,4-13C-succinate, or 2,3-13C-succinate or succinate + 13C-bicarbonate without or with cephaloridine. Activities of pyruvate dehydrogenase, glucose 6-phosphatase and citrate synthase were also diminished by cephaloridine. In conclusion, the absence of inhibition of mitochondrial uptake of succinate in intact rabbit renal proximal tubules strongly suggests that results obtained with isolated mitochondria do not necessarily apply to mitochondria in intact cells and, therefore, should be interpreted with great caution. These results also suggest that mitochondrial functions should be studied in intact cells.

10% , 25% and 50% PGMC treatment groups were 2.2, 2.8, and 2.6, respectively. Based on oral LD50, dermal LD50 and inhalation LC50, PGMC was classified as non-cytotoxic, but Optifree Replenish MPS was non-cytotoxic, and (ii) reduces respiration. In the present study, we have re-examined this problem using isolated rabbit renal proximal tubules. For these, tubules were incubated for 4 hours in oxygenated Krebs-Henseleit buffer containing either 1,4-13C-succinate, or 2,3-13C-succinate or succinate + 13C-bicarbonate without or with cephaloridine. Activities of pyruvate dehydrogenase, glucose 6-phosphatase and citrate synthase were also diminished by cephaloridine. In conclusion, the absence of inhibition of mitochondrial uptake of succinate in intact rabbit renal proximal tubules strongly suggests that results obtained with isolated mitochondria do not necessarily apply to mitochondria in intact cells and, therefore, should be interpreted with great caution. These results also suggest that mitochondrial functions should be studied in intact cells.

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was not considered to be a dermal sensitizer at any of these concentrations. The results of the LLNA provide further support for the safe use of high purity PGMIC in personal care and other products.

PS 500 VALPROATE REDUCES NEURITE OUTGROWTH BUT REVERSIBLE IN HUMAN SY5Y NEUROBLASTOMA CELLS.

Y. Qian, Y. Zheng and E. Tiffany-Castiglioni, Texas A&M University, College Station, TX.

Valproate (VPA) is a commonly prescribed mood stabilizer. However, emerging evidence indicates that VPA administration frequently causes the reversible syndrome of parkinsonism and cognitive decline (P/CID) in patients. The mechanism of this phenomenon is unknown. In this study, we used human SY5Y neuroblastoma cells as a neuronal model to investigate the effects of VPA on neurite outgrowth and neurofilament expression. Data showed that the treatment with VPA (0-1.0 mM) for 3 days significantly reduced cell proliferation at 1.0 mM and neurite outgrowth length at both 0.5 and 1.0 mM in human SY5Y neuroblastoma cells, but not in human CCF-astrocytoma cells. A prolonged treatment with 0.5 mM VPA in neuroblastoma cells revealed a reduced neurite outgrowth starting at day 3 through day 6. In comparison, the same treatment significantly induced a reduced cell proliferation starting at day 4 through day 6. In parallel to these alterations, both mRNA and protein levels of neurofilament 160 (NF160) were significantly reduced, starting at day 2 and day 3, respectively, by the treatment. Interestingly, the reduced neurite outgrowth by VPA could be completely reversed by the co-administration with nerve growth factor (NGF, 10 ng/ml) for 16 hrs or by the removal of VPA from the medium after 2 days. NF160 protein levels also rebounded to control levels by VPA removal, but not by NGF treatment. These data suggest that VPA-modified NF160 expression was probably involved in the protection and the reversal of neurite outgrowth, consistent with VPA-induced reversible syndrome of P/CID in patients.

PS 501 28-DAY ORAL (GAVAGE) TOXICITY STUDY WITH ETHYLMETHANESULFONATE (EMS) IN THE RAT.

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The purpose of this study was to assess the general toxicity of EMS in a subacute toxicity study in Wistar rats. EMS was administered daily by oral gavage at doses of 0, 20, 60 and 189 and 500 mg/kg/day for 28 days. Various animals were used for recovery and exposure assessment. The incidence and severity of findings were generally dose-dependent occurring at doses of 60 mg/kg/day or above. Decreases in food consumption and body weight were the dose-limiting effects. The starting top dose of 180 mg/kg/day had to be ceased after 5 days due to severe weight loss. After a 9-day treatment pause animals were re-dosed at 120 mg/kg/day. Organ toxicity was characterized by decreased red and white blood cell counts, hematocrit, and hemoglobin. Fatty atrophy of bone marrow, and involution of the thymolymphatic system. Effects on sexual organs were lower organ size for testes, epididymides, seminal vesicles, prostate, and uterus. The main histopathological findings in testes were tubular atrophy and single germ cell necrosis, and in epididymides reduced spermatico-spermatozoon. Other target organs were the pancreas (acinar cell vacuolation), thyroid gland (follicular cell hypertrophy), and salivary gland (secretory depletion of convoluted ducts). Reversibility was observed for the effects on food consumption and body weight and for findings which are most likely secondary to changed physiological conditions (e.g. thyroid gland and pancreas findings, thymus involution). Findings typically attributable to the genotoxic effect of EMS persisted at least in the high dose group (e.g. hematopoiesis, lymph node and testis findings). The systemic exposure to EMS was monitored by hemoglobin ethylene- line adduct measurement. The low dose of 20 mg/kg/day was the No-Observed-Adverse-Effect Level (NOAEL).

PS 502 A REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING ASSAY IN RATS WITH HIGH PURITY MONOOCTYLTIN TRIS(2-ETHYLHEXYL)ERMAPHTOCACTATE (MOTEC).

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Monooctyltin compounds have been previously tested in reproductive studies using mixtures of monooctyltin and diocyltin compounds. These studies were performed using a mixture with high content (85%) monooctyltin trichloride (MOTC) and diocyltin chloride (DOTC) (11%). The NOAEL for maternal toxicity was set at approx. 0.6 mg/kg bw/day because of decreased thyroid weight and lymphoid depletion. The NOAEL for fertility and developmental effects was approx. 6 mg/kg bw/day as a consequence of increased post-implantation losses and decreased pup viability. A mixture with high content of DOTC (>94%) and low MOTC (2.2%) was also evaluated in a comparable study showing the same immunotoxicity and fertility effects. We therefore investigated whether the toxicity observed in both fertility studies was the consequence of monooctyltin or diocyltin content. We evaluated MOTEC (CAS No. 27107-89-7) with high purity (>97%) containing less than 0.5% diocyltin bis-2-ethylhexylmercaptoacetate (DOTE), in a reproduction/developmental toxicity screening assay in rats. Groups of 12 rats/six received MOTEC at 0, 200, 500 and 1250 mg/kg diet throughout the pre-mating period, during the mating and post-mating periods until final sacrifice (28 days) for the males, and through pre-mating (2 weeks) and mating periods, during pregnancy and lactation, until day 4 post-partum inclusive, for the females. Microscopic examination was performed on the testes, epididymides, seminal vesicles, prostate, ovaries, uterus and thymus. There were no toxic effects on parental and female fertility. No effects were recorded on fertility or on pre- and post-natal development. In this study, the NOAEL for parental toxicity, fertility and developmental toxicity is approx. 72 and 9 mg MOTEC/kg bw/day for male and female animals respectively. This indicates that immunotoxicity and effects on fertility observed in previous fertility studies are likely to be the consequence of the diocyltin concentration.

PS 503 SUBCHRONIC TOXICITY OF CITRONELLYL NITRILE IN RATS.

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The subchronic toxicity of citronellyl nitrile (CAS No. 51566-62-2), a widely used fragrance ingredient, was evaluated in a 90-day oral toxicity study in rats. Male and female rats (10/sex/dose) were gavaged once daily with citronellyl nitrile in corn oil at dose levels of 0, 10, 30, 100 or 300 mg/kg/day. Control animals received the vehicle alone. Observations included morbidity and mortality assessments, body weights, food consumption, estrous cycle and ophthalmology. Hematology, blood chemistry and urinalysis were evaluated at weeks 7 and 13. A complete necropsy was conducted on all animals. There were no unscheduled deaths and there were no clinically observable signs of toxicity. There were no adverse effects on bodyweights, food consumption, or hematotoxicology and there were no treatment related ocular effects and no treatment-related effects on female estrous cycles. A statistically significant increase in total protein was observed in high-dose females and a statistically significant increase in albumin levels was observed in high-dose-males. No macroscopic abnormalities were detected at necropsy. A statistically significant increase in absolute and relative liver weights was noted in both sexes in the high-dose group and in males in the 100 mg/kg/day dose group. Marginal centrilobar hepaticocyte enlargement was observed in both sexes in the high-dose group and in 2 males and 1 female in the 100 mg/kg/day dose group but was considered to be adaptive in nature. A higher incidence of lower grades of severity of adipose infiltration in the marrow was noted in high-dose females but was not statistically significant and was considered to be a marginal effect as there were no corresponding hematological changes. Based on these findings, the No Observed Effect Level (NOEL) was concluded to be 30 mg/kg. The No-Observed Adverse Effect Level (NOAEL) was concluded to be 300 mg/kg/day as the liver changes identified histopathologically were considered to be adaptive in nature and the bone marrow changes observed in high-dose females were marginal and were not accompanied by any hematological changes.

PS 504 FRAMEWORK FOR THE SAFETY ASSESSMENT OF MATERIALS USED IN AUTO INTERIORS.

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Next to homes and offices, we spend the most time in automobiles: individuals spend approximately 97 min/day inside a vehicle (Tsang and Klepeis, 1996; Klepeis et al. 2001). Time spent inside a vehicle range from 12 min at the 5th percentile to 770 min at the 95th percentile. The Suppliers Partnership for the Environment (SP), an innovative collaboration between automobile manufacturers, their suppliers, and the U.S. Environmental Protection Agency (EPA) has formed a Chemical Issues Work Group to provide a forum for discussing emerging chemical issues within the automotive supply chain. The purpose of the Workgroup is to share best practices and no treatment-related effects on female estrous cycles. A statistically significant increase in total protein was observed in high-dose females and a statistically significant increase in albumin levels was observed in high-dose-males. No macroscopic abnormalities were detected at necropsy. A statistically significant increase in absolute and relative liver weights was noted in both sexes in the high-dose group and in males in the 100 mg/kg/day dose group. Marginal centrilobar hepaticocyte enlargement was observed in both sexes in the high-dose group and in 2 males and 1 female in the 100 mg/kg/day dose group but was considered to be adaptive in nature. A higher incidence of lower grades of severity of adipose infiltration in the marrow was noted in high-dose females but was not statistically significant and was considered to be a marginal effect as there were no corresponding hematological changes. Based on these findings, the No Observed Effect Level (NOEL) was concluded to be 30 mg/kg. The No-Observed Adverse Effect Level (NOAEL) was concluded to be 300 mg/kg/day as the liver changes identified histopathologically were considered to be adaptive in nature and the bone marrow changes observed in high-dose females were marginal and were not accompanied by any hematological changes.
consists of conservative (“worst case”) assumptions regarding airborne chemical levels and the availability of residues on exposed interior surfaces. Tier II employs mathematical models to estimate the dynamics of air and surface levels in a car under various conditions. Tier III uses actual analytical determinations of airborne and interior surface levels (both total and transferable) to estimate occupant exposure. The exposure estimates are used to conduct a risk characterization for the appropriate endpoints. We demonstrate the application of this framework from hazard identification through risk assessment with example chemicals.

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Humans are exposed to numerous chemicals through sources such as foods, medicines and consumer products. Consumer safety is of paramount importance, yet detailed toxicological data may be lacking for chemicals present in consumer products at low levels. This represents a challenge in assessing consumer safety at a time when there is increasing public and regulatory pressure to reduce animal testing and increasingly rely on information from alternative methodologies. However, validated alternatives to repeated dose toxicity tests in animals are unlikely to be available in the near future. This may be overcome by the applying the Threshold of Toxicological Concern (TTC), an increasingly accepted tool used in the absence of chemical-specific toxicity data. The concept relies on information from the general toxicity database to establish exposure thresholds for groups of chemicals, below which there would be no appreciable risk to human health. Using the TTC framework, this investigation examined whether consumer exposure to low level ingredients present in air fresheners was of negligible risk. Air fresheners may contain natural oils, solubilizers, and/or gelling components amongst other ingredients. Groups of chemicals for which the TTC approach is not considered suitable (e.g., heavy metals, high potency carcinogens) are not used as ingredients in air fresheners. It was assumed that the low level ingredients released from the said product belonged to structural Class III, the most conservative of the Cramer Classes, and consumer exposure was modeled using RIVM’s ConsExpo model on the basis of measured product emission rates. An additional uncertainty factor was applied to account for extrapolation from the oral to the inhalation route. It was concluded that consumer exposure to ingredients present at concentrations below 0.1% in air fresheners was below the limit set for Class III compounds and consequently did not present a safety concern for systemic endpoints.

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Allergic contact dermatitis is a delayed-type hypersensitivity reaction induced by small reactive chemicals (hapten). Currently, the sensitizing potential of chemicals is usually identified on the basis of animal studies, such as the local lymph node assay (LLNA) or Guinea Pig tests. There are, however, increasing public and political concerns regarding the use of animal testing for the screening of new chemicals. Consequently, the development of in vitro models for predicting the potential of new chemicals is receiving widespread interest. The COLIPA Skin Tolerance project team currently collaborate with a number of academic research groups to increase and apply our understanding of the molecular and cellular events occurring during the acquisition of skin sensitization. At present fundamental and applied research is being funded in the following key areas; chemistry/peptide binding, skin metabolism, skin bioavailability of skin sensitizing chemicals; evaluation of different biomarkers for Dendritic cell activation by chemical sensitizers; and T cell proliferation. Knowledge gained from this research is being used to support the development and pre-validation of novel in vitro approaches for the identification and characterization of skin sensitizing chemicals. At present three in vitro test methods (Direct peptide reactivity assay, MUSST and hCLAT) are being evaluated for their potential to predict skin sensitization potential within COLIPA interlabatorial ring trials.


Triclosan (Ingasan®; 5-Chloro-2-(2,4-dichlorophenox) phenol) has a long history of safe use as an antimicrobial in home and personal care products and has been extensively studied to ensure it is acceptable and safe for its intended uses. The majority of the human health-related test results for triclosan (TCS) are confidential to Ciba, thus speculation and unsubstantiated statements appear regularly indicating triclosan should be avoided, removed from consumer products, or raise concerns for severe adverse health effects. With this poster, we present an overview of our key hazard data for TCS, exposure estimates for consumer-used products containing TCS (up to 0.5%), and a risk characterization calculation and assessment for human health. Data are available on metabolism and pharmacokinetics for TCS including ADME studies in hamsters, mice, rats, and humans. These data indicate humans exposed to TCS are best represented by hamsters as a laboratory model, in which we established a N0AEL = 75 mg/kg/d from a lifetime study wherein liver and kidney changes defined the effects. TCS is readily absorbed, fully metabolised, and eliminated (no bioaccumulation) from the body of humans and all other mammalian species used. Total exposure to TCS from personal care products gives a range of systemic estimated doses (SEDs) depending on the types of products included. Several risk calculations will be presented, but as an example: including exposure to the full range of products containing TCS gives a human SED of about 0.245 mg/kg/day. The Margin of Safety (MoS) is about 300, which is derived against the hamster lifetime NOAEL: [75 mg/kg/d x 0.245 = 306]. With 100 considered as minimally acceptable using animal data, we find triclosan is safe for the intended uses. Other exposure based estimates give higher MoS and also show TCS exceeds acceptance criteria for safe use in personal care products.


Allergy to hair dyes is an important topic for the safety of hair dye products. QRA (quantitative risk assessment) is the predicted knowledge of the allergenic potency and the dose response characteristics of the ingredient as well as the assessment of the consumer exposure level. We introduce an approach for experimentally measuring the skin exposure to hair dye actives. Specifically, we suggest assessing the consumer exposure level based on exposure data available from skin penetration studies. These in vitro studies (OECD guideline 428) provide direct quantification of the concentrations found on and in the skin and will be referred to as the measured consumer exposure level (mCEL) at a distinct use condition under realistic use conditions. For example, the dermal load for p-phenylenediamine was 22 µg/cm² determined after application of a typical hair dye product containing 2% PPD under use condition (30 min exposure time and rinsing with water and shampoo). We conclude that this approach allows for a more robust and accurate QRA for hair dyes.

J. P. Tailhardat10.

Extracts of natural rubber latex (NRL) medical devices (e.g., condoms) typically produce a positive response in in vitro cytotoxicity assays; however, clinical use of these devices is associated with a very low incidence of adverse effects. The positive response seen in cytotoxicity assays complicates the interpretation of these results in the preclinical biological evaluation of NRL-containing medical devices. To differentiate devices with the potential to produce adverse effects in vivo from those with an adequate safety profile, dilution cutoff values (e.g., 1:8) have been proposed to determine an acceptable level of toxicity of the extracts in cytotoxicity tests. The goal of this study is to provide an empirical basis for dilution cutoff values for acceptable levels of in vitro cytotoxicity. Sections of commercially available male condom lubricated NRL (polyurethane, and 1 lubricated NRL with spermicide) were extracted (3 cm²/ml) with RPMI-1640 media +/- 10% FBS for 24 hrs at 37°C. Extracts from NRL tubing were used as positive controls. Dilutions (1:1 to 1:20) of the extracts were sterile filtered, applied to confluent 24-hour-old cultures of L929 fibroblasts and RAW 264.7 macrophages, and incubated for 24 hours. Results for the cytotoxicity of the extracts were calculated as the mean of triplicate experiments.


Natural rubber latex (NRL) medical devices (e.g., condoms) typically produce a positive response in in vitro cytotoxicity assays; however, clinical use of these devices is associated with a very low incidence of adverse effects. The positive response seen in cytotoxicity assays complicates the interpretation of these results in the preclinical biological evaluation of NRL-containing medical devices. To differentiate devices with the potential to produce adverse effects in vivo from those with an adequate safety profile, dilution cutoff values (e.g., 1:8) have been proposed to determine an acceptable level of toxicity of the extracts in cytotoxicity tests. The goal of this study is to provide an empirical basis for dilution cutoff values for acceptable levels of in vitro cytotoxicity. Sections of commercially available male condom lubricated NRL (polyurethane, and 1 lubricated NRL with spermicide) were extracted (3 cm²/ml) with RPMI-1640 media +/- 10% FBS for 24 hrs at 37°C. Extracts from NRL tubing were used as positive controls. Dilutions (1:1 to 1:20) of the extracts were sterile filtered, applied to confluent 24-hour-old cultures of L929 fibroblasts and RAW 264.7 macrophages, and incubated...
SAFETY ASSESSMENT OF OXIDATIVE DEGRADATION PRODUCTS IN HYDROCORTISONE.

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Hydrocortisone, a commonly used anti-inflammatory and anti-pruritic agent, was demonstrated to be susceptible to oxidation, thus requiring qualification of the resultant degradation products. A toxicological assessment was conducted to determine the toxicological potential (if any) associated with these degradation products. The likely degradation products were identified as 17-deoxy-21-dehydrohydrocortisone and 21-dehydrohydrocortisone under neutral conditions and the etio-acid (an oxidative product), 17-ketosteroid, and the glycolic acid under basic conditions. Hydrocortisone formulations containing clay minerals (ingredients common to topical formulations) were implicated as pro-antigens in allergic reactions caused by corticosteroid preparations; however, this is a known adverse reaction of hydrocortisone itself. These 2 degradation products also contain a ketoaldehyde which is comparable to the alkyl aldehyde group, for which a structural alert for genotoxicity has been identified; however, most other chemicals with this alert are short chain aldehydes such as formaldehyde. Bulki er structures like that of 21-dehydrohydrocortisone and 17-deoxy-21-dehydrohydrocortisone could potentially buffer or diminish any potential genotoxic effects. Since clay minerals generally contain surface ferric iron, the potential for accelerating the oxidative degradation of drugs should be considered whenever clay minerals and drugs susceptible to oxidation are combined.

SAFETY ASSESSMENT OF OXIDATIVE DEGRADATION PRODUCTS IN HYDROCORTISONE.

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RESEARCH AND DEVELOPMENT OF IN VITRO STUDIES FOR DOSIMETRY ASSESSMENT: A COST-BENEFIT ANALYSIS.

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K.C. Koo.

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In vivo dermal exposure was studied for comparison with in vitro dermal exposure. A cost-benefit analysis was conducted to identify the optimal in vitro model for dust exposure assessment. One of the major drawbacks of an in vitro dermal exposure study is the cost associated with animal use. The cost associated with animals and hormone use was taken into consideration when evaluating the cost-benefit of various in vitro models. The purpose of the study was to establish the most cost-effective in vitro dermal exposure assessment model.

COMPARISON OF SEVERAL COSMETIC PRESERVATIVES FOR MILDNESS.


Mary Kay Inc, Dallas, TX.

A significant number of personal care product consumers believe that they have sensitive skin. However, the concept of sensitive skin is not always consistent. It is therefore important to evaluate objective skin irritation in addition to subjective skin irritation forms in formulations specific on clinically-diagnosed sensitive skin. The method comprises of 2 parts. In part 1, we used data from the EpiOcularTM assay to predict ocular irritation potential of products. In part 1, we used data from the EpiOcularTM assay to predict ocular irritation potential of various preservatives. The EpiOcular assay results are an organotypic model of human corneal epithelium cultured from normal human keratinocytes using serum free medium making it an excellent choice to determine the irritancy of their products without using animals. In part 2, 14-day cumulative irritation test and sensory irritation tests were performed on human subjects with sensitive skin to compare various combinations of preservatives in sensitive skin formulations. A comparison of the in vitro data and in vivo data was performed to assess the utility of the tissue model to screen potential preservative systems specifically for sensitive skin formulations.

OPTIMIZATION OF IN VITRO MICRONUCLEUS TEST WITHOUT CYTOCHALASIN B TO EVALUATE TOTAL PARTICLE MATTER OF CIGARETTE SMOKE.


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In the current OECD draft guideline (3rd version) for in vitro micronucleus test (MN), some options were cited for several parameters (e.g. cell line, presence or absence of cytochalasin B (CB)). On the other hand, Health Canada (TS03 2nd edition) specifies CHO cells and the without-CB condition for MN to evaluate the total particle matter (TPM) of cigarette smoke. CORESTA in vitro toxicity task force recommends to include in vitro genotoxicity test using mammalian cells (MN, CA or MLA) in biological test methods for TPMs, and arranged a collaborative study of MN for TPMs. However, experimental condition for MN has not been studied in detail. Therefore, we examined the conditions of CB using CH/LU cells, and tried to find the optimal parameters for the evaluation of TPMs. Furthermore, we evaluated the accuracy and discrimination capability for the test condition using various TPMs. The number of cells with micronucleus did not vary between the with-CB and without-CB condition at the dose range without severe cytotoxicity. In that dose range, the frequencies of mitotic cells are almost the same to that of solvent control under the without-CB condition. It is indicated that, under the without-CB condition, the micronucleus induction by the TPMs can be evaluated sufficiently. The optimal condition of parameters which we found under the without-CB condition (3-h treatment and 21-h for recovery time) is: 5.2×103 cells/mL for initial number of cells, 24 hours for preculture and 1.5% for the S9 concentration. Under this condition, it was observed that the dose responses of the micronucleus induction by three different TPMs (blue cured, burley and 3R4F) were clearly discriminated, and furthermore, the order of the activities was maintained with and without S9 condition. In conclusion, the optimal condition of in vitro micronucleus test using CH/LU cells without cytochalasin B for TPMs was established from this experiment.

OPTIMIZATION OF IN VITRO MICRONUCLEUS TEST WITHOUT CYTOCHALASIN B TO EVALUATE TOTAL PARTICLE MATTER OF CIGARETTE SMOKE.

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IN VIVO DERMAL EXPOSURE ASSESSMENT: RESOLVING THE DISPOSITION OF BOUND SKIN RESIDUES FOR LANNATE® 20SL.

W. J. Harrison and K. S. Bentley.

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Nearly a decade ago, Thongsinthusak et al. (1999) published an exponential saturation model to estimate absorption of bound skin residues (BSR) in rats using cumulative excretion data. Use of a modeling approach was intended to avoid repeating the in vivo dermal exposure study with additional animals and longer recovery times. In the ten years since Thongsinthusak, little has been published on the applicability of the modeling approach. Nonetheless, the European Commission guidance, SANCO/222/2000 (2004), recommends use of modeling as part of "expert judgment on disposition of BSR. We have evaluated BSR disposition from an original rat in vivo dermal study terminated at 66 h post-exposure with modeling and a follow-up in vivo dermal study terminated 42 days post-exposure. Radioactivity was exposed for 6 h to Lannate® 20SL (suspension liquid) containing methomyl active ingredient (a.i.), either a spray dilution (5 g a.i./L) or the neat formulation (200 g a.i./L) at a rate of 10 μL/cm2 to a 10.5 cm2 area on the back; at h po, residual formulation was removed by washing with soap and water. Excreted were collected during the exposure and post-exposure periods. For rats held until 66 h post-exposure, 17.8% and 6.04% of the applied dose was absorbed and 39.3% and 6.09% remained as BSR for the spray dilution and neat formulation, respectively. On the other hand, residual formulation was removed by washing with soap and water. Excreted were collected during the exposure and post-exposure periods. For rats held during the 66 h post-exposure, 17.8% and 6.04% of the applied dose was absorbed and 39.3% and 6.09% remained as BSR for the spray dilution and neat formulation, respectively. On the other hand, residual formulation was removed by washing with soap and water. Excreted were collected during the exposure and post-exposure periods. For rats held during the 66 h post-exposure, 17.8% 6.04% of the applied dose was absorbed and 39.3% 6.09% remained as BSR for the spray dilution and neat formulation, respectively. On the other hand, residual formulation was removed by washing with soap and water. Excreted were collected during the exposure and post-exposure periods. For rats held during the 66 h post-exposure, 17.8% 6.04% of the applied dose was absorbed and 39.3% remained as BSR for the spray dilution and neat formulation, respectively. Overall, absorption of the spray dilution, based on the 66 h post-exposure, was comparable to the model-predicted value at least higher than the model-predicted value at least higher than the 42-day results; the model estimated absorption and the 42-day results for the neat formulation were comparable to the 66 h study. These results support the conclusion that the BSR retained in the skin following a 6 h dermal exposure to Lannate® 20SL, does not become systematically available.

POST-MARKET CHALLENGES FOR SAFETY ASSESSMENT OF HERBAL PRODUCTS.

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In Canada herbal products are regulated as natural health products (NHP). Not all NHPs on the Canadian market are currently authorized for sale; however a phased-in approach ensures that all marketed NHPs will be authorized in 2010. Post-market (PM) assessment is critical for improving the sale of all marketed products
and can influence pre-market requirements for NHPs. PM safety assessment of herbal products includes signal detection and clinical/toxicological assessment. Regulators can face difficult risk mitigation (RM) decisions due to uncertainty in the clinical and toxicological database for herbal products. Efforts at decreasing uncertainty can lead to better informed RM decisions. As part of the NHP Regulations, manufacturers must report serious adverse reactions (AR) to Health Canada (HC); practitioners are encouraged to do so voluntarily. Published reports of herbal ARs are also monitored. Challenges in clinical assessment of AR information include issues of quantity and quality. HC has taken steps to improve AR reporting: the development of on-line educational modules, and issuing publications aimed at health care practitioners and the public. Targeted stimulation of reporting has led to increased information and mitigation of risk (eg black cohosh). Scientific assessment of safety signals is also hampered by quantity and quality of toxicological information. Verification of plant species in published case reports and toxicological studies is important for determining cause and effect; however, this information is often lacking, making accurate associations difficult. With the current lack of toxicological information for many herbs, predictive toxicology can be of some use in the safety assessment of individual psychochemicals; however, herbal products are generally complex mixtures of psychochemicals. Thus, data on isolated constituents needs to be taken in context with studies using whole plants or plant extracts, such as those being conducted by the NTP. Despite challenges, combined clinical and scientific assessment of herbal safety can provide meaningful information on which to base appropriate RM activities.

515 EFFECT OF SIMULATED RESPIRATORY ACIDOSIS AND HYPERTHERMIA ON THE HEMOLYTIC EFFECT OF MATERIAL EXTRACTS.

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Hemolysis testing of medical device materials is typically conducted using erythrocytes (RBCs) from healthy animals or humans (e.g., ISO 10993-4, ASTM F756); however, various disease states can potentiate the toxic effect of compounds released from medical device materials. Use of assay conditions that replicate the physiological conditions found in disease states may result in biocompatibility testing of device materials under more clinically relevant conditions than those defined in standard test methods. The goal of this study is to determine if in vitro simulated respiratory acidosis (hypoxia, acidosis, hypercapnia) and hyperthermia potentiate the hemolytic effect of material extracts. Positive (latex, neoprene, PVC, BUNA rubber) and negative (silicone) control materials were extracted at 37°C in ethanol:PBS (50:50) for 24 hrs using the surface area/volume ratios defined in ISO 10993-12. Extracts were incubated with washed cow and rat erythrocytes (Hct 1-5%) under normothermic (37°C) or hyperthermic (40°C) conditions for 4 hrs in control (pH 7.4) or CO2-gassed (pH 6.8) PBS. Hyperthermia potentiated the toxicity of all material extracts in both cow and rat RBCs. Simulated respiratory acidosis potentiated the toxicity of some but not all material extracts in either cow or rat RBCs. A synergistic hemolytic effect was produced by hyperthermia and acidosis for neoprene extracts in cow and rat RBCs. Other extracts produced synergistic effects in cow (PVC) but not rat, and in rat (latex, BUNA rubber) but not cow RBCs incubated under hyperthermic and acidotic conditions, suggesting that interactive hemolytic effects produced under these conditions are species dependent. Since the hemolytic effects produced by material extracts incubated in vitro under normal physiological conditions can differ from those seen under conditions consistent with disease states, the clinical relevance of the results of hemolysis tests conducted using the ISO and ASTM standards should be carefully considered for devices used in critically ill patients.

516 DETERMINING THE DISTRIBUTION OF VX IN GUINEA PIG TARGET ORGANS FOLLOWING A SINGLE SUBLETHAL SUBCUTANEOUS OR PERCUTANEOUS EXPOSURE.


Physiologically based pharmacokinetic-pharmacodynamic (PBPK-PD) models have become useful analytical tools to interpret data from chemical warfare nerve agent (CWA) exposure scenarios for risk assessments. These models rely upon accurate databases to compare the uptake and clearance of similar CWA doses across various routes of administration. A sensitive method for determining exposure to the chemical warfare nerve agent VX has been developed in which the biomarker ethyl methylphosphonothioate (VX-G) is used as a dose metric for determining tissue distribution and kinetics of VX in guinea pigs following sublethal exposures via subcutaneous (s.c.) or percutaneous (p.c.) routes. The VX-G is measured in select target organs following treatment with fluoride ion using isotopic dilution gas chromatography tandem mass spectrometry (GC-MS/MS). The aforementioned analyte and a deuterated analog were isolated via solid phase extraction and chemical ionization in the monitoring mode. The method has been applied to the analysis of blood, skin (from the exposure site), liver, lung, and kidney tissues from guinea pigs exposed to 0.1 LD50 s.c. or p.c. dose of VX taken at 2, 4, 6, 24, and 48 hours post exposure. For the subcutaneous exposure, VX-G levels in general showed a decreasing trend in time with the highest concentrations in liver and kidney tissues. For the percutaneous exposures, VX-G levels were highest in the skin tissues which decreased exponentially over 48 hours. These results are consistent with previous studies on p.c. application of lethal doses of VX on anesthetized and atropinized guinea pigs where the concentrations of VX in blood rose over a six hour period and then slowly decreased with implications that VX was still circulating at toxicologically relevant levels.

517 EFFICACY OF NOVEL PYRIDINIUM OXIMES FOR POTENTIAL ACTIVITY IN THE CENTRAL NERVOUS SYSTEM FOR REACTIVATING PHOSPHORYLATED ACETYLCOLINESTERASE.

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Organophosphate (OP) poisoning resulting in the inhibition of acetylcholinesterase (AChE) traditionally has been treated with atropine sulfate and an oxime, such as 2-PAM. Currently available oximes have limited abilities to cross the blood-brain barrier, resulting in little, if any, reactivation of AChE in the brain. This study involves the testing of pyridinium oximes that have been synthesized to increase the phosphorylating potential of the oximes. The test compounds are the propyl group containing a phosphinatomethylphosphonate (PIMP; IC50=36nM), leaves AChE phosphorylated with the same moiety as the nerve agent sarin, but degrades quickly in aqueous solution to prevent re-inhibition of reactivated AChE in vivo assay. Rat brain homogenate was treated with PIMP to yield about 80% inhibition, followed by 0.1mM oxime for 30 min, and AChE activity was measured by a discontinuous spectrophotometric assay. Reactivation varied among the oximes (34-81%), with 2-PAM yielding 89% reactivation. Lipophilicity, reflecting the potential for crossing the blood-brain barrier, was determined by n-octanol/water partition coefficients and varied among the oximes with some up to 30 fold greater than 2-PAM. The partition coefficient for 2-PAM was 0.006, and those for some of the oximes were up to 0.138. A few of the novel oximes showed combined higher lipophilicity along with reactivation potential approaching that of 2-PAM, suggesting that they may be effective reactivators in the brain. Supported by Defense Threat Reduction Agency: 1.E0056_08_AHB_C.

518 DETERMINATION OF LCT50 IN RATS EXPOSED TO NEBULIZED NERVE AGENTS.


Studies were designed to determine the LC50 of the organophosphate nerve agent VX (O-ethyl S-(2-diisopropylaminoethyl) methylphosphonothioate) and its iso- mer VR using a consistent inhalation delivery system. Nerve agents pose a threat to the respiratory tract with exposure resulting in acute lung injury and death. The determination of toxicity by inhalation is important for the rational development of these in vitro countermeasures. This study was designed to deliver nebulized diluted nerve agents in a dose-response manner to determine the extent of lethality of these non-volatile agents. Male rats (240-270g) were anesthetized intramuscularly with 10mg/kg xylazine and 90 mg/kg ketamine. Following anesthesia, rats were intubated with a glus endotracheal tube (ET) and placed in a glove box. The ET was connected to a closed circuit nebulizer system (Aeroneb, Aerogen, Inc.) that delivered a particle size of <2.0 μm and was in series between the ventilator and the ET. Nerve agents were delivered by a small animal ventilator set for a volume of 2.5 mL, 0.60 breaths/minute and VR was nebulized in 0.1mM solutions for 30 minutes with inhalation concentrations ranging from 6.25 to 800 μg/kg over a 10 min exposure time period. Lethality by inhalation occurred either during the 10 min exposure period or less than 15 min after the start of exposure. Survivors were euthanized at 24 h post-exposure. LC50 estimates (95% confidence intervals) were obtained from the se
result of a single seizure-inducing dose of RDX. Supported by Army subcontract N01-AI-40095.

**519 EVIDENCE OF RDX-INDUCED BRAIN INJURY USING PERIPHERAL BENZODIAZEPINE RECEPTOR (PBR)/TRANSLOCATOR PROTEIN 18KDA (TSPO) QUANTITATIVE AUTORADIOGRAPHY.**

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Hexahydro-1,3,5-trinitro-1,3,5-triazine is a synthetic, high-impact, stable chemical known as Rapid Detonation Explosive (RDX) that has been used in munitions since World War II. Humans can be exposed during manufacture or when RDX leaks into groundwater from an unexploded ordnance. The primary symptoms of acute oral exposure to high doses in humans is transient and reversible seizures. Seizures have also been reported in experimental animals. These effects in humans are often reported in occupational settings with high RDX airborne exposures. Studies have correlated measurements of RDX in the tissue of animals with frank seizures but little work has been done on the effects of RDX at the molecular or cellular level. The peripheral benzodiazepine receptor (PBR), recently renamed translocator protein 18 kDa (TPSO) has been used as a biomarker of brain injury in a variety of animal models (Chej and Guillaire, Pharm. Ther. 118: 1-7, 2008). In this work, male Sprague-Dawley rats (165-185 g) were either given a single oral dose (60 mg/kg) of RDX suspended in water or vehicle. Within 3 hours, 21 out of 24 animals had tonic-clonic convulsions. At 1, 3, 5, 7, and 10 days after RDX administration, animals were euthanized, brains harvested and fresh frozen at -80 degrees. Autoradiography of [3H]-R-PK11195 binding to PBR/TSPO was performed in RDX-treated and control brains. We found increased [3H]-R-PK11195 specific binding to PBR/TSPO in all animals dosed with RDX relative to controls. In particular, significantly increased levels of [3H]-R-PK11195 binding were measured in the cingulate cortex, hippocampus, and cerebellum. This work quantifies the effects of RDX in specific areas of the mammalian brain and suggests widespread brain injury as a result of a single seizure-inducing dose of RDX. Supported by Army subcontract W91ZLK-07-P-2160 and ES07062 to TRG.

**520 ACUTE TOXICITY, TOXICOGENETICS, AND HISTOPATHOLOGY OF INHALED RICIN IN BALB/C MICE AND SPRAGUE-DAWLEY RATS.**

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Ricin is a ribosome-inactivating protein derived from the castor bean. Because of its ease of dissemination and potential for human morbidity following exposure, the CDC has classified ricin as a Category B bioterrorist agent. Most likely routes of human exposure are inhalation and ingestion. The purpose of this study was to compare the acute toxicity, lung clearance and histopathology of inhaled ricin in rats and mice. For the acute toxicity studies, 5 groups of 6 animals of each species were exposed nose-only to ricin aerosols (1 μM MMAD). Targeted deposited doses for the acute toxicity studies were 0.12 – 1.2 μg/kg. The observation period was 7 days, with tissues collected for histopathological evaluation at necropsy. The calculated median lethal doses for rats and mice were 0.15 and 0.36 μg/kg, respectively. For rats, mean survival time was ±2 days, except for the lowest dose tested, where mean survival time was 6.2 days. Concentrations of ricin in rat lung from all but the lowest dose group ranged from 23 – 93 ng. For mice, survival time was 2 (highest dose) to 7 days (two lowest doses), with 6 – 9 ng ricin/lung found up to 3 days post exposure. Pulmonary lesions included hemorrhage, bronchiolitis, and vascu
tis. Respiratory tract clearance and tissue distribution were assessed at 6 time points, up to 72 hours post exposure of rats and mice to approximately 95 ng ricin/lair for 20 min and 40 min. For rats, initial lung burdens following 20 and 40 min exposures were 52 ± 7.7 and 145 ± 25 ng/lung (45 ± 10, and 110 ± 25.2 ng/lung respectively. The initial lung burden following a 40 min exposure in mice was 12.6 ± 7.7 ng/lung (88.5 ± 65.9 ng/lung). Half times for elimination of ricin from the lung were less than 24 hours for both species. Results of our studies indicate that ricin is extremely toxic following inhalation, that ricin is eliminated from the lung rapidly, and that responses of rats and mice are similar. Research conducted under NIAID contract N01-AI-40095.

**521 ACETYLCHOLINESTERASE ACTIVITY IN GUINEA PIG TISSUE AFTER SUB-LETAL VX EXPOSURE: COMPARISON OF PERCUETANEOUS VS SUBCUTANEOUS EXPOSURE ROUTES.**

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The nerve agent VX is a potent inhibitor of acetylcholinesterase (AChE). In contrast to the G-agents that are volatile and primarily an inhalation threat, VX has low volatility, is readily absorbed through the skin and is primarily a percutaneous (PC) threat. Data on the toxicity of PC exposure to VX are limited to exposures in anesthetized animals. Since anesthesia may affect the absorption of VX, it is important to evaluate kinetics in un-anesthetized animals. We and other laboratories observed that there is a delay in maximum concentration of VX (or fluoride ion generated G-analog of VX) and a much longer plasma half-life compared to G-agents. While VX inhibition of blood AChE was robust, VX inhibition of butyrylcholinesterase was minimal. We currently report on the time-course of AChE inhibition in tissues following a single sub-lethal and asymptomatic VX dose (0.4 LD50), administered by PC or subcutaneous (SC) route to un-anesthetized guinea pigs. Tissue activities of AChE were determined between 2-48 hr after exposure. Compared to saline control animals, following SC exposure, significant inhibition occurred in heart and brain 2-48 hr and in lung and eye 2-24 hr. No significant inhibition was observed in diaphragm or kidneys. In the diaphragm, AChE activity was variable, and there was no significant trend for levels to be inhibited at 2 and 6 hr after exposure. However, following PC exposure, AChE inhibition was delayed, with inhibition in heart 6-24 hr and brain and lung 2-24 hr. There was no significant inhibition observed in eye, kidneys or diaphragm, although there was a non-significant trend for reduced AChE activity in diaphragm at 24 hr. These data, along with VX-G concentration in tissue, will be used in mathematical models of absorption, distribution, elimination and tissue response to nerve agents to improve human toxicity estimates.

**522 ACUTE TOXICITY, TOXICOGENETICS, AND HISTOPATHOLOGY OF INGESTED RICIN IN BALB/C MICE.**

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Because of its ease of dissemination and potential for human morbidity following exposure, the CDC has classified ricin as a Category B bioterrorist agent. Most likely routes of human exposure from a terrorist attack are ingestion and inhalation. The purpose of this study was to compare the acute toxicity, GI tract clearance, tissue distribution, and histopathology of ricin administered to BALB/c mice by gavage. For the acute toxicity studies, 5 groups of 10 mice were administered ricin at 44.7, 7, 11.7, 25 or 28 mg/kg. Mice were observed for 7 days for morbidity and mortality. Tissues were harvested from all animals for quantitation of ricin and evaluation of morphologic changes. The calculated LD50 (95% confidence interval) was 12.1 mg/kg (8.44-17.0 mg/kg). Survival time was dose dependent, ranging from 3.3 ± 1.8 to 6.5 ± 1.6 days for the highest and lowest dose groups, respectively. The primary target organs for ricin induced toxicity were the gastrointestinal tract (apoptosis, congestion, mucosal/submucosal granulocytic infiltrates), liver (apoptosis, congestion, hepatic cytoplasmic vacuolization, sinusoidal granulocy
tic leucocytosis), spleen (lymphocytic/hematopoietic apoptosis, neutrophilic leucocytosis), and pancreas (apoptosis, congestion, granulocytic infiltrates). The kidney was less frequently affected (congestion, vacuolization). Notably, similar lesions were present in mice surviving 7 days, even at the lowest dose tested. Results were verified in a follow-up experiment with a second ricin preparation. Ricin clearance and tissue distribution were assessed at 7 time points post dosing. Ricin concentration in stomach tissue was 19.2 ± 14.4 μg/g (97.8 ± 65.5 μg/g tissue) 0.25 hour and 60.2 ± 77.7 ng/lung (576 ± 519 ng/g tissue) 48 hours post dosing. In conclusion, ingested ricin is several orders of magnitude less toxic by ingestion than by inhalation. Lesions persist in animals surviving seven days post exposure. Ricin clears rapidly from the stomach, but quantifiable levels persist up to 2 days post dosing. Research conducted under NIAID contract N01-AI-40095.

**523 NEUREGULIN-1 PROTECTS AGAINST PARAOXON-INJECTED APOTOSIS IN HIPPOCAMPAL SLICE CULTURES.**

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Organophosphorous (OP) pesticides cause significant death worldwide each year and OP nerve agents continue to represent a credible terrorist threat in military combat and against civilian populations. Current post-exposure medical countermeasures against OPs are useful in preventing mortality, but are insufficiently effective in protecting the CNS from seizures and permanent injury. Therefore, new and
more effective medical countermeasures must be developed to facilitate better emergency treatment and protection of civilians and military personnel following exposure to OPs. The use of neuroprotective and anti-inflammatory compounds, in combination with the current antidotal drugs, has been shown to decrease toxic symptoms and inflammation-induced brain damage. The objective of this study is to evaluate the therapeutic benefit of neuregulin-1 (NRG-1), a novel neuroprotective, anti-inflammatory compound, alone or as a complement to the standard therapy for OP poisoning. Hippocampal slice cultures derived from postnatal rat pups were exposed to varying concentrations of paraoxon for 24 hr beginning on the fifth day in vitro. Two markers of neuronal damage were assessed: 1) apoptosis, which was measured by fluorometric detection of caspase-3 activity; and 2) oxidative damage, which was assessed by western blot analyses of oxidized proteins. Oxidative damage was not detected but significant increases in caspase-3 activity were observed at 0.01 and 10 μM paraoxon. Addition of NRG-1 at 0.1 and 3 μM significantly reduced paraoxon-induced apoptosis. These findings suggest that NRG-1 represents a novel neuroprotective agent that may be useful in optimizing treatment of individuals exposed to acutely toxic levels of OPs. This work was supported by NIH (NS 057993).

524 2-DICHLOROETHYL ETHYL SULFIDE STIMULATES ARACHIDONIC ACID METABOLISM IN MOUSE SKIN.

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The chemical warfare agent sulfur mustard induces blister formation and severe inflammatory reactions in the skin. In the mouse ear vesicant model (MEVM), sulfur mustard rapidly induces edema and inflammation as well as acanthosis and hypergranulosis. In previous studies we demonstrated that in the MEVM, 2-chloroethyl ethyl sulfide (CEES), a model sulfur mustard vesicant, rapidly induces the production of the antioxidant enzyme oxygenase-1, as well as an inducible nitric oxide synthase and the proinflammatory mediators IL-1β and IL-6. In the present studies we determined if CEES modulated production of prostaglandins and leukotrienes, two important classes of arachidonic acid-derived proinflammatory lipid mediators. CEES (65 μM) induced the biosynthesis of both prostaglandin E2 (PGE2) and leukotriene B4 in the skin. This was associated with increases in mRNA expression of COX-2 and PGE2 synthase, two enzymes mediating production of PGE2. Leukotrienes are derived from arachidonic acid via 5-lipoxygenase (5-LOX); CEES decreased expression of 5-LOX mRNA, but increased expression of 5-LOX activating protein (FLAP), a critical protein upregulating leukotriene biosynthesis. CEES also decreased mRNA for leukotriene A4 hydrolase and leukotriene C4 synthase, as well as 12-lipoxygenase. Taken together these data show that in the MEVM, CEES modulates both the prostaglandin and leukotriene biosynthetic pathways and generates inflammatory mediators. PGE2 and LTβ4 agonists may be important therapeutics for sulfur mustard-induced tissue injury. Supported by NIH grants CA100994, CA093798, ES004738, NIH grants CA100994, CA093798, ES004738, ES05022, GM034310 and AR059073.

525 THE EFFECTS OF LOW DOSE SARIN AT SYMPTOMATIC AND ASYMPTOMATIC DOSES ON BEHAVIOR AND CATHECOLAMINE LEVELS IN MICE.

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The differences between symptomatic and asymptomatic doses of the chemical warfare agent sarin (GB) at the time of dosing are significant. Using two different types of exposure we are able to investigate the differences behaviorally and neurochemically between the two doses. In the first method of exposure we used the carbamate esterase inhibitor CBP1 to enhance the effects of GB on the mouse and allow a lower dose of GB to be administered. In the second group, a low asymptomatic dose, animals are given 2 doses of sarin 1 day apart. The first group of animals were given 1.5 mg/kg CBP1 followed an hour later 0.2 LD50 GB and the second group was given 2 doses of 0.4 LD50 GB a day a part (asymptomatic). The 2 doses of 0.4 LD50 was asymptomatic and the CBP1 plus GB produced significant acetylcholinesterase inhibition, seizure and a 35% mortality rate. The animals underwent a neurobehavioral battery before being euthanized for HPLC, neurochemi-
guinea pig model is in progress. Our next goal is to evaluate the efficacy of PON1 against inhaled soman and sarin in the guinea pig model of microinstillation inhalation exposure.

528 THERAPEUTIC EFFECT OF REACTIVATION OF SARIN-INHIBITED ACETYLCHOLINESTERASE (ACHE) IN THE CENTRAL NERVOUS SYSTEM (CNS) BY THE TERTIARY OXIME MONOSONITROSOCETONE (MINA) IN GUINEA PIGS.


The nerve agent sarin is a potent inhibitor of the enzyme ACHE which results in excessive accumulation of acetylcholine (ACh) and overstimulation of central and peripheral cholinergic receptor sites. Treatment of sarin poisoning consists of the anti-cholinergic atropine to block the effects of excessive ACh and an oxime such as 2-pyridinium aldodoxime (2-PAM) to reanimate agent-inhibited ACHE. Oximes such as 2-PAM are quaternary drugs that provide negligible reactivation of CNS ACHE. The present study investigated the capacity of the tertiary oxime MINA to 1) reactivate peripheral tissue, blood and brain ACHE inhibited by sarin; 2) terminate sarin-induced seizures; 3) prevent neuropathology and the behavioral consequences of sarin exposure; and 4) protect against the lethal effects of sarin. MINA (22-139 mg/kg, IM) administered 15 min after 1LD50 of sarin resulted in a dose-dependent reactivation of peripheral tissue, blood and brain ACHE after 60 min, whereas 2-PAM (25 mg/kg, IM) reactivated only peripheral tissue ACHE. MINA (18-56 mg/kg, IM) administered from 0 to 10 min after a 2LD50 sarin challenge resulted in a dose-dependent blockade of electrophysiological seizures, neuropathology and behavioral depletion. These sequelae were not blocked by 2-PAM treatment. Seizure prevention or termination preserved neurobehavioral functioning in tests of shuttlebox avoidance, acoustic startle, and open field locomotion. MINA (35 or 60 mg/kg, IM) administered alone, with atropine (0.5 mg/kg), or with atropine and 2-PAM 1 min after seizure challenge increased the 24-hr LD50 of sarin 5- to 15-fold compared to 1- to 3.5-fold for atropine, 2-PAM or atropine plus 2-PAM treatment. The results show that reactivation of brain ACHE by the tertiary oxime MINA can mitigate the lethal and adverse neurobehavioral consequences of sarin intoxication and reinforce the importance of restoring ACHE activity in the CNS following nerve agent intoxication.

529 EVALUATION OF DELAYED TREATMENT WITH NOVEL NERVE AGENT ANTICONVULSANTS AND THEIR EFFECTS ON BRAIN NEUROTRANSMITTERS IN A RAT MODEL.


Nerve agents are potential threats for use in terrorist attacks. Administration of standard antidote drugs (atropine, pralidoxime (2-PAM), diazepam) to civilian casualties in a terrorist attack will be substantially delayed. This study developed a delayed nerve agent treatment model and evaluated standard treatments plus novel anticonvulsant adjuncts. Rats were surgically prepared with electroencephalographic (EEG) recording electrodes and a microdialysis guide cannula directed at the basolateral amygdala (BLA) or the piriform cortex (PC). A week later the animals were challenged with the nerve agent soman (180 ug/kg, SC). EEG was monitored for seizure onset and 20 min after seizure onset the animals received standard medical countermeasures — 0.45 mg/kg atropine sulfate, 25 mg/kg 2-PAM and 2.0 mg/kg diazepam — either alone or with an adjunctive drug. The animals were monitored 6 hr after exposure. Microdialysis samples were collected at 15-min intervals at a flow rate of 1.5 ul/min during this time. At 24 hr the animals were anesthetized and perfused with saline followed by 10% formalin. The brains were examined to determine cannula placement and evidence of neuropathology. In the absence of an adjunct drug, all animals continued to display seizure activity for more than 4 hr after administration with focal countermeasures and 12 of 16 (75%) were still seizing at 24 hr; all animals displayed marked weight loss and extensive brain pathology. Treatment with the anticholinergic procyclidine (5.6 mg/kg) as an adjunct to standard medical countermeasures stopped seizure activity 10.8 +/- 0.2 min after administration in 14 of 22 (64%). Animals in which seizures were stopped by procyclidine had no brain pathology and lost significantly less weight than those where seizures were not controlled by procyclidine treatment. Adjunct treatment with procyclidine can control nerve agent-induced seizures even under delayed treatment conditions.

530 MINOCYCLINE AS A NOVEL THERAPEUTIC TO ORGANOPHOSPHOROUS NERVE AGENT POISONING.


Exposure to chemical warfare nerve agents results in the accumulation of synaptic acetylcholine, exciting both peripheral and central cholinergic receptors. The resultant acute cholinergic crisis induces the synaptic release of alternative neurotransmitters, leading to widespread neuronal stimulation in the CNS. This progressive condition causes excitotoxic neuron injury via increased intracellular calcium and mitochondrial dysfunction, which is further exacerbated by initiation of the proinflammatory response. The focus of this study was the ability of minocycline (MIN) to mitigate neural injury. MIN has been shown to be neuroprotective during global and focal brain ischemia, traumatic brain injury, endothelin-1-induced vasconstriction, intracerebral hemorrhage, and seizure-induced brain injury (Domercq and Matute, 2004). Rats were injected with HI-6 30 min before delivery of 1.6 LD50 of soman and atropine. Following seizure onset, rats were injected with diazepam and either MIN (22.5, 45 or 90mg/kg) or vehicle. The neuroprotective efficacy of MIN was evaluated using EEG recording and neuropathology. In the initial phase, we are assessing whether MIN treatment affects 1) tissue injury and clinical response to soman exposure, 2) soman-induced increases in permeability of the blood brain barrier, and 3) the release of pro-inflammatory cytokines. We have determined that 90 mg/kg MIN reduces seizure severity at 24 hrs post-exposure. H&E and Fluoro-Jade B staining suggest that this dose decreases cell deaths in the hippocampus and piriform cortex. If MIN is in fact minimizing excitotoxic neuron injury, we hypothesize that it may decrement the proinflammatory response, attenuate BBB permeability, downregulate the release of the inflammatory cytokines and reduce the presence of apoptotic markers. Our initial data suggest that MIN inhibits neuron injury in specific brain regions at 90 mg/kg, with potential to decrease seizure severity and brain injury by downregulating mediators of inflammation and apoptosis.

531 IN VIVO PHARMACOKINETIC EVALUATION OF A NOVEL CESIUM (CS) DECORPORATION AGENT IN RATS.

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Novel decorporation agents are being developed to protect against radiological terrorist attacks (i.e. dirty bombs). Nano-engineered solid sorbents for chelation therapy were developed and validated in vivo. These sorbents, known as the self-assembled monolayer on mesoporous supports (SAMS@MN), are hybrid materials where differing organic moieties are grafted onto mesoporous silica (SiO2). For cesium (Cs) a Cu-Fc-EDA-SAMMS was evaluated for in vivo decorporation and experiments were conducted comparing the performance of the SAMMS vs. insoluble Prussian Blue. Three groups of jugular cannulated S-D rats (6/treatment) were evaluated. Group I was administered Cs (~40 mg/kg) by intravenous (iv) injection and oral gavage; Group II was administered prebound SAMMS-Cs by oral gavage; and Group III was orally administered Cs (~0.06 μg/g/kg) followed by 0.1 g of either SAMMS or Prussian Blue. Following dosing the rats were maintained in metatbolism cages for 72 hr and blood, urine and fecal samples were collected for Cs analysis (gamma counting). Rats were humanely euthanized and liver, stomach, small and large intestine were analyzed. Orally administered Cs was well absorbed (~100% relative to iv dose), and the pharmacokinetics (blood, urine, feces & tissues) were very similar. For both exposures the urine and feces accounted for 20% and 3% of the dose, respectively. The prebound SAMMS-Cs was retained primarily within the feces (72% of the dose), with ~1.4% detected in the urine, suggesting that the Cs remained tightly bound to SAMMS, and is extensively eliminated in the feces. SAMMS & Prussian Blue both effectively chelated available Cs in the gut with feces accounting for 80-88% of the administered dose, while less than 2% was detected in the urine. This study suggests that the functionalized Cs derivative can be effective at reducing Cs exposure.

532 EFFECTS OF 4-PYRIDINEALDOXIME (4PA) ON NERVE AGENT-INHIBITED ACETYLCHOLINESTERASE ACTIVITY IN GUINEA PIGS.


The oxime methoxime (MMB-8) is being proposed as a replacement for 2-pyridinium aldodoxime (2-PAM) as an antidote to reactive nerve agent-inhibited ACHe activity and alleviate toxic and lethal consequences of nerve agent exposure. 4PA is...
a break-down product and a probable metabolite of MMB-4. The interaction of 4PA with AChE in vivo may complicate the pharmacological consequence of nerve agent therapy. Therefore, the purposes of this study were to determine in guinea pigs the capacity of 4PA to affect the toxicity of the nerve agent sarin and/or to interact with AChE activity in blood, brain, and peripheral tissues. Animals were pre-treated with atropine methyl nitrate (AMN) (1.0 mg/kg, im) 15 min prior to sarin exposure to minimize peripheral toxic effects. AMN is a peripheral acting muscarinic receptor blocker that does not affect AChE activity. Animals were challenged with 1.0 x LD50 dose of sarin. 4PA (5.5, 7.0, or 14.0 mg/kg) was given im at 15 min (at a time of maximum brain, blood and tissue AChE inhibition by 1.0 x LD50 dose of sarin) after sarin exposure. A saline/saline control group and a sarin-exposed/saline-treated group were also included. At 15 min (before 4PA administration) and 58 min (before termination of the experiment) after sarin injection cholinergic toxic signs were scored. Blood, brain regions, and peripheral tissues were collected at 60 min after sarin exposure for analysis of AChE activity. 4PA did not modify the severity of sarin-induced toxicity score at a time point. The AChE activity observed at 60 min after sarin exposure was not significantly affected by any of the doses of 4PA administered at 15 min following sarin challenge. The present findings indicated that 4PA at the doses tested in this study does not influence (reduce or potentiate) the toxicity of sarin or the AChE activity inhibited by sarin.

**433 GENOMIC ANALYSIS OF PIRIFORM CORTEX AND HIPPOCAMPUS FOLLOWING EXPOSURE TO SARIN.**

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Organophosphorus nerve agents, such as sarin, are known to inhibit acetylcholinesterase; however, the biological pathways involved in nerve agent-induced neurodegeneration are not well understood. To determine these pathways, we analyzed gene expression changes following sarin exposure. Male Sprague-Dawley rats were challenged with 1.0 x LD50 sarin, using saline as a vehicle. One minute after seizure onset, the animals were treated with atropine sulfate and 2-pyridine aldoxime methachloride (2-PAM). Thirty minutes later, they were given diazepam. Control and experimental animals were killed 30 minutes later (post-exposure day (PED) 8). Following a 5-minute habituation period, the rats were given 15-tone (85 dB, 10s) shock (1.0 mA, 2s) pairings with 60-second intervals. Fear conditioning was conducted on post-exposure day (PED) 8. Following a 3-minute habituation period, the rats were presented with 7 tones (85dB, 10 s) with 60 seconds between pairings. Twenty-four hours after conditioning the rats were returned to the test chamber and freezing behavior was recorded. Freezing behavior was measured, 48 hours after conditioning in a novel context. Following a 5-minute habituation period, the rats were presented with 7 tones (85dB, 10 s) with 60 seconds between trials. Rats exposed to GD or saline froze for 40-60% of each minute when placed in the conditioning chambers. When presented with the tone, rats exposed to 1.2 LD50 GD froze less than 1.0 LD50 GD and saline- treated animals. The present study indicates that fear conditioning potentially could be used as a behavioral assessment of neurotoxicity resulting from GD exposure. Evaluation to compare neurotoxicity of the GD-exposed rats with performance in the fear conditioning tasks is ongoing.

**434 A COMPARISON OF THE SENSITIVITY OF DIFFERENT STRAINS OF MICE TO SARIN AND KAINIC ACID SEIZURES.**


Soman (GD), an organophosphorus nerve agent, irreversibly inhibits acetylcholinesterase leading to an accumulation of acetylcholine at central and peripheral sites. The effects of cholinergic hyperactivity include hypersecretion, respiratory distress and convulsions. Current therapies increase survival rates but do not necessarily offer protection from seizure-associated brain pathology and performance deficits. The amygdala and hippocampus are affected in animals that seize extensively. Both regions are believed to play important roles in the development and expression of fear conditioning. The fear conditioning tasks are evaluated as possible behavioral correlates of the observed neurotoxicity. Male rats were implanted with a telemetry transmitter which continuously collects electroencephalographic activity. The rats were exposed to GD (1.0, 1.2 LD50) or saline followed 1 minute later with injections of atropine (2 mg/kg, im) and the oxime Hi-6 (93.6 mg/kg, im). Diazepam (10 mg/kg, sc) was administered 30 minutes after seizure onset or 50 minutes post-exposure in rats that did not seize. Fear conditioning was conducted on post-exposure day (PED) 8. Following a 3-minute habituation period, the rats were given 15-tone (85 dB, 10s) shock (1.0 mA, 2s) pairings with 60 seconds between pairings. Twenty-four hours after conditioning the rats were returned to the test chamber and freezing behavior was recorded. Freezing behavior was measured, 48 hours after conditioning in a novel context. Following a 5-minute habituation period, the rats were presented with 7 tones (85dB, 10 s) with 60 seconds between trials. Rats exposed to GD or saline froze for 40-60% of each minute when placed in the conditioning chambers. When presented with the tone, rats exposed to 1.2 LD50 GD froze less than 1.0 LD50 GD and saline- treated animals. The present study indicates that fear conditioning potentially could be used as a behavioral assessment of neurotoxicity resulting from GD exposure. Evaluation to compare neurotoxicity of the GD-exposed rats with performance in the fear conditioning tasks is ongoing.

**435 EFFECTS OF SOMAN EXPOSURE ON CONTEXTUAL AND CUED FEAR CONDITIONING IN RATS.**


Soman (GD), an organophosphorus nerve agent, irreversibly inhibits acetylcholinesterase leading to an accumulation of acetylcholine at central and peripheral sites. The effects of cholinergic hyperactivity include hypersecretion, respiratory distress and convulsions. Current therapies increase survival rates but do not necessarily offer protection from seizure-associated brain pathology and performance deficits. The amygdala and hippocampus are affected in animals that seize extensively. Both regions are believed to play important roles in the development and expression of fear conditioning. The fear conditioning tasks are evaluated as possible behavioral correlates of the observed neurotoxicity. Male rats were implanted with a telemetry transmitter which continuously collects electroencephalographic activity. The rats were exposed to GD (1.0, 1.2 LD50) or saline followed 1 minute later with injections of atropine (2 mg/kg, im) and the oxime Hi-6 (93.6 mg/kg, im). Diazepam (10 mg/kg, sc) was administered 30 minutes after seizure onset or 50 minutes post-exposure in rats that did not seize. Fear conditioning was conducted on post-exposure day (PED) 8. Following a 3-minute habituation period, the rats were given 15-tone (85 dB, 10s) shock (1.0 mA, 2s) pairings with 60 seconds between pairings. Twenty-four hours after conditioning the rats were returned to the test chamber and freezing behavior was recorded. Freezing behavior was measured, 48 hours after conditioning in a novel context. Following a 5-minute habituation period, the rats were presented with 7 tones (85dB, 10 s) with 60 seconds between trials. Rats exposed to GD or saline froze for 40-60% of each minute when placed in the conditioning chambers. When presented with the tone, rats exposed to 1.2 LD50 GD froze less than 1.0 LD50 GD and saline- treated animals. The present study indicates that fear conditioning potentially could be used as a behavioral assessment of neurotoxicity resulting from GD exposure. Evaluation to compare neurotoxicity of the GD-exposed rats with performance in the fear conditioning tasks is ongoing.

**436 H2AX FORMATION IN THREE-DIMENSIONAL HUMAN SKIN AND CELL MODELS FOLLOWING IN VITRO EXPOSURE TO SULFUR MUSTARD.**


Sulfur mustard (2,4-dichlorodioxyl sulfoxide, SM) is a cytotoxic chemical warfare agent known for its vesicating properties. SM exposure to human tissue results in DNA damage, cell and tissue death. To elucidate the genotoxic effects of SM, the presence of γ-H2AX foci was determined in several skin models. These included normal human epidermal keratinocytes (NHEK) and two commercially available multicellular skin tissue constructs, EpiDerm™ and EpiDermFTM (MarTek Corp.). γ-H2AX is a phosphorylated derivative of the H2AX histone and is tightly bound to double strand DNA break sites, making γ-H2AX an important indicator of genotoxic injury. NHEK, EpiDerm™ and EpiDermFTM constructs were exposed to 0, 50, 100 and 300 μM concentrations of SM. Two and 24 hrs following SM exposure, models underwent immunohistochemistry staining using a mouse anti-γ-H2AX antibody with propidium iodide (PI) as a nuclear stain. Our results show that SM exposure leads to the formation of γ-H2AX foci in the in vitro models at 24 hrs compared to the untreated controls. This in vitro model of early DNA damage will aid in the development of therapeutic compounds to prevent cell death following SM exposure.

**437 POLYURETHANE SPONGES CONTAINING ADDITIVES FOR DECONTAMINATING GUINEA PIGS EXPOSED TO CUTANEOUS SOMAN OR VX.**

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Polyurethane sponges were very effective for the decontamination of skin following acute exposure to chemical warfare agents. The sponges were designed for ease of use and the ability to detoxify or inactivate chemical warfare agents. Detoxifying
This research was supported by the Defense Threat Reduction Agency – Joint GD-induced SE and greater understanding of the neuroinflammatory process may play a key role in the progressive brain pathology observed following GD-induced SE, a strong chemotactic response originates primarily in neurons and microglia. IL-1β, TNF-α, and MCP were tested against each of these conditions. Additionally, serum samples were collected from rats exposed to the neuropathological mechanisms for these effects remain to be determined, but there are clear implications for developing medical countermeasures to chemical warfare agents prevents further contamination and toxicity and extends the life of the sponge, because it can be used multiple times. Decontamination efficacy was measured by survival of guinea pigs exposed to soman (GD) and VX. GD or VX was applied to skin on the lateral flank of anesthetized guinea pigs and was removed with a wiping motion 1 minute after application. To measure destruction of GD and VX in the sponge over a 5 hour period, samples from the sponge were added to known concentrations of acetylcholinesterase (AChE). The amount of GD or VX removed by the sponge but not neutralized was measured by residual inhibition of AChE. When testing the effectiveness of oximes, sponge solutions contained 50 mM oxime in phosphate buffer. The oximes TMB-4, 2-PAM, BMO and HI-6 were tested against GD challenge and HI-6 was found to be the most effective, increasing the median lethal dose (MLD) by a factor of 7. Solutions of 30% tetryramine or 10% triglyme were also tested against GD challenge; and each increased the MLD by a factor of 8. All additional experiments were conducted using mixtures of oximes and tetryramine. The combination of tetryramine and HI-6 was very successful, increasing the MLDs of GD and VX by a factor of 110. The results at GD and VX were neutralized in the sponges correlated well with the increased MLDs observed. In summary, the small, self-contained polyurethane sponges will provide immediate use for either the individual or buddy in the field with little additional training and enhance force protection and mobility.

**539 RAPID RADIATION EXPOSURE ASSESSMENT ACCOMPANIED VIA A HEMATOXYLIC MATRIX**

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The human health concerns associated with radiological incidents, whether accidental or intentional, are well documented. However our ability to respond to a clinical level is limited by the lack of technologies that can rapidly and reliably assess the level of radiation that a victim has received. Building on the established response of lymphocyte frequencies to irradiation (IR), the Hematotoxicity Response Matrix (HARM) assay employs flow cytometry to provide data on the absolute number and apoptotic signature of multiple hematopoietic cell types in peripheral. Undiluted whole blood is placed directly into a staining solution that labels cellular nucleic acid content and mitochondrial membrane potential (MMP). Proof-of-principle experiments in mice demonstrated the ability of the HARM assay to assess the response of several blood cell populations to radiation exposure. These studies were extended by validating the method of cell identification using phenotypic signatures and using HARM-based blood cell counting to a standard hematology analyzer. Blood samples collected from rats exposed to 6 Gy, 15 Sv total body irradiation revealed dose- and time-dependent reductions in lymphocyte and nucleocyte frequencies and elevations in the MIF-based apoptotic signature in these populations. Additional experiments using untreated nonhuman primate and human samples demonstrate the cross-species compatibility of the reagents used and analytical approach. Application of statistical modeling revealed that the combinatorial use of multiple endpoints improved the dose estimating ability of the HARM assay. Our findings lay the groundwork to suggest that this method could play an important role in the triage of individuals involved in radiological incidents.

**540 EVALUATION OF A CAPSAICIN ANALOG AS A MODULATOR OF INFLAMMATORY CYTOKINE RELEASE IN HUMAN TISSUE MODELS**

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We studied the mechanism of toxicity of the chemical vesicant sulfur mustard (2, 2'-dichlorodiyethyl sulfide, SM) for the purpose of developing medical countermeasures to chemical threat agents. A major feature of SM tissue injury is inflammation. Capsaicin is known to block neurogenic inflammatory pathways. The capsaicin drug has been shown to have limited ability to prevent lung injury in the mouse ear vesicant model. They have been used with cell culture models to inhibit inflammatory cytokine release and reduce cellular death. The novel capsaicin analog KR-24095 was obtained from the KRICT. Development of a potential therapeutic compound required additional information on tissue sensitivity to capsaicin analogs. We acquired data in vitro. The following results indicate that the same compound attenuates the inflammatory response due to SM. Thus, a capsaicin analog may serve a dual purpose of being anti-inflammatory and protecting against SM-induced cell death.

**541 DEVELOPMENT OF A PULMONARY INJURY MODEL OF INHALED SULFUR MUSTARD**


Sulfur mustard (SM) poses a considerable potential threat as a weapon or terrorism agent due to its ease of synthesis and ability to be easily distributed in a manner that would allow for a mass casualty situation. Appropriate animal models are required to understand mechanisms of effect and to validate therapeutic agents. We compared inhalation of both vapor and aerosol forms of SM in female F344 rats. Due to the extreme reactivity, nose-only inhalation of SM vapors resulted in severe nasal damage with minimal injury in the conducting Airways and nose in the deep lung. An aerosol formulation of SM in ethanol delivered by nose-only inhalation induced injury in the lung but still caused severe injury in the nasal cavity. IL-2, -8, -15, -IL-6, -IL-2, -1β, IFN-γ, TNF-α, and GM-CSF induced by 300 µM SM was reduced in both the bronchial cells and resident microglia to areas of brain damage. The induction of this inflammatory response may play a key role in the progressive brain pathology observed following GD-induced SE and greater understanding of the neuroinflammatory process may help develop better therapeutic strategies to mitigate the effects of GD exposure. This research was supported by the Defense Threat Reduction Agency – Joint Science and Technology Office, Medical S&T Division.
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1pharmacology and toxicology, wright state university, Dayton, OH and 2Applied
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group compare to toxic challenge alone in dentate gyrus.

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group compare to toxic challenge alone in dentate gyrus.
DOXYCYCLINE AND TOBRADEX SUPPRESS RABBIT CORNEAL BASEMENT MEMBRANE ZONE DAMAGE INDUCED BY 2-CHLOROETHYL ETHYL SULFIDE.


Pharmacology and Toxicology, Rutgers University, Piscataway, NJ, “Air Force Research Laboratory, Wright-Patterson AFB, Dayton, OH,” Environmental and Occupational Medicine, UMDNJ, Robert Wood Johnson Medical School, Piscataway, NJ, “Environmental Health Science, New York Medical College, Valhalla, NY,” Pharmacaceutics, Rutgers University, Piscataway, NJ, “Chemistry, Lehigh University, Bethlehem, PA” and Battelle, Biomedical Research Center, Columbus, OH.

A rabbit corneal organ culture model system was used to evaluate the effects of anti-oxidative and steroid countermeasures against 2-chloroethyl ethyl sulfide (CEES), a model sulfur mustard vesicant. In this system, airlifted corneal organ cultures were exposed to CEES, plus or minus countermeasure, and then analyzed 24 hr later by electron microscopy to evaluate the junction between the epithelial basement membrane and the anterior stroma. In unexposed controls the lamina lucida and lamina densa were distinct, as were hemidesmosomes, anchoring filaments, and anchoring fibrils. A 200 n mole CEES exposure for 2 hr caused a range of phenotypes. Some areas looked normal and intact, while adjacent regions showed vesicant effects ranging from swelling and loss of clarity of the hemidesmosomes to basal lamina integrity loss. In more damaged areas, the electron density of the hemidesmosomes faded, and the cell membrane between the hemidesmosomes retracted from the degrading basal lamina. The anterior stroma looked littered from basement membrane degradation. Corneas treated for 22 hr with doxycycline after CEES exposure were markedly improved, although in some areas the hemidesmosomes still looked swollen. The basal lamina in most sections had a clear lamina lucida and lamina densa, similar results were found with Tobradex, although extensive detachment of epithelial cells from the stroma between the hemidesmosomes was frequently observed. Despite this, the lamina lucida and lamina densa were, for the most part, distinct. Supported by NIH grants EY009056 and AR055073.

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Involvement of BNIP3-Mediated Intracellular Calcium Mobilization in Cyanide-Induced Cell Death.

Zhang, L., Li, H., Heaverley, X., Zhang, J., Boorwitz and G. Iomn. MCMP, Purdue University, West Lafayette, IN.

Cyanide is a potent neurotoxin that can induce neurodegeneration in select brain areas. Sublethal cyanide intoxication can result in dopaminergic neuronal cell death in substantia nigra and has been linked to a Parkinsonism-like syndrome. In the current study, the mechanism of cyanide-induced apoptosis was determined in a dopaminergic cell line, Mes 23.5. In response to cyanide, BNIP3, a BH-3 only Bcl-2 family protein, localized in both endoplasmic reticulum (ER) and mitochondria. Elevated BNIP3 in ER caused by cyanide mobilized calcium from ER to mitochondria. This increase in mitochondrial calcium was blocked by Ru360, a mitochondrial Calcium uniporter inhibitor. The excess mitochondrial Calcium facilitated loss of mitochondrial membrane potential (ΔΨm) and resulted in cell death. Either Ru360 or cyclopiazonic A, a mitochondrial permeability transition pore inhibitor, preserved ΔΨm and provided protection from cyanide-induced cell death. Also, in response to cyanide, Bax was activated and co-localized with BNIP3 in ER and mitochondria. Forced overexpression of BNIP3 resulted in activation of Bax, whereas gene silencing of BNIP3 with RNA interfering strategy reduced Bax activity. Knockdown of Bax prevented BNIP3-mediated Calcium transfer from ER to mitochondria, thereby blocking cyanide-induced mitochondrial dysfunction and apoptosis. These findings indicate that in response to cyanide, Bax acts downstream of BNIP3 to facilitate ER Calcium release and subsequent mitochondrial Calcium overload, thereby inducing a mitochondria-mediated cell death. (This work was supported by National Institutes of Health grant ES 04140).

Radioprotection by Oral Administration of Genistein in Mice Receiving Total Body Irradiation.

Landauer, M. R., Davis, T. A. and Mog, S. R. 1, 2, 3 Armed Forces Radiobiology Research Institute, Bethesda, MD and 2 Naval Medical Research Center, Silver Spring, MD.

Medical radiation countermeasures have applications in clinical oncology, space travel, radiation site cleanup and radiological terrorism. In the present studies, mice were pre-treated with oral genistein and exposed to a lethal dose (8.75 Gy) of cobalt-60 gamma radiation. Mice were gavaged with genistein for 4 or 6 days, either once a day (QD) with 10 mg day/mouse, or twice a day (BID) with 5 mg/mouse. Mice that received genistein for 4 or 6 days QD, had survival rates of...
60% and 55%, respectively, and were not significantly different from the vehicle control group (30%). When gavaged BID for 4 or 6 days, survival rates for genistein-treated mice were 75% and 85%, respectively, significantly higher than vehicle-treated mice (50%). Based on the survival data, a separate group of mice were administered genistein (5 mg/mouse, BID) for 6 days prior to 6 Gy irradiation and evaluated for hematological recovery. Mice treated with genistein had an increase in bone marrow cellularity and a more rapid recovery of white blood cells, neutrophils, platelets, red blood cells, and reticulocytes than time-matched vehicle-treated mice. These data indicate that oral administration of genistein prior to irradiation protects mice from lethal doses of radiation by resulting in accelerated recovery of bone marrow and reducing the severity of pancytopenia in irradiated mice.

552 DISRUPTION OF KERATINOCYTE - BASEMENT MEMBRANE (BM) COMPONENT INTERACTIONS BY SULFUR MUSTARD (SM) ANALOGUES.

M. P. Shakarjian1, 4, A. M. Vetrano1, 3, J. P. Marrow and reducing the severity of pancytopenia in irradiated mice.

553 INHALATION OF 2-CHLOROETHYL ETHYL SULFIDE (CEES, HALF MUSTARD) RESULTS IN MARKED INJURY AT MULTIPLE LEVELS OF THE UPPER AND LOWER RESPIRATORY TRACTS.


Sulfur mustard (bis(2-chloroethyl) sulfide, SM) is an alkylating chemical warfare agent. Exposure to SM and the SM analog 2-chloroethyl ethyl sulfide (CEES, half-mustard) results in acute injury to the skin, eyes, and respiratory tract. Rats were exposed to 300 mg/liter of CEES in ethanol for 15 minutes under anesthesia and subsequently sacrificed 18 hours following exposure. Lungs were lavaged with two 5 ml passes of saline for subsequent biochemical analyses or inflation fixed at a 20 cm pressure with 4% paraformaldehyde in PBS for histopathology studies. Nasal cavities were either lavaged with two 500 ml passes of saline or retrograde flushed with 1 ml 4% paraformaldehyde through the naresphynx and fixed for histopathology. In the lower airways, bronchoalveolar lavage (BAL) protein was significantly increased as compared to ethanol controls indicating alveolar-capillary or airway-capillary leak (p<0.05). Proximal tracheobronchial regions revealed variable response of the middle meatus region near the septum as a result of CEES exposure. Combined, these data indicate significant damage specific regions of the upper and lower airways as a result of exposure to the SM analog CEES.

554 CUMULATIVE REPRODUCTIVE EFFECTS OF IN UTERO ADMINISTRATION OF A MIXTURE OF TEN ‘ANTIANDROGENS’ IN MALE SD RATS: SYNERGY OR ADDITIVITY?

L. E. Gray1, C. Rider1, 2, and J. Furr1, 2. Endocrinology Branch, NIEHS, U.S. EPA, Research Triangle Park, NC and 1. NCSS/EPA Cooperative Research Grant, North Carolina State University, Raleigh, NC.

In 1996, the USEPA was charged under FQPA to consider the cumulative effects of chemicals in their risk assessments. We are conducting studies to provide a framework for assessing the cumulative effects of antiandrogens. In the current study, ten “antiandrogenic” chemicals were administered orally to pregnant rats on gestational days 14-18 and the reproductive development of the male offspring is being evaluated. Data were analyzed to determine if the chemicals behaved in a response, integrated or dose-additive fashion. The experimental design is a fixed ratio mixture of ten chemicals with a control group, a top dose group and five dilutions of the top dose (100%, 80, 60, 40, 20, 10 and 0% of the top dose). The top dose included dosages of each chemical that are at or below the no observed effect level for hypospadias induction (vinclozolin - 50 mg/kg, procymidone - 50 mg/kg, linuron - 40 mg/kg, prochloraz - 60 mg/kg/d and six phthalate esters: DEHP, DBP, BBP, DiBP, DiHP - 150 mg/kg/d/phthalate and DPP – 50 mg/kg/d). F1 male rats displayed retained female-like areolae at 14 days of age and reduced anogenital distance (AGD) at 3 days of age in the 20% and 40% groups and above, respectively. F1 female rat AGD was unaffected. In addition, malformed external genitalia were seen at puberty in males in the 40% and above groups. Dose addition was the only model that accurately predicted the malformations in the top three dose groups (observed and dose addition=100%; integrated addition=0-16%; response addition=0%) or retained nipples in the top four dose groups. The observation that dose but not response- or integrated-addition models accurately predict the effects of chemical mixtures that act via diverse mechanisms suggests that dose additivity may commonly occur among chemicals that disrupt the same signaling pathway at different molecular sites during sexual differentiation. This abstract does not necessarily reflect EPA policy.

555 IN UTERO EXPOSURE TO THE FUNGICIDE PROCYMIDONE AND DIBUTYL PHTHALATE PRODUCE DOSE-ADDITIVE DISRUPTIONS OF MALE RAT SEXUAL DIFFERENTIATION.

A. K. Mochkin1, 2, K. M. Howsheld1, C. R. Blystone1, 2, J. Furr1, 2, C. V. Rider1, 2, and L. E. Gray1. Reproductive Toxicology Division, NIEHS, ORB, U.S. EPA, Research Triangle Park, NC and 1. Molecular Biomedical Sciences, NC State University, Raleigh, NC.

Procymidone (PRO) and dibutyl phthalate (DBP) alter male rat sexual differentiation by disrupting the androgen-signaling pathway via distinctly different cellular mechanisms of toxicity. DBP inhibits fetal Leydig cell androgen production whereas PRO binds AR and blocks androgen action in the target tissues. Here, we report on two studies in which pregnant rats were treated with a mixture of PRO and DBP. We hypothesized that these chemicals would induce adverse effects on reproductive development in a dose-additive fashion. In the 1st study, SD rats were gavaged on gestational day 14-18 with either corn oil, 50 mg/kg/d PRO, 500 mg/kg/d DBP or 50 mg/kg/d PRO + 500 mg/kg/d DBP. The 2nd study was a fixed ratio study with a control group, a top dose group mixture (PRO at 150 mg/kg/d and DBP at 1125 mg/kg/d) or seven dilutions of the top dose (83, 67, 50, 33, 17, 10, 8, or 4%). In F1 male rats, the mixtures resulted in dose-related reductions in AGD, increased nipple retention and malformations, and reduced reproductive tissue weights. Further, these changes were accurately predicted by dose- but not by response addition models indicating that these chemicals did not interact independently. We also found a dose-related increase in testicular interstitial cell adenomas and hyperplasia in the 67% and above groups and seminiferous tubular degeneration in the 30% and above groups; effects that could not be modeled given the absence or low incidence of similar lesions in the individual PRO and DBP studies.
Despite different mechanisms, coadministration of DBP and PRO produced dose-additive effects on androgen-dependent tissues. The data suggest that having a common mechanism should not be a requirement for conducting a cumulative risk assessment; rather it should be based on producing similar adverse effects. This abstract does not necessarily reflect USEPA policy.

**PS 556** DEFINING THE BORDERS OF DOSE ADDITION: MIXTURE EFFECTS OF 2, 3, 7, 8-TCDD, 3,3,4,4-Tetrachlorodibenzop- 
Dioxin (TCDD) and 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) disrupts male rat reproductive tract differentiation. However, they act via different modes of toxicity and induce distinct postnatal phenotypes. DBP exposure decreases anogenital distance (AGD), and induces nipple retention and undescended testes by reducing fetal hormone levels. TCDD delays the onset of puberty, but does not reduce fetal androgen levels. Both DBP and TCDD induce epididymal agenesis and decrease sperm counts. Our hypothesis is that observed responses for endpoints targeted by only one of the chemicals would be response additive: driven by the active chemical, while those targeted by both chemicals would be dose additive: both chemicals would contribute predictably to the observed effects. Pregnant Sprague-Dawley rats were dosed with either vehicle, DBP (500 mg/kg/d on gestational days (GD) 14-18), TCDD (2 mg/kg on GD 16), or both DBP and TCDD (High Mixture Dose (HMD) = 500 mg/kg/d DBP + 2 mg/kg TCDD) or (Low Mixture Dose (LMD) = 150 mg/kg/d DBP + 1.3 μg/kg TCDD). Male offspring are being assessed for reproductive development. The data generally support our hypothesis, reflecting a dose-dependent TCDD-driven delay in puberty (4d delay in LMD and a 6-8d delay with TCDD or HMD) and presence of a temporary thread along the frenulum of the penis prepuce (10% in LMD and 20-40% with TCDD or HMD) and a dose-dependent DBP-driven increase in areolae retraction (2 areolae/male in LMD and 5-5 with DBP or HMD). No clear trends among the treatments were evident with AGD, pup loss, or maternal weight gain. Additional endpoints include reproductive organ weights and malformations. The results from this study will contribute to determining the limits of dose additivity among reproductive toxicants. Funding provided by NCSU/EP A Cooperative Training Program.

**PS 557** DOSE ADDITION PREDICTS THE EFFECTS OF A MIXTURE OF FIVE PHTHALATE ESTERS TO INHIBIT FETAL TESTOSTERONE PRODUCTION AND GENE EXPRESSION, AND POSTNATAL REPRODUCTIVE DEVELOPMENT IN THE SPRAGUE-DAWLEY RAT.


Exposure to some phthalate esters (PE) during sexual differentiation induces reproductive malformations in male and female rats. In the male fetus, these lesions result from phthalate-induced reductions in testicular testosterone (T) production and insulin-like hormone 3 (insl3) levels. We hypothesized that dose addition modeling would predict the effects of co-administration of five PEs [benzyl butyl (BBP), di(n)butyl (DBP), diethylhexyl (DEHP), diisobutyl (DiBP) and dipentyl (DPP)] on fetal testicular T production and gene expression since they act via a common mode of toxicity. First, we characterized the dose response effects of six individual PEs on gestation day (GD) 18 testicular T production following exposure to rats on GD8-18. BBP, DBP, DEHP, and DiBP and DBP were equipotent (ED50 = 441 mg/kg), and DPP was about 3-fold more potent (ED50 = 130.9 mg/kg); diethyl phosphatase (DPP) and TCDD (High Mixture Dose (HMD) = 500 mg/kg/d DBP + 2 mg/kg TCDD) or (Low Mixture Dose (LMD) = 150 mg/kg/d DBP + 1.3 μg/kg TCDD). Male offspring are being assessed for reproductive development. The data generally support our hypothesis, reflecting a dose-dependent TCDD-driven delay in puberty (4d delay in LMD and a 6-8d delay with TCDD or HMD) and presence of a temporary thread along the frenulum of the penis prepuce (10% in LMD and 20-40% with TCDD or HMD) and a dose-dependent DBP-driven increase in areolae retraction (2 areolae/male in LMD and 5-5 with DBP or HMD). No clear trends among the treatments were evident with AGD, pup loss, or maternal weight gain. Additional endpoints include reproductive organ weights and malformations. The results from this study will contribute to determining the limits of dose additivity among reproductive toxicants. Funding provided by NCSU/EP A Cooperative Training Program.

**PS 558** THE IMPACT OF NICOTINE EXPOSURE ON THE PHARMACOKINETICS AND PHARMACODYNAMICS OF CHLORPYRIFOS: IMPLICATIONS FOR DRUG/CHEMICAL MIXTURES.

S. Lee, T. S. Post, A. L. Busby and C. Timchalk. Biological Monitoring and Modelling, Pacific Northwest National Laboratory, Richland, WA.

Chlorpyrifos (CPF) is the most widely used organophosphorus (OP) insecticide to control agricultural pests. CPF is bioactivated mainly in the liver by CYP450-mediated desulfuration to its oxon metabolite (CPF-oxon), which elicits neurotoxicity by inhibiting cholinesterases (ChE). CPF also undergoes dearylation to form 3,5,6-trichloro-2-pyridinol (TCPy). The routine use of tobacco products may modify key metabolizing systems which will further impact the metabolism of OPs. The objective of this study was to evaluate the impact of repeated nicotine administration on CPF pharmacokinetics and pharmacodynamics in male S-D rats. The influence of repeated nicotine exposure on the in vivo metabolism of CPF and the level of inhibition in ChE activities in plasma, brain and diaphragm were investigated. Animals (250-300 g) were administered 1) both 1 mg nicotine/kg/day (s.c. at nape) and 5 mg CPF/kg/day (in corn oil, po) for 7 days, 2) 1 mg nicotine/kg/day for 7 days and a single dose of 35 mg CPF/kg (po) on 7th day, no-nicotine-controls were given 3) 5 mg CPF/kg/day for 7 days, or 4) 35 mg CPF/kg once. All the animals were sacrificed at 1, 4, 8, 12 or 24 h post-last dosing of CPF. For both (single and repeated) CPF exposures, Cmax and AUC of blood TCP were increased (~2-fold) indicating a possible increase of CYP450-mediated metabolism of CPF to TCPy, which is consistent with previous in vitro data. The most significant changes in ChE activity due to nicotine pretreatments were in the brain following the daily administration of 5 mg CPF/kg, where less inhibition of ChE in the brain from nicotine groups (~71% of naive) compared to chlorpyrifos-only controls (~42% of naive) were observed. But there were no differences in plasma and diaphragm ChE activities due to nicotine pretreatment. The results of this study suggest that repeated nicotine exposure (i.e., from smoking), related to smoking abstinence and pharmacodynamics of CPF (Funded by CDC/NIOSH grant R01-OF03629).

**PS 559** CRITICAL ANALYSIS OF LITERATURE ON LOW DOSE SYNERGY FOR USE IN SCREENING CHEMICAL MIXTURES FOR RISK ASSESSMENT.

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Scientists and regulators need a better understanding of the consequences of co-exposures to substances at environmentally relevant concentrations. From a safety perspective, it is essential to know if synergistic interactions can occur at these dose levels, which are typically low compared to those used in toxicological studies. Our objective was to determine if sufficient data were available to derive a synergistic factor or criteria that could be applied in a Threshold of Toxicological Concern (TTC)-based approach to screen and prioritize environmental chemical mixtures. A literature search was conducted to identify studies that demonstrated synergy in mammalian test systems, with emphasis on synergism at doses close to the individual chemicals’ NOELs or LOELS. The search identified 204 unique chemicals by in-depth critical review of 90 unique references; these were compiled into a database. Based on study selection criteria, a quantitative estimate of low dose synergy was found in only a limited number of studies. Calculations of interaction magnitude were included in eleven articles, reflecting seven research programs. Their approaches differed, as did their estimates of magnitude. While the range of reported values is quite wide, from 1.1 to 107, these values are not comparable with each other because definitions of synergy and methods used to quantify synergy were different across studies. Methods varied in terms of the null hypothesis, response measured, departure point (dose at which the effect was observed), consideration of the slope of the dose-response curve, and difference between experimental results and an estimate based on the assumption of additivity. Before such data can be integrated into a TTC approach, it is recommended that: synergy be defined in terms of departure from dose additivity; uniform procedures be developed for assessing synergy at low exposures; and points of departure for calculating synergy be standardized.
Organophosphorus (OP) insecticides are widely used inhibitors of acetylcholinesterase (AChE) in both target and non-target species. Surveys have revealed the presence of urinary OP insecticide metabolites in large numbers of random survey participants. The use of many OP insecticides for a variety of applications results in many people being exposed to multiple OP insecticides repeatedly. The common mechanism of toxicity for OP insecticides requires the evaluation of risk for multiple OP exposures. A reverse dose-mixture - or mathematical model could predict the exposure scenario through the use of biomarkers such as blood AChE inhibition data or urinary metabolites. These experiments have acquired the biomarker data that could be used for subsequent model development. Rats were exposed orally to chlorpyrifos (7.5mg/kg) and diazinon (60mg/kg) alone or a mixture of ½ the individual doses or an equal mixture of the individual doses every 4 days over a 15 day period. Brain, plasma and red blood cell (RBC) AChE and liver and serum carbonylsterase (CbsE) activities were determined spectrophotometrically. AChE inhibition was dose related, with peak inhibition in the brain of about 20% for the individual compounds and the lower dose mixture, and about 40% in the higher dose mixture 24 hours after exposure, with plasma and RBCs showing greater inhibition than in the brain. Partial recovery of AChE activity occurred between exposures; therefore, there was only a slight increase in inhibition with repeated exposures and inhibition was relatively greater in brain than in blood, suggesting faster recovery of activity in blood than in brain. CbsE activity was greatly inhibited 24 hours after dosing (about 80%) with partial recovery occurring between exposures. These data sets along with urinary metabolite levels will be used to calibrate a reverse dosimetry model. Supported by EPA STAR grant RD 83345101.

**560** ACETYLCHOLINESTERASE INHIBITION FROM REPEATED EXPOSURES TO A MIXTURE OF TWO ORGANOPHOSPHORUS INSECTICIDES (CHLORPYRIFOS AND DIAZINON) IN RATS.

E. C. Meek, A. Coban, M. K. Davis, K. H. Lednum, S. X. Guo-Ross and J. E. Chambers, Center for Environmental Health Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS.

Metals and metalloids are widespread pollutants that are present in the environment. Metals can enter organisms through various routes, including drinking water, food, and air. They can accumulate in the body, potentially leading to adverse health effects. The objectives of this study were to evaluate the toxic effects of a mixture of two metals, lead (Pb) and cadmium (Cd), on the human adrenocortical cell line H295R. The human adrenocortical cell line H295R shares the pathways for aldosterone and cortisol synthesis with the normal adrenal gland and is a valuable model to test adrenal endocrine disruption. Herein, the effects of the imidazoles on adrenal hormone secretion and steroidogenic gene expression were investigated after exposure to individual chemicals and binary and tertiary mixtures. H295R cells were treated for 24 hours with PRO, KET and IMA at non-toxic concentrations (10 nM), individually and as mim. Control and aldosterone levels in cell culture medium were analysed by ELISA. Expression of key genes in the steroidogenic pathway was analysed by quantitative RT-PCR. A dose dependent inhibition of cortisol secretion was evident after PRO, KET and IMA treatment, both individually and as mixtures. For aldosterone secretion, a biphasic response was shown with a maximal two-fold increase following PRO treatment. The non-linear dose response for aldosterone was also observed following mixture treatment. The IC50 for the effect on cortisol were similar for the individual chemicals and the mixture. Gene expression analysis indicated that the aldosterone pathway specific gene CYP11B2 was up-regulated while other steroidogenic genes were down-regulated after imidazole treatment. In summary, the effects of the individual chemicals are similar to the effects following mixture exposure. We conclude that the H295R cells are a promising model to study endocrine disrupting effects of chemicals and mixtures.

**561** ENVIRONMENTALLY REALISTIC MIXTURES OF THE FIVE REGULATED HALOACETIC ACIDS EXHIBIT CONCENTRATION-DEPENDENT DEPARTURES FROM DOSE ADDITIVITY.


DBPs as a mixture. The objective of this study was to evaluate environmentally relevant HAA mixtures under an assumption of dose addition. Mixtures were tested at an equimolar mixing ratio (EQ) and at ratios representing disinfection by postchlorination (CL) or preozonation/postchlorination (OZ/CL). The 5 individual HAAs (chloroacetic, dichloroacetic, trichloroacetic, bromoacetic (BA), dibromoacetic acid) and the HAA mixtures were evaluated in a Chinese Hamster Ovary cell chronic cytotoxicity assay at a minimum of 10 concentrations each. The individual HAA data were used to develop a smooth additivity model consistent with Berenbaum’s definition of additivity. The fitted models provided reasonable fits to the experimental data. The slope of the fitted dose-response curve for each individual HAA was negative and statistically significant, indicating that cell density decreased significantly with increasing HAA concentration. BA was markedly more toxic than the other regulated HAAs. The overall test for departure from dose additivity was significant (p<0.001) for each mixture. Individual dose levels (with Hochberg’s correction applied, alpha=0.05) demonstrated less-than-additive (antagonistic) departures from additivity in the higher portion of each mixture dose-response curve (EQ, OZ, CL). In addition, the lowest two concentrations of the EQ mixture exhibited statistically significant greater-than additive departures from additivity. In summary, all 3 HAA mixtures exhibited less-than-additive toxicity in the higher dose region but only the EQ mixture, the least environmentally realistic, exhibited detectable greater-than-additive toxicity. (This abstract may not specify the desirability functions for neurochemical (blood and brain cholinesterase activity) and behavioral (motor activity, gait score, tail-pinch response score) endpoints were

**562** MIXTURE EFFECTS OF THREE IMIDAZOLE FUNGICIDES ON ADRENAL HORMONE SECRETION IN THE HUMAN ADRENOCORTICAL CELL LINE H295R.

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Toxicological studies have focused on effects of single chemicals, but exposure to mixtures is more likely. There is a need for test systems that can address and predict effects of mixtures. Prochloraz (PRO), ketcnazole (KET) and imazalil (IMA) are imidazole fungicides that are known to bind un-specifically to cytochrome P450s (CYP). Adrenal steroidogenesis is depending on a number of CYPs and is therefore a likely target of these compounds. The human adrenocortical cell line H295R shares the pathways for aldosterone and cortisol synthesis with the normal adrenal gland and is a valuable model to test adrenal endocrine disruption. Herein, the effects of the imidazoles on adrenal hormone secretion and steroidogenic gene expression were investigated after exposure to individual chemicals and binary and tertiary mixtures. H295R cells were treated for 24 hours with PRO, KET and IMA at non-toxic concentrations (10 nM), individually and as mim. Control and aldosterone levels in cell culture medium were analysed by ELISA. Expression of key genes in the steroidogenic pathway was analysed by quantitative RT-PCR. A dose dependent inhibition of cortisol secretion was evident after PRO, KET and IMA treatment, both individually and as mixtures. For aldosterone secretion, a biphasic response was shown with a maximal two-fold increase following PRO treatment. The non-linear dose response for aldosterone was also observed following mixture treatment. The IC50 for the effect on cortisol were similar for the individual chemicals and the mixture. Gene expression analysis indicated that the aldosterone pathway specific gene CYP11B2 was up-regulated while other steroidogenic genes were down-regulated after imidazole treatment. In summary, the effects of the individual chemicals are similar to the effects following mixture exposure. We conclude that the H295R cells are a promising model to study endocrine disrupting effects of chemicals and mixtures.

**563** PROBABILISTIC ANALYSES OF UNCERTAINTY IN MIXTURE TOXICITY SUGGEST THAT ADDITIVE AND INDEPENDENCE MODELS MAY PRODUCE SIMILAR ESTIMATES OF TOXICITY.

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Toxicologists have debated when additive or independence models should be used to evaluate the toxicity of mixtures. In a recent study of the toxicity of mixtures of anthropogenic compounds found in surface waters, probabilistic analyses of the uncertainty in the predictions of toxicity for additive and independence models were performed. The analyses used Monte Carlo modeling, published distributions of the inter-chemical variation in safety factors, and the mixture-specific relative proportions of each component. Traditional deterministic models of additive and independence toxicity were also performed. As expected, independence models either predicted the same or lower toxicities (larger estimates of safe doses) than the additive models. Deterministic models the ratios of the safe levels of the mixtures predicted by the two models ranged from 1.0 to 2.9 and averaged 1.5. For the probabilistic versions of models, the ratios of the lower 90% confidence limits of the safe levels for the mixtures ranged from 1.0 to 1.2 and averaged 1.1. The reason for the closer agreement between the probabilistic versions of the models is due to statistical processes that cause deterministic additive models to overestimate, and deterministic independence models to underestimate mixtures’ toxicities. Specifically, additive models assume all mixture components will always be as toxic as their standards while independent models assume that no mixture component will have a higher toxicity than its standard. In the probabilistic models of mixture toxicity these assumptions are relaxed and as a result the two approaches produce results that are more similar. This analysis demonstrates the value of performing probabilistic modeling in the investigation of the risks posed by mixtures and suggests that the decisions concerning additivity versus independence may not be as critical an issue when probabilistic models are used.

**564** EVALUATING NEUROTOXICITY OF A MIXTURE OF FIVE OP PESTICIDES USING A COMPOSITE SCORE.

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The evaluation of the cumulative effects of neurotoxic pesticides often involves the analysis of both neurochemical and behavioral endpoints. Multiple statistical tests on many endpoints can greatly inflate Type I error rates. Multiple comparison adjustments are often overly conservative leading to reduced power to detect effects of interest. Furthermore, identification of the most sensitive endpoint may be chemically dependent so that neurotoxicity may be most evident on a per animal basis by evaluating many endpoints. Use of a composite score focuses the inference and avoids inflated type I error rates. Coffey et al (2007) describe the development of an evaluation method based on desirability functions for the many types of outcomes measured in neurobehavioral toxicology experiments. Our objective was to evaluate the neurotoxicity of a mixture of five pesticides (Moser et al, 2005). In particular, the desirability functions for neurochemical (blood and brain cholinesterase activity) and behavioral (motor activity, gait score, tail-pinch response score) endpoints were
calculated for selected chemical (acetate, dextran, dimethane, chlorprophyn, and malathion) and mixture dose response data, and a composite score of neurotoxicity was determined. Both an additivity model using single chemical data and a model for an environmentally-relevant fixed-ratio mixture were estimated. Focusing in the low dose region using a 5%, 10% and 20% benchmark responses (BMRs), departure from additivity was found at the 20% BMR with all five pesticides in the mixture; additivity was observed at the lower BMRs. Using the same relevant mixing ratio but without the most common chemical, malathion, we found departure from additivity at all three BMRs. Thus, malathion significantly influences the interactions of the remaining chemicals in the mixture. This methodology is therefore useful in evaluating the overall neurotoxicity of pesticide mixtures. This is an abstract of a proposed presentation and does not reflect US EPA policy.

565 JOINT TOXICITY OF CHEMICAL MIXTURES OF BENZENE, TRICHLOROETHYLENE AND METHYL MERCUERY NOT SIGNIFICANTLY DIFFERENT FROM INDIVIDUAL CHEMICALS IN F344 RATS, FOLLOWING PER ORAL EXPOSURE.

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Benzene (200 or 400 mg/kg), mercury methyl (0.25 or 0.75 mg/kg) and trichloroethylene (500 or 1500 mg/kg), three important and extensively characterized environmental pollutants were studied individually and as their mixtures in male F344 rats, following per oral exposure. Several toxicity endpoints such as body/organ weights (kidneys, liver, spleen, thymus); clinical chemistry (plasma glucose, albumin, creatinine); and hematological parameters (red blood cells, white blood cells, lymphocytes) were studied. A simple classification scheme was used to categorize the observed effects into the following groups: induced by individual chemical components and their mixtures (additive); induced by individual components, but not their mixtures (antagonistic); and induced by mixtures, but not by individual components (synergistic or new). The results show subtle mixture-induced toxicity shifts in several endpoints. Decrease of plasma creatinine was observed in binary or ternary mixtures while plasma albumin levels increased in contrast to the individual components. Thus, the endpoints affected by binary and ternary mixtures generally matched those affected by the individual chemicals but were different in observed severity. In fact, none indicated a lower threshold for joint toxicity than their individual components. The implications of these findings will be discussed using a systems approach to provide insights to potential underlying mechanisms of the overall toxicity of mixtures. (Supported by American Chemical Council LRI, RSK-0103).

566 IN VITRO CHARACTERIZATION OF METABOLIC INTERACTIONS BETWEEN N-HExANE, TOULENE, CYCLOHEXANE AND ISOOCITAN.

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The objective of this study was to characterize the metabolic interactions occurring between four volatile organic compounds (VOCs) associated with petroleum products, namely n-hexane (H), toluene (T), cyclohexane (C) and isooctane (I). More specifically, using liver microsomal preparations from the rat, we studied the potential inhibition of metabolism of T by H, C, and I, as well as the inhibition of H metabolism by T, C, and I. First, measurements of medium-air partition coefficients (Pm:a) were made to allow estimation of inhibitor and substrate concentrations in the incubation medium during the metabolism studies. Pm:a values obtained for H, T and C were 0.9322, 0.2286, and 2.2318, respectively. Then, by measuring disappearance rates in sealed glass vials by headspace gas chromatography after incubations of 45 min for T and 4 min for H, metabolic rates were determined for these two compounds incubated alone (3 concentrations) or in presence of inhibitors (5 concentrations). Regression analyses indicate that inhibition of T by H, C, and I is best described by competitive mechanism and the inhibition constants (Ki) are 0.48, 1.28 and 2.35 μM for H, C, and I, respectively. The mechanisms best describing inhibition of H metabolism were uncompetitive inhibition for T (Ki=1.25 μM) and non-competitive inhibition for C (Ki=0.39 μM) and I (Ki = 1.54 μM). These in vitro data on metabolic interactions between the VOCs can be integrated with physiologically based pharmacokinetic models for predicting the internal dose resulting from mixed exposure to these chemicals. Supported by ExxonMobil and NSERC.

567 URBAN PARTICULATE MATTER ACTIVATES AKT IN HUMAN LUNG CELLS.

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The normally picturesque Cache Valley in northern Utah gained national attention by achieving, during winter atmospheric inversions, some of the highest PM2.5 concentrations ever measured in the United States. PM is associated with a variety of cardiopulmonary diseases and mortality. We and others have shown that PM2.5 induces the inflammatory response in cultured human lung cells, through mechanisms associated with Akt activation, such as induction of ER stress, activation of NF-κB, calpain, and heat shock protein (Hsp)-27, apoptosis suppression, and inflammatory cytokine release. This study was undertaken to determine whether Cache Valley PM2.5 and PM10 would cause Akt activation in human lung cells. BEAS-2B cells were treated with either PM2.5 and PM10 (12.5 and 25 μg/ml) at time intervals up to 24 hr. PM treatment resulted in Akt activation as evidenced by phosphorylation at Thr308, and Ser473. PM-treated cells showed phosphorylation of Akt related proteins PDK, and PTEN while that of GSK-3β was reduced compared to control cells. Increases in p53 protein were observed at 1 hr post-treatment but not 24 hr. NF-κB activation was observed at 1 hr and 24 hr, but IkB phosphorylation occurred at 24 hr only. PM treatment led to the release of the NF-κB related inflammatory cytokines IL-1α, and IL-6 and the chemokine IL-8 at 6 and 24 hr. These effects were independent of IRAK-1 phosphorylation, indicating a MyD88 receptor independent mechanism. Treatment with calpain inhibitor leupeptin limited Akt phosphorylation to Ser473 and reduced release of IL-1α, IL-6 and IL-8. Thus, it is possible that calpain or similar proteases are involved in PM-induced activation of Akt leading to release of inflammatory cytokines. There were no consistent differences in these molecular events with respect to particle size. In short, our data indicate that Akt activation may play an important role in the inflammmogenic response to PM exposure in human lung cells. Supported in part by the Marriner S. Eccles Foundation.

568 COMPARATIVE HEALTH EFFECTS OF INHALED DIESEL AND GASOLINE EXHAUST, WOOD SMOKE, AND “DOWNWIND” COAL EMISSIONS.

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Rats and mice were exposed 6 hr/day, 7 days/wk by inhalation to multiple dilutions of diesel engine exhaust, gasoline engine exhaust, hardwood smoke, or a mixture simulating downwind coal emissions. The identical health response protocols were aimed at providing a database allowing comparisons among source emissions and identification of causal pollutant species. Body and organ weights, hematology, serum chemistry, bronchoalveolar lavage and histopathology were evaluated in F344 rats exposed 1 wk or 6 mo. Body and organ weights and micronuclei in circulating erythrocytes were evaluated in A/J mice exposed 1 wk or 6 mo. Clearance of bacteria from lung was evaluated in C57BL6 mice exposed 1 wk. Heart rate and electrocardiogram were evaluated in SHR rats exposed 1 wk. Vascular responses were evaluated in ApoE-/- mice exposed 7 wk. Development of respiratory allergic responses was evaluated in BALB/c mice after in utero + postnatal exposure. Exacerbation of existing allergic responses was evaluated in BALB/c mice exposed 3 days. To place results on the same scale for comparisons among variables and exposures, an “effect size” (ES) parameter was calculated for each variable by dividing the exposure-response slope estimate by its standard error. The sign of ES reflected the direction of difference from control, and the magnitude reflected the significance of the effect on the same scale for all variables. Combining the magnitudes of ES with judgements on whether increases or decreases of each variable were “adverse” allowed semi-quantitative comparisons among exposure atmospheres. Overall, engine exhaust elicited stronger adverse responses than wood smoke or coal emissions, and coal emissions elicited the fewest adverse responses. Research conducted by the National Environmental Respiratory Center with government and industry support. Contents do not represent the views of any sponsor.

569 ATMOSPHERIC GAS-PARTICLE INTERACTIONS CAUSE A RAPID SHIFT IN TOXICITY FROM THE GAS PHASE TO THE PARTICLE PHASE.

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Researchers have attempted to determine whether particulate matter (PM) is inherently toxic, or if its toxicity can be attributed to pollutants it absorbs from the air around it. Previous experiments have measured significant changes in the chemical
composition of both gases and particles as air pollution mixtures age in sunlight, usually with an accompanying increase in toxicity. In general, these reactions create oxidized species which are less volatile than their parent compounds and are, therefore, more likely to partition to particulate matter. As a result, PM can act as a vehicle to deliver air toxics into the deep lung. Here, the long residence time of PM can greatly affect the dose of the VOCs absorbed by the body. This study is designed to investigate whether gas-particle partitioning can cause a non-toxic aerosol to become toxic. Using a continuously stirred tank reactor (CSTR), human epithelial cells were exposed to mixtures of gases and aerosols in the system. The CSTR was coupled to two in vitro exposure systems EAVES (Electrostatic Aerosol In Vitro Exposure System) and GIVES (Gas In Vitro Exposure System), which allow gas and particle phase exposures to occur separately. Moreover, the exposure system maintains equilibrium between phases until delivery to the cells. Four exposure mixtures were used: clean air, mineral oil aerosol in clean air, VOCs alone (toluene/hexane and acrolein), and mineral oil aerosol mixed with VOCs. While no toxicity was measured with either of the exposures in clean air, the VOCs alone elicited a significant response from the cells exposed to the gas phase pollutants. When VOCs were mixed with the aerosol, the gas-phase response decreased, and the response from the cells exposed to the particles alone increased. Therefore, we were able to see a shift in toxicity from the gas phase to the particle phase and, as the shift occurs in a matter of seconds, these findings have important ramifications for understanding the toxicity of PM across the environment.

570 ETS AND HORMONE EFFECTS ON AIRWAY RESPONSES IN A MODEL OF ALLERGIC ASTHMA.

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Women have a higher incidence of adult onset asthma, COPD, and certain forms of lung cancer. All of these lung pathologies have been linked to tobacco smoke exposure. In the current study, we hypothesized that progesterone would enhance and testosterone would dampen allergic inflammation and that this would be mediated, in part, by regulation of antioxidant levels in the lung. Female 6 week BALB/c ovariectomized mice were implanted with placebo, progesterone, or testosterone time-release pellets. The pellets were exposed in environmentally relevant levels of ETS for 6 weeks (6hrs/day, 5 days/week). Half of the animals were sensitized and exposed to house dust mite allergen (HDMA) aerosols for two weeks. Endpoints included bronchoalveolar lavage (BAL) for cytokine analysis followed by lung fixation in 1% paraformaldehyde or airway hyperresponsiveness testing to methacholine challenge. IL-12p40, a subunit with antagonist activity to the heterodimer IL-12, is known to be upregulated in the lung in response to allergen exposure. IL-12p40 and its antagonist IL-12p35 were measured by ELISA. At the end of the study, the lungs were harvested for analysis of antioxidant levels. The results indicated that progesterone enhanced and testosterone decreased IL-12p40 and IL-12p35. Additionally, we collected serum and measured testosterone, estradiol, and progesterone levels. These data indicated that the enhanced IL-12p40 and IL-12p35 levels were not due to circulating hormones. Therefore, the increased IL-12p40 and IL-12p35 levels were likely due to local production in the lung. These findings have important ramifications for understanding the role of ETS in asthma.

571 SIMULTANEOUS EXPOSURE TO MULTIPLE AIR POLLUTANTS INFLUENCES ALVEOLAR EPITHELIAL CELL ION TRANSPORT.


Purpose. Air pollution sources generally release multiple pollutants simultaneously and yet, research has historically focused on the source-to-health linkages of individual air pollutants. We recently discovered that de novo endometriosis is present in up to 10% of reproductive age women and is defined as the presence of endometrium-like tissue in ectopic sites outside the uterus. The biologically active estrogen, estradiol, is essential for growth of endometriosis and synthesized locally in endometriosis from cholesterol through six serial enzymatic conversions. Aromatase and other necessary steroidogenic genes are expressed in vivo in endometriotic stromal cells enabling this single cell type to synthesize estradiol from cholesterol de novo. SF1 acts as a master-switch downstream of the PGE2-cAMP pathway and coordinates induction of the steroidogenic genes, steroidogenic acute regulator protein (StAR), side chain cleavage, 3β-hydroxysteroid dehydrogenase (HSDO) type II, 17-20-desmolase (P450c17) and aromatase in endometriotic stromal cells. The PGE2-dependent inductions of StAR and aromatase mRNA levels were the highest in comparison with other steroidogenic genes. In contrast, normal endometrium or endometrial stromal cells do not biosynthesize estradiol. Both endometriosis and normal endometrium contain the enzyme 17β-HSD-1 that catalyzes the final step that is the conversion of estrone to estradiol. What separates normal endometrium from endometriosis, however, is the inappropriate SF1 expression in endometriosis activates multiple steroidogenic genes. The clinical significance of local estradiol synthesis in endometriosis was exemplified by successful treatment of this disease using aromatase inhibitors. SF1 is the key determinant of steroidogenic gene expression in endometriosis; its mRNA levels in endometriotic stromal cells is ~12,000 times higher than those in endometrial cells. We identified a classic Cpg island that occurred in the SF1 promoter and regulates its activity. This region was heavily methylated in normal endometrial stromal cells and unmethylated in endometriotic stromal cells. Thus, inappropriate SF1 expression in endometriosis activates multiple steroidogenic genes resulting in local production of estradiol in this pathologic tissue.

572 OVERVIEW: BIOLOGICAL IMPORTANCE OF AROMATASE (CYP19) EXPRESSION AND FUNCTION.

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During the past five years, advancements have been made in our understanding of the regulation of aromatase (CYP19) gene expression and function in humans and wildlife. This cytochrome P450 microsomal enzyme is responsible for the conversion of androgens to estrogens, and is essential for maintaining estrogen homeostasis within multiple target tissues for both males and females. The expression of the human CYP19 gene is regulated through multiple promoters and co-factors that are target tissue specific. Thus, local regulation of estrogen concentrations within various target tissues may vary significantly from that observed in circulating serum. To date, the ability of environmental chemicals to inhibit the catalytic activity of aromatase, as well as resulting adverse effects on reproductive function, have been clearly demonstrated. However, specific effects on aromatase gene expression following exposure to environmental chemicals, and the physiological impact on local and systemic levels of estrogen, remain to be elucidated. In addition, the presence of elevated aromatase expression in hormone-dependent cancers underscores the need for determining if environmental chemicals can modulate the activity and expression of aromatase in humans and wildlife. To highlight these important findings, the session will provide an overview of the current state of knowledge regarding the regulation of aromatase (CYP19) gene expression, describe the toxicological consequences of altered aromatase expression and function in humans and wildlife, and present novel approaches for identifying environmental chemicals that disrupt the homeostasis of estrogen biosynthesis via this mode-of-action.

This abstract does not necessarily reflect EPA policy.
S 574 AROMATASE REGULATION IN BREAST CANCER.
Estrogen biosynthesis from \(C_{19}\) steroids is catalysed by the enzyme aromatase cytochrome P450. Regulation of aromatase expression is complex, involving at least 10 alternative promoters that are used in a tissue-specific manner. Aromatase is expressed in breast adipose tissue through the use of a distal promoter (pl4) that is regulated by cytokines and glucocorticoids. In the presence of breast tumor, however, aromatase is over-expressed in response to tumor-derived factors that induce the proximal, CAMP-responsive promoter II (pl2, normally active in ovary), leading to increased estrogen production that supports tumor growth and development. Nuclear receptors are central to promoter II activity. In the ovary, expression from this promoter requires the monomeric orphan receptor SF-1. However, SF-1 is not expressed in breast adipose or cancer tissue. Instead, other receptors including LRH-1 and ERs positively regulate aromatase expression, in balance with inhibitory receptors such as COUP-TF and PPARY. Although many of these receptors are ligand-responsive, their activities are potentially altered by endocrine disrupting chemicals. This could provide a link between environmental factors and local aromatase activity, in turn influencing breast cancer susceptibility.

S 575 UNDERSTANDING THE EFFECTS OF ATRAZINE ON STEROIDOGENESIS IN WISTAR RATS.
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The effects of environmental chemicals on the catalytic activity of steroidogenic enzymes, including aromatase, have been well documented. However, specific effects of environmental chemicals on the expression of genes within the steroidogenic pathway, and the physiological impact on local and systemic concentrations of steroids have not yet been clearly demonstrated in laboratory animals. Recent in vitro studies have shown that atrazine (ATR), a chlorotriazine herbicide, induces aromatase gene expression in several human cell lines and may be associated with a stimulatory effect on NR5A receptors (SF-1, LRH-1) and second messenger signaling. Thus, these studies support the hypothesis of a specific cellular target through which ATR and potentially other environmental chemicals may alter aromatase expression, as well as other enzymes within the steroidogenic pathway. Using in vivo and in vitro methods, we have evaluated the effects of ATR and metabolites of atrazinogenesis. ATR (50 mg/kg/day, 3 days, oral) caused a significant dose dependent increase in serum progesterone (P4), estrone and estradiol in male Wistar rats. However, no significant changes were observed in serum testosterone activity or CYP19 mRNA in the brain or gonads, suggesting that alternative mechanisms such as substrate availability may be responsible for the elevated serum estrogens. To further investigate, we characterized the effects of ATR on steroidogenesiss in rat granulosa cell cultures using different concentrations and exposure times. As with the in vivo studies, a consistent increase in P4 was observed, indicating that ATR (10 and 50 μM) does impact steroidogenesis in these cells. However, ATR did not significantly alter estradiol production. Additional studies are ongoing to determine the cellular mechanisms through which ATR induces P4 synthesis in this culture system. Thus, these studies support the hypothesis that ATR may have a direct effect on the steroidogenic pathway.

This abstract does not necessarily reflect U.S.EPA policy.

S 576 DIFFERENTIAL REGULATION OF AROMATASE ISOFORMS AND TISSUE RESPONSES TO ENVIRONMENTAL CHEMICALS IN FISH.
As in mammals, aromatase plays a basic role in fish reproduction, but unlike most mammals, fish have two distinct isoforms. One isoform, 450aromA, predominates in ovaries and controls circulating levels of estrogens that are critical to female differentiation and development. Another isoform, 450aromB, prevails in brains and controls local aromatization of testosterone into estrogen, controlling sex-specific reproductive behavior. The two isoforms, derived from distinct genes, have unique regulatory \(5\)-flanking promoter regions. Degradation of mRNA for the two isoforms differ. In addition, differences in amino acid sequences between isoforms relate to different levels of activity. The isoforms also have different phosphorylation sites that regulate their activity, suggesting distinct sensitivity to phosphorylation. Because of these differences, we investigated whether chemicals that modulate aromatase activity affect the two isoforms differently. Endocrine-disrupting chemicals (EDCs) have been shown to modulate aromatase activity. We compared the effects of four EDCs on reproduction and aromatase activity in brains and gonads of a marine fish, \textit{Tautogolabrus adspersus}, treated \textit{in vivo}. Testicular aromatase activity was low and not affected by any EDC treatment. Treatment with estradiol or ethynylestradiol significantly decreased aromatase activity in ovary and male brain, and resulted in lower egg production, viability and fertility. Treatment with octylphenol maleate significantly increased aromatase activity and decreased egg viability and fertility. An aromatase inhibitor, androstradiol, significantly reduced male and female brain aromatase activity, as well as egg production and fertility, but had no effect on ovarian aromatase activity. Overall, our results demonstrate that different EDCs can affect the activity of brain and ovarian isoforms differently. Furthermore, changes in reproductive parameters were associated with alterations in the activity of either isoform. This abstract does not necessarily reflect EPA policy.

S 577 EVALUATION OF (LPTA®) CD-1-TG(CYP19-LUC)-XEN MICE AS A BIOLUMINESCENT RESEARCH TOOL FOR THE IN VIVO STUDY OF ENDOCRINE DISRUPTORS.
There is increasing concern that chemicals widely used in commercial and food products, and enter the environment, are affecting the endocrine system of humans and wildlife. These chemicals are suspected of causing endocrine-related diseases, such as reduced fertility, impaired fetal/child development, and hormone-dependent cancers of the breast, ovary, testis and prostate. A number of bioluminescent mouse models have recently been developed as possible tools to assess the in vivo biological potency of potential endocrine disruptors, as effects on gene expression can be monitored in real-time using an imaging system, without the need to sacrifice the mice. A potentially useful model is the Cyp19-luc mouse (Caliper LifeSciences), which expresses luciferase under control of a gonadal aromatase promoter. Aromatase converts androgens to estrogens, and its dysregulation is associated with various pathologies. Overexpression is associated with the development of hormone-dependent cancers, whereas reduced expression/inhibition results in bone loss and infertility in females and decreased sperm production in males. Aromatase is increasingly recognized as an important target for endocrine disrupting chemicals. We are currently evaluating the Cyp19-luc mouse model (line 125) for its suitability as an in vivo tool for the study of chemicals that disrupt aromatase expression. Females expressed luciferase almost exclusively in the ovaries; males expressed it in the testes and epididymides. Luciferase expression increased as the reproductive system matured in both sexes, doubling between day 21 and 140 of age. In females, 5 IU rCG increased expression about 6-fold after 24h after it returned to baseline levels. Dexamethasone did not affect luciferase expression in either sex, whereas forskolin, a stimulant of cyclic-AMP synthesis, increased expression about 3.5-fold after 3 days. Our results indicate that the luciferase transgene, similar to the aromatase gene, is stimulated by activators of the cAMP-dependent protein kinase A pathway in Cyp19-luc mice.

S 578 GENOMIC, NON-GENOMIC AND EPIGENETIC MECHANISMS OF NUCLEAR HORMONE RECEPTOR ACTION.
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Our understanding of how nuclear hormone receptors respond to steroid hormones, endocrine disruptors and xenobiotics is rapidly evolving. Nuclear hormone receptors, which were once thought to act as simple transcription factors primarily through interaction with the DNA, are now appreciated to have equally important activities as activators of non-genomic signaling cascades within the cell and to interact with protein modifiers, such as arginine and lysine methyltransferases, to induce epigenetic modifications of chromatin in a lipid-, receptor-, co-activator and gene-specific fashion. Because of the importance of this class of receptors for many toxic responses, including those induced by xenostrogens and xenobiotics, this session will highlight cutting-edge discoveries being made in this area that are having a major impact on the discipline of toxicology.

S 579 TRANSCRIPT PROFILING OF ESTROGEN-RESPONSIVE GENES.
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Global analysis of gene expression can provide significant information about the molecular basis of normal and abnormal biological function. Virtually every change in biological function is accompanied by changes in gene expression and the transcript profile has sufficient specificity as to be diagnostic of particular mechanisms
of action. It also provides support for the generation of hypotheses about the role of specific genes in a particular biological response. We have evaluated the effects of estrogens on gene expression in the rodent reproductive system. Exposure of rats to estrogens of varying potency produces a characteristic transcript profile that is sex- and life-stage-specific. Changes in the expression of steroid metabolizing genes are consistent across sex and stage, but other genes, e.g., transcription factors, have much greater specificity. We have carried out a temporal analysis of gene expression in the juvenile rat uterus after a single dose of an estrogen. Gene expression responses are consistent with what can be observed at a histological level. Early changes involve genes that are responsible for the cell proliferation, inhibition of apoptosis and fluid imbibition. This is followed by changes in genes that affect the state of cellular differentiation. Subsequently, there is an increase in expression of genes associated with inflammation and tissue remodeling, as well as an upregulation of apoptotic genes as the uterus returns to a pre-stimulus state. The expression of specific genes can be organized into models of coordinated gene action that can be stated as testable hypotheses. We have made progress in evaluating gene expression in a uterine adenoma cell line, for which the estrogen-driven transcript profiles appear sufficiently similar to in vivo that it appears that this system can be used to test hypotheses about the role of specific genes in the uterine response to estrogens. Gene expression analysis has utility as a predictor of mechanism of action and has the potential to be a significant adjunct to the molecular understanding of biological responses.

580 NON-CLASSICAL ER/SP ACTIVATION OF ESTROGEN RESPONSIVE GENES.

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17β-Estradiol (E2) binds the estrogen receptor (ER) and activates both genomic and non-genomic signaling pathways that increase or decrease gene expression. The classical genomic pathway involves E2-induced formation of an ER dimer that modulates gene expression through interactions with estrogen responsive elements (EREs) in target gene promoters. Research in this laboratory has focused on the non-classical genomic pathway in which E2 activates ER-specificity protein (Sp)-mediated induction or repression of genes in breast cancer cells by interacting with GC-rich sites located in EREs in the promoters of Sp genes. Analysis of Sp-2-dependent gene expression in breast cancer cells transfected with a non-specific oligonucleotide or a cocktail (Sp) of small inhibitory RNAs for Sp1 (Sp1), Sp3 (Sp3), or Sp4 (Sp4) demonstrates that E2 induced 62 and repressed 134 genes. Moreover, analysis of this data shows that over 62% of all genes induced or repressed by E2 were Sp-dependent. E2, pharmacological selective ER modulators (SERMs), and xenosteroids also activate ER/Sp-dependent gene expression, and results of comparative activation of wild-type and variant ER indicate differences among xenosteroids, suggesting that these compounds are also SERMs. Results of in vitro studies in breast cancer cells also show that activation of ER/Sp is complex and dependent on ligand structure, ER subtype (ERα or ERβ), Sp protein, promoter, and cell context. In vivo studies with transgenic mice expressing a GC-rich luciferase construct show that E2/Sp is also activated or repressed in multiple tissues by E2 and other estrogenic compounds and this further confirms the importance of this non-classical estrogenic (ER-dependent) pathway.

581 LIFE, DEATH, AND TRANSFORMATION: MOVEMENTS, REPEATS, AND NCRNAS.

M. Rosenfeld. Howard Hughes Medical Institute, University of California San Diego, San Diego, CA. Sponsor: C. Walker.

The nuclear receptor (NR) superfamily of transcriptional regulators plays a central role in developmental homeostasis and diseases processes, and has been extensively studied as a model to identify the molecular mechanisms responsible for spatial and temporal control of gene expression. Additional regulatory and sensor strategies have altered our views about mechanisms of gene regulation and disease. While the role of liganded nuclear receptors in mediating coactivator/corepressor exchange is well established, nuclear hormone-dependent regulation of chromosomal organization in the three-dimensional space of the nucleus to diverse signaling events is emerging as a major parallel strategy to achieve integrated transcriptional responses. A key role for the modulation of nuclear architecture in orchestrating regulated gene expression programs in the mammalian nucleus, using previously unsuspected classes of sensor molecules. Thus, a network of covalent modifications and their corresponding gene fusions play important roles in many diseases of developmental and in initial steps in cancers, which is particularly well-characterized for leukemias, but now identified as a more general event in solid tumors. The molecular basis for these tumor translocation events is quite incompletely understood, although the prevailing model instituted by events in yeast, is that random translocations are selected by conferring growth advantage. We will present a model for the molecular logic underlying tumor translocation events, linking these translocations to evolutionarily-conserved strategies, with the liganded nuclear receptor (AR) serving as a driver of non-random and tissue-specific chromosomal translocations by inducing both transcriptional repression of the recombination sites, and based on local chromatin modification, serial recruitment of genotoxic stress induced enzymes that cause a synergistic enhancement of extended DSβ at these sites in response to genomic insults, and NHEJ-dependent tumor translocations. In parallel, life, death and cell fate decisions can be observed to avoid massive apoptotic death in response to genotoxic stress. While many of the regulatory mechanism controlling DNA damage/repair and apoptosis are well characterized, the precise molecular strategies that adjudicate DNA repair or apoptosis remain incompletely understood. A posttranslational modification that can modulate the ability of H2AX to function as an active determinant of repair/survival versus apoptotic responses to DNA damage through regulated differential recruitment of either DNA repair or pro-apoptotic factors, revealing a switch that modulates survival/cell death decisions during mammalian organogenesis will be presented.

582 REGULATION OF TRANSCRIPT BY PROTEIN ARGinine METHyLtransferases.

M. Bedford. MD Anderson Cancer Center, Smithville, TX. Sponsor: C. Walker.

The covalent marking of protein arginine residues by methyl groups can promote recognition by a binding partner or a modulation in biological activity. A small family of eukaryotic gene products that catalyze methylation reactions (PRMTs) work in conjunction with a changing cast of associated subunits to recognize specific targets in the cell for modification. Physiological roles for protein arginine methylation have been established in signal transduction, RNA splicing, transcriptional control, DNA repair, and protein translocation. Using different proteomic approaches, we have identified a number of PRMT substrates and methylarginine-dependent protein-protein interactions. This has helped us understand the molecular mechanisms behind the diverse biological roles of arginine methylation.

Recent developments in our knowledge of how arginine methylation regulates transcription will be presented.

583 IN VITRO MODELS OF HUMAN TOXICITY PATHWAYS.

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For toxicity testing and assessment programs to address the large numbers of substances of potential concern, a paradigm shift in the assessment of chemical hazard and risk is needed that takes advantage of advances in molecular toxicology, computational sciences, and information technology. This shift represents an evolution of toxicology from an observational science, to a predictive science built upon mechanism-based, biological observations in vitro. The progress in developing robust, quantitative in vitro models of human toxicity pathways with the potential to replace the current reliance on in vivo animal data will be presented. These in vivo models identify cellular and molecular responses associated with critical biological pathways, which can result in adverse health effects when sufficiently perturbed by chemical exposure. One of the challenges to this in vitro approach is selecting appropriate cell types and endpoints that provide a sufficient battery for predicting the response of relevant toxicity pathways. Results from these high-throughput screening (HTS) assays are being linked to historical toxicological test results to facilitate a transition in testing paradigms. The results from five laboratories and programs wherein the congruence of science, technology and regulatory need have produced initial successes in creating in vivo models of human toxicity pathways will be highlighted. Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.

584 EVALUATION OF THE TOXCAST SUITE OF CELLULAR AND MOLECULAR ASSAYS FOR PREDICTION OF IN VIVO TOXICITY.

K. A. Hauk. U.S. Environmental Protection Agency, Research Triangle Park, NC.

Measurement of perturbation of critical signaling pathways and cellular processes using in vitro assays provides a means to predict the potential for chemicals to cause injury in the intact animal. To explore the utility of such an approach, a diverse collection of human in vitro cellular assays was used to profile the activity of 320 bioactive compounds, having known toxicities, from the EPAs TestCast Program plus a chemical library. The assays consist of a wide variety of endpoint and were used for both cell lines and primary cells. Some systems included bio-transformation capacity in the form of phase I and II enzymes or primary human
hapatocytes. Results demonstrated that chemicals could be categorized with respect to mechanisms of toxicity including mitochondrial dysfunction, microtubule disruption, oxidative stress, nuclear receptor activation, DNA damage and other mechanisms. These toxicity phenotypes sometimes recapitulated the intended mechanism of action against target species for pesticide active ingredients, and other times reflected apparently unrelated activities. Together with activity profiles generated from high-throughput, biochemical screening assays against over 200 specific molecular targets, correlations with in vivo toxicity endpoints from the EPA Toxicology Reference Database have been made. Initial toxicity predictive in vivo toxicity endpoints illustrate the utility of including both phenotypic cellular assays along with specific molecular targets in building predictive toxicity models. Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.

585 USE OF NUCLEAR REPORTER ASSAYS TO INVESTIGATE SPECIES DIFFERENCES IN TOXICITY.

Nuclear receptors (NRs) are a diverse family of transcription factors that play a role in physiological and toxic processes. For some compounds, variation in toxicity between species may be related to differential activation of the NRs as well as differences in receptor splice variants within a species or tissue. For example, the critical role of the Constitutive Androstane Receptor (CAR) in the generation of mouse liver tumors by phenobarbital (PB) and 1,4-bis(3,5-Dichloropyridyloxy)benzene (TCPOBOP) has been demonstrated by the absence of tumors in Car-/- mice (Yamamoto, 2004; Huang, 2005). A reporter assay in HEK293-T fibroblasts was developed with one of four different CAR ligand-binding domains (mouse CAR-1, rat CAR-1, human CAR-1 or human CAR-3) fused to the DNA binding domain of yeast Gal4. A luciferase reporter plasmid under control of the Gal4 response element was also present. Using a number of model substrates at a range of concentrations, phenobarbital produced no increase in luciferase activity in any of the four CAR variants, as would be predicted for an indirect CAR activator. However, mCAR was specifically activated 6-fold by TCPOBOP whereas no activation was observed for hCAR-1, hCAR-3 or rCAR. The model hCAR activator CITCO produced a 60-fold increase with hCAR-3, but no response with the other three forms including hCAR-1. Androstane had the profile of an inverse agonist with all forms of CAR except for hCAR-3. These experiments and further literature references point to the possible species differences in responsiveness to different substrates by CAR, which could have bearing on differential toxicity.

586 TOWARDS NEW IN VITRO TOXICOLOGY STRATEGIES FOR DECISION MAKING: ACUTE TOXICITY AS A CASE STUDY.

In Europe, the REACH legislation and the 7th amendment to the European Cosmetic Directive are challenging for the safety assessment of chemicals. Both regulations are strong incentives for seeking alternative methods in hazard/risk assessment. Although single in vitro assays are valuable in providing mechanistic insight into toxicity pathways, they cannot address the complexity of events leading to toxicity in vivo. According to the 7th amendment to the cosmetic directive, 4 in vivo endpoints will be phased out starting in March 2009: skin irritation, eye irritation, genotoxicity and acute toxicity. While there are a number of alternatives for the former 3 endpoints, clues for replacing acute toxicity remain scarce. Previous initiatives like the Multi-centre Evaluation of In Vitro Cytotoxicity (MEIC), have pointed to the possibility of using in vitro cytotoxicity data to predict lethal systemic toxicity in vivo. Towards this end, L’Oreal has invested a lot of effort in the past two years trying to build up a strategy for the development and the implementation of in vivo acute toxicity. A proof of concept approach has been performed using a set of compounds with well documented values of LD50. These compounds have been selected to cover different in vivo toxicity range, as defined by the LD50 values and also a variety of mechanisms of toxic action. Other selection criteria relate to the target species and the route of administration: data generated in rats treated by gavage have been used to assess the correlation between the in vitro derived data and LD50 values. Preliminary statistical analysis has led to encouraging results. This is an exploratory work and further investigations such as assessing the relevance of the in vitro results in an Integrated Testing Strategy (ITS) workflow, are still in progress. This research project is in line with the strategy used by the US EPA for the ToxCast program. It is part of L’Oreal’s commitment to contribute to the research effort needed for building the new paradigm of the “toxicity testing in the 21st century”.

587 THREE-DIMENSIONAL HUMAN CELLULAR AND METABOLIZING ENZYME MICROARRAYS FOR HIGH-THROUGHPUT TOXICITY SCREENING.
J. S. Dordick1, 2, D. S. Clark1, 3, M. Lee1, R. Avani1, J. Ryan1, M. G. Hogg1, B. Eker1, S. Bun1 and A. Ramasubramanian1, 2, Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, NY. 1Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, NY. Sponsor: D. Dix.

A miniaturized 3D cell-culture microarray (the Data Analysis Toxicology Assay Chip, or DataChip) has been developed for high-throughput toxicity screening of drug candidates and their cytochrome P450-generated metabolites. The DataChip consists of human cells encapsulated in 20 ml alginate gel spots arrayed on a functionalized glass slide for spatially addressable screening against multiple compounds. Similar dose responses were obtained with the DataChip and conventional 96-well plate assays, demonstrating that the near 5,000-fold miniaturization does not influence the cytotoxicity response. In combination with the MetaChip, which mimics the Phase I and II metabolism of the liver, the DataChip may therefore enable toxicity analyses of drug candidates and chemicals, and their metabolites, at throughputs compatible with the increased need for rapid, animal-free testing.

588 MICROSCALE LIVER MODELS FOR DRUG DEVELOPMENT AND TOXICOLOGY SCREENING.
S. R. Khetani1 and S. Bhattacharya1, 2, Hupnos Corporation, Marlford, MA, 1Health Sciences and Technology / Electrical Engineering and Computer Science, Massachusetts Institute of Technology, Cambridge, MA and 2Medicine, Brigham and Women’s Hospital, Boston, MA. Sponsor: D. Dix.

Drug-induced liver disease is the leading cause of pre-launch and post-market attrition of pharmaceutical compounds. Environmental compounds can also cause drug-induced liver disease. Species-specific variations in liver-specific molecular pathways necessitate supplementation of animal data with in vitro assays to assess human responses to compounds. Isolated primary hepatocytes are considered to be the gold standard for evaluating drug disposition in vitro. However, hepatocytes from various species exhibit a precipitous decline in viability and phenotypic functions under conventional culture conditions. We have utilized microtechnology tools to optimize and miniaturize into a multi-well format in vitro models of the rodent and human livers. Specifically, primary hepatocytes were organized into colonies of prescribed, empirically optimized dimensions and subsequently surrounded by supportive stroma. Hepatocytes in these micropatterned culture systems retained in vivo-like morphology, expressed liver-specific genes, metabolized compounds using active Phase I/II drug metabolism enzymes, secreted diverse liver-specific products, and displayed functional bile canaliculi for several weeks. We demonstrate utility of our platform in drug development by quantifying drug-drug interactions and susceptibility to a panel of clinical hepatotoxins. In particular, we show that cultures responded to both continuous and intermittent treatment with prototypical drug inducers, showing elevated CYP450 activities when treated with drugs and recovery to basal enzyme levels within 72 hours following inducer removal. We also show that micropatterned co-are amenable to high content imaging readouts, such as those used to evaluate mechanisms of hepatotoxicity. In the future, improved in vitro models of liver tissue could eliminate problematic compounds earlier in drug discovery towards creating safer drugs for patients.

589 NITRATIVE AND OXIDATIVE STRESS IN TOXICOLOGY AND DISEASE.
R. Roberts1 and W. Slikker2, 1Safety Assessment, AstraZeneca, Macclesfield, United Kingdom and 2NCTR, U.S. FDA, Jefferson, AR.

Persistent inflammation and the formation and actions of reactive oxygen species play pivotal roles in tissue injury during disease pathogenesis and as a reaction to toxicant exposures. The associated oxidative and nitrate stresses promote diverse biological reactions including neurodegenerative disorders, cancer, atherosclerosis and stillbirth. These effects occur via sustained cell proliferation and cell death and, in some cases, via induction of a pro-angiogenic environment. Exposure to ozone, a ubiquitous urban air pollutant, leads to the generation of reactive oxygen and nitrogen species in lung macrophages, inducing inflammatory genes which play a key role in subsequent tissue damage. Similarly, the developing brain is susceptible to anesthetic-induced injury; recent studies have indicated that genes along the oxidative stress pathway are altered by this anesthetic treatment. In addition to a role in damage to developing brain, inflammation and oxidative stress are implicated in Parkinson’s disease (PD), a neurodegenerative disease characterized by the loss of dopamine neurons. Recent data suggests a mechanistic link between oxidative stress and elevated levels of DOPAL, a neurotransendogenous to dopamine neurons. Such work has significant implications for the development of therapeutics and...
Oxidative stress plays a key role in the pathogenesis of diseases such as asthma and COPD, and in lung injury following exposure to air pollutants. We have previously demonstrated that alveolar macrophages (AM) and inflammatory mediators including nitric oxide (NO) generated via inducible NO synthase (NOSII) and TNF can contribute to the pathogenesis of tissue injury. The regulation of these mediators is regulated by the transcription factor NF-κB. Our findings that NF-κB p50/52 mice are unable to generate inflammatory mediators and are protected from O3-induced lung injury demonstrate a critical role of this protein. In further studies we analyzed mechanisms regulating NF-κB activation in the lung after O3 inhalation.

Treatment of WT mice with ozone (0.8 ppm, 3 h) results in a rapid increase in NF-κB activity in the lung after O3 inhalation.

Inflammatory breast cancer (IBC) is the most lethal form of breast cancer, with little understood about this disease to ensure its detection and effective treatment. Patients with IBC are commonly misdiagnosed, have a significantly higher incidence of locoregional recurrence, with a 5-year overall survival rate significantly lower compared to patients with non-IBC locally advanced breast cancer. The mechanisms underlying IBC in vivo phenotype of IBC have not been clearly defined. Our recent studies provide first time evidence that IBC is associated with upregulation of genes and proteins that regulate nitrosative and oxidative stress.

The human SUM149 IBC breast tumor cells and IBC patient tumors express very high levels of the inducible isoform of the cyclooxygenase enzyme, COX-2, with concomitant production of prostaglandin E2 (PGE2). The COX-2 enzyme is a bifunctional enzyme and produces oxygen free radicals as a byproduct of its peroxidatic function. SUM149 IBC breast tumor cells also produce large amounts of nitric oxide and peroxynitrite, which is a very labile and active free radical that can rapidly damage DNA. We have found that selective inhibitors of COX-2, prostanoid receptor antagonists, and nitric oxide inhibitors significantly reduce the levels of oxidative and nitrosative stress in SUM149 IBC breast tumor cells as well as alter the phenotype of the IBC breast tumor cells. Taken together, these observations suggest that targeting these pathways can effectively diminish the ‘pro-inflammatory’ microenvironment that may contribute to the genetic instability and aggressive phenotype of IBC.
oxidative stress translates into selective degeneration of DA neurons. Recent data have suggested a link between oxidative stress and disruption of the cellular metabolism of an endogenous neurotoxin derived from DA. Catabolism of DA by monoamine oxidase (MAO), a process implicated in oxidative stress and inflammation, initially yields the toxic and protein-reactive intermediate 3,4-dihydroxyphenylacetaldehyde (DOPAL), which is further metabolized to 3,4-dihydroxyphenylacetic acid via aldehyde dehydrogenase (ALDH) or to 3,4-dihydroxyphenylethanol via reductases. Previous studies have demonstrated the high sensitivity of ALDH toward 4-hydroxynonenal (4HNE) and malondialdehyde (MDA), aldehydes generated via lipid peroxidation and associated with inflammation. The hypothesis of this research is that aldehydes generated via oxidative stress inhibit DA catabolism, yielding elevated levels of DOPAL, a neurotoxin inflammation. The hypothesis of this research is that aldehydes generated via oxidative stress inhibit DA catabolism, yielding elevated levels of DOPAL, a neurotoxin inflammation.

Both epidemiological and animal studies suggest various pesticide exposures can implicate dopaminergic neuronal survival or death: implications in Parkinson’s disease. Parkinson’s disease (PD) is the second most common aging-related neurodegenerative disorder characterized by loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), resulting in irreversible motor symptoms including bradykinesia, and rigidity. Although the etiology of idiopathic PD, which accounts for at least 90% of all PD cases, has been elusive, epidemiological studies suggest a correlation between increased risk for PD and occupational exposure to pesticides. Consequently, studying environmental and rodent models with pesticides has been useful in the investigation of PD pathogenesis. However, despite extensive research in the past, molecular mechanisms underlying dopaminergic neuronal death associated with PD remain incompletely defined. Recent studies using several pesticides including rotenone, paraquat, and chlorpyrifos as models to investigate transduction mechanisms that regulate dopaminergic neuronal death will be presented. Furthermore, idiopathic PD is most likely caused by multiple factors, including a complex interaction between genes and the environment. Interestingly, mutations in LRRK2 found in dominant familial PD have also been found in idiopathic PD, and the penetrance of LRRK2 mutations is incomplete. Thus, LRRK2 may provide an example whereby the onset and progression of PD may depend on gene-environment interactions. Recent advances in the role of LRRK2 mutations, many of which increase its kinase activity, in dopaminergic neuronal death are also important to address. Data is available on signal transduction pathways that promote dopaminergic neuronal survival and should be of general interest to scientists studying neurodegeneration, neurotoxicology, and signal transduction. Molecular mechanisms of toxicity, and occupational and public health. Presentation of this data is likely to accelerate understanding of dopaminergic neuronal survival or death: implications in Parkinson’s disease. Parkinson’s disease (PD) is the second most common aging-related neurodegenerative disorder characterized by loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), resulting in irreversible motor symptoms including bradykinesia, and rigidity. Although the etiology of idiopathic PD, which accounts for at least 90% of all PD cases, has been elusive, epidemiological studies suggest a correlation between increased risk for PD and occupational exposure to pesticides. Consequently, studying environmental and rodent models with pesticides has been useful in the investigation of PD pathogenesis. However, despite extensive research in the past, molecular mechanisms underlying dopaminergic neuronal death associated with PD remain incompletely defined. Recent studies using several pesticides including rotenone, paraquat, and chlorpyrifos as models to investigate transduction mechanisms that regulate dopaminergic neuronal death will be presented. Furthermore, idiopathic PD is most likely caused by multiple factors, including a complex interaction between genes and the environment. Interestingly, mutations in LRRK2 found in dominant familial PD have also been found in idiopathic PD, and the penetrance of LRRK2 mutations is incomplete. Thus, LRRK2 may provide an example whereby the onset and progression of PD may depend on gene-environment interactions. Recent advances in the role of LRRK2 mutations, many of which increase its kinase activity, in dopaminergic neuronal death are also important to address. Data is available on signal transduction pathways that promote dopaminergic neuronal survival and should be of general interest to scientists studying neurodegeneration, neurotoxicology, and signal transduction. Molecular mechanisms of toxicity, and occupational and public health. Presentation of this data is likely to accelerate understanding of dopaminergic neuronal survival or death: implications in Parkinson’s disease.

S 595 NOVEL SIGNALING MECHANISMS THAT REGULATE DOPAMINERGIC NEURONAL SURVIVAL OR DEATH: IMPLICATIONS IN PARKINSON’S DISEASE.

Z. Xia, University of Washington, Seattle, WA. Parkinson’s disease (PD) is the second most common aging-related neurodegenerative disorder characterized by loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), resulting in irreversible motor symptoms including bradykinesia, and rigidity. Although the etiology of idiopathic PD, which accounts for at least 90% of all PD cases, has been elusive, epidemiological studies suggest a correlation between increased risk for PD and occupational exposure to pesticides. Consequently, studying environmental and rodent models with pesticides has been useful in the investigation of PD pathogenesis. However, despite extensive research in the past, molecular mechanisms underlying dopaminergic neuronal death associated with PD remain incompletely defined. Recent studies using several pesticides including rotenone, paraquat, and chlorpyrifos as models to investigate transduction mechanisms that regulate dopaminergic neuronal death will be presented. Furthermore, idiopathic PD is most likely caused by multiple factors, including a complex interaction between genes and the environment. Interestingly, mutations in LRRK2 found in dominant familial PD have also been found in idiopathic PD, and the penetrance of LRRK2 mutations is incomplete. Thus, LRRK2 may provide an example whereby the onset and progression of PD may depend on gene-environment interactions. Recent advances in the role of LRRK2 mutations, many of which increase its kinase activity, in dopaminergic neuronal death are also important to address. Data is available on signal transduction pathways that promote dopaminergic neuronal survival and should be of general interest to scientists studying neurodegeneration, neurotoxicology, and signal transduction. Molecular mechanisms of toxicity, and occupational and public health. Presentation of this data is likely to accelerate understanding of dopaminergic neuronal survival or death: implications in Parkinson’s disease.

S 596 SYNERGISTIC EFFECTS OF RISK FACTORS THAT CONVERGE ON DOPAMINERGIC SYSTEMS: IMPLICATIONS FOR PARKINSON’S DISEASE.

D. A. Corv-Slecha 1 and M. Thiruchelvam 2. 1Environmental Medicine, University of Rochester Medical School, Rochester, NY and 2Environmental and Occupational Health Sciences Institute, Piscataway, NJ. Both epidemiological and animal studies suggest various pesticide exposures can serve as risk factors for the Parkinson’s disease (PD) phenotype. Humans are commonly exposed to multiple pesticides that act on brain nigrostriatal dopaminergic (DA) systems, a scenario likely to foster interactions. Based on this, we postulated a multiple-hit model which hypothesizes that concurrent exposure to multiple pesticides, either other risk factors acting on DA systems at different target sites may actually enhance vulnerability relative to single risk factors or to multiple risk factors acting by a single mechanism, by constraining the ability to evoke compensatory or homeostatic processes. The combined paraquat (PQ) and maneb (MB) model of the PD phenotype was developed based on this premise. Synergisms and/or potential observed with the model, as demonstrated e.g., in nigral DA neuron loss, include effects of combined PQ+MB relative to PQ or MB alone. Moreover, PQ+MB effects are enhanced by altered generic background (human α-synuclein mutations), aging, male gender, and early developmental exposures. Protection against PQ+MB is afforded by protein-reactive and over-expression of gluthathione peroxidase 1 and superoxide dismutase. Developmental exposure of males to PQ+MB results in a progressive loss of DA nigral neurons across the life-span and enhances vulnerability associated with adult exposures, confirming subtle neurotoxicity revealed by large-scale epidemiological studies implicating increased risk factors that converge on the nigrostriatal DA system will assist in further testing the multiple hit hypothesis and in refining models of the PD phenotype. Such an evolution should permit more focused hypothesis testing in epidemiological and clinical studies.

S 597 MITOCHONDRIAL COMPLEX I INHIBITION IS NOT REQUIRED FOR DOPAMINERGIC NEURON DEATH INDUCED BY ROTENONE, MPP+, OR PARAquat.

Z. Xia, University of Washington, Seattle, WA. Inhibition of mitochondrial complex I is one of the leading hypotheses for dopaminergic neuron death associated with Parkinson’s disease (PD). To test this hypothesis, genetically, we used a yeast strain lacking Complex I, a gene encoding a subunit required for complete assembly and function of complex I. Deletion of Ndufs4 gene abolished complex I activity in midbrain mesencephalic neurons cultured from embryonic day (E) 14 mice, but did not affect the survival of dopaminergic neurons in culture. This presentation will present data suggesting that dopaminergic neuron death induced by rotenone, MPP+, or pararquat in culture does not require complex I inhibition and bring into question the role of complex I inhibition in PD pathogenesis. Signal transduction mechanisms involving JNK/ERK, MAP kinases, both in vitro and in vivo, regulating dopaminergic neuron death and survival using the rotenone and pararquat models will also be discussed.

S 598 NOVEL ROLES AND REGULATION OF SURVIVAL FACTOR MEF2 IN DOPAMINERGIC NEURONS.

Z. Mao, Departments of Pharmacology and Neurology, Emory University, Atlanta, GA. Sponsor: Z. Xia. Mitochondrial dysfunction has long been hypothesized to contribute to the pathogenesis of Parkinson’s disease (PD). MEF2 family of transcription factors have been implicated in promoting the survival of several types of CNS neurons. This presentation will discuss a role for the MEF2 proteins in neuroprotection of dopaminergic neurons and in the regulation of mitochondria function. It will present evidence to support MEF2-mediated regulation of mitochondria as a direct target for known PD-inducing toxins such as MPP+ and Rotenone. How these mechanisms may be modulated by genetic and environmental toxic factors associated with PD will also be discussed. These studies reveal a novel site of action for PD toxins and may provide insight concerning neuroprotective therapies for PD treatment.

S 599 LRRK2 SIGNALING AND NEURONAL DEATH.

M. R. Cookson, Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, MD. Sponsor: Z. Xia. Dominant mutations in Leucine rich repeat kinase 2 (LRRK2) are the single most common cause of inherited Parkinson’s disease (PD). The gene product is a large multi-domain protein that has several protein-protein interaction domains surrounding a central enzymatic region that includes GTPase (ROC) and kinase domains separated by an intervening COR (C-terminal of ROC) domain. Definitively pathogenic mutations are found in all of the catalytic regions and may be clustered here, although there are a few mutations in the protein-protein interaction domains. Several groups have shown that LRRK2 is an active kinase, and we have recently shown that it is also a minimal dimer that autophosphorylates within each dimer unit. Some mutations (such as the common G2019S variant) seem to increase kinase activity directly, while others may influence regulation of the protein activity. For example, mutations in the GTPase domain (e.g. R1441C) appear to decrease GTP turnover activity. Because the GTP bound form of LRRK2 is more active than the GDP bound form, this group of mutations is likely to increase the length of time that LRRK2 is in the higher activity state. Therefore, our current model is that many of the mutations impinge on kinase activity although they may do this in different ways. Whether this is true of all mutations remains to be proven. These mutations are a key contributor to the toxic effects of increased expression of LRRK2 at least in acute experiments in vitro. The key questions, which will be discussed in this presentation,
NOVEL PKC SIGNALING IN OSTEOARTHRITIS AND OSTEOARTHRITIC NEUROTOXICITY MODELS.

A. Kanthasamy. Biomedical Sciences, Iowa State University, Ames, IA.

Presentation description: Chronic exposure to neurotoxic pesticides has been implicated in PD pathogenesis. However, the cell death mechanisms underlying pesticide-induced dopaminergic neuronal degeneration have not yet been defined. This talk will present evidence for the possible role of novel PKC isozyme aberrant signaling in the initiation and promotion of pesticide-induced dopaminergic degenerative processes. A translational approach for intervention of the activation of the proapoptotic kinase will also be discussed. Epidemiological and case control studies have shown a strong association between neurotoxic chemical exposure and the development of idiopathic Parkinson’s disease (PD). However, understanding cellular and molecular mechanisms of the etiopathogenesis and selective nigrostriatal degeneration in PD is a formidable challenge. Recently, we uncovered a novel apoptotic pathway involving caspase-3 dependent proteolytic cleavage of a protein kinase C isozyme PKCδ that mediates apoptosis in cell culture models of PD following exposure of the organochlorine pesticide diazinon or the neurotoxic metal manganese. Exposure to these agents resulted in activation of the mitochondrial dependent apoptotic cascade, starting from cytochrome C release to caspase-3 activation, in a time- and dose-dependent manner. Interestingly, environmental toxins induced caspase-3 dependent proteolytic cleavage of native PKCδ into a 41 kDa catalytic subunit and a 38 kDa regulatory subunit to persistently activate the kinase. The classic Parkinsonian toxin MPTP also induced proteolytic activation of catalytic subunit and a 38 kDa regulatory subunit to persistently activate the kinase C isoform PKCδ generation in PD is a formidable challenge. Recently, we uncovered a novel apoptotic pathway involving caspase-3 dependent proteolytic cleavage of a protein kinase C isozyme PKCδ that mediates apoptosis in cell culture models of PD following exposure to the organochlorine pesticide diazinon or the neurotoxic metal manganese. Exposure to these agents resulted in activation of the mitochondrial dependent apoptotic cascade, starting from cytochrome C release to caspase-3 activation, in a time- and dose-dependent manner. Interestingly, environmental toxins induced caspase-3 dependent proteolytic cleavage of native PKCδ into a 41 kDa catalytic subunit and a 38 kDa regulatory subunit to persistently activate the kinase. The classic Parkinsonian toxin MPTP also induced proteolytic activation of catalytic subunit and a 38 kDa regulatory subunit to persistently activate PKCδ CRM, or PKCδ siRNA protected against dopaminergic toxins-induced apoptotic cell death, confirming the proapoptotic function of PKCδ in dopaminergic neurodegeneration. This talk will describe some recent findings pertaining to feedback regulation and subcellular localization of PKCδ and translational strategies (supported by NIH grants ES10586 and NS38644).

REGULATION OF DRUG TRANSPORTERS IN DISEASE STATES AND ITS TOXICOLOGICAL AND CLINICAL IMPLICATIONS.

J. E. Manautou1 and N. J. Cherrington2. 1University of Connecticut, Storrs, CT and 2University of Arizona, Tucson, AZ.

Drug transporters play an important role in the uptake, distribution and elimination of pharmaceuticals, environmental contaminants and endogenous compounds. In the last decade, considerable interest has been centered on regulation of drug transporters and on how chemicals and disease states alter their expression. It is clearly important to understand the pharmacological and toxicological consequences of changes in drug transporter function. Both induction and repression of transporter expression have been documented with exposure to clinical drug metabolizing enzyme inducers, treatment with target organ toxicants and under a variety of pathological conditions. Many of these changes are mediated by transcription factors, including CAR, PXR, Nrf2, as well as cytokines and related inflammatory mediators. Therefore, it is important to highlight the recent knowledge gained on how transporter expression changes during non-alcoholic steatohepatitis and drug-induced hepatotoxicity, as well as regulation of blood brain barrier transporters and its implications to the management and/or treatment of central nervous system disorders. Finally, this session will address the molecular regulatory mechanisms involved and the potential functional consequences, and understanding how changes in transporter expression or function may be involved in drug-drug interactions and the implications of these effects in drug development and the clinical setting.

REGULATION OF ABC TRANSPORTER EXPRESSION AT THE BLOOD-BRAIN BARRIER.

D. S. Miller1, A. M. Hartz2 and B. Bauer3. 1Laboratory of Pharmacology, NIH/NIAMS, Research Triangle Park, NC, 2Department of Biochemistry and Molecular Biology, Medical School, University of Minnesota, Duluth, MN and 3Department of Pharmaceutical Sciences, College of Pharmacy, University of Minnesota, Duluth, MN. Sponsor: NIH/NIAAA, Grant Number: R01AA020741.

The blood-brain barrier, which resides within the brain capillary endothelium, is an exceptionally effective barrier to the delivery of therapeutic drugs to the central nervous system (CNS), limiting pharmacotherapy of major CNS diseases, including, neurodegenerative diseases, epilepsy, brain cancer and neuro-AIDS. Two elements are largely responsible for these barrier properties: tight junction between endothelial cells and high expression of ATP-driven drug efflux pumps. Of all the transporters expressed in the brain capillary endothelium, P-glycoprotein (MRP1, P-gp) is clearly the major obstacle to drug delivery. We have focused on understanding the signals and mechanisms that modulate P-gp expression and activity in brain capillaries. The overall goals are to understand how barrier transport function is altered in disease and to identify signaling elements that can be targeted to improve drug entry into the CNS. Using a combined in vitro/vivo approach (intact brain capillaries from rats and mice as well as from treated animals), we have identified...
tified multiple extracellular and intracellular signals that modulate P-gp. Several ex- tended signaling pathways have been mapped. Three pathways are triggered by the brain’s innate immune response, two by xenobiotic-nuclear receptor (RXR, CAR) interactions, one by oxidative stress and one by seizures. Several pathways share common elements (TNF-alpha-receptor1, endothelin B receptor, nitric oxide synthase, protein kinase C), suggesting a regulatory network. Signaling is also spatially complex, involving autocrine/paracrine elements and the cytoskeletal machinery. Multiple steps in signaling are potential therapeutic targets that could be used to adjust P-gp activity in the clinic. Finally, some of these pathways modulate expression of other ABC transporters (BCRP, MRPI, MRP2, MRP4) as well as xenobi- otic metabolizing enzymes.

S 605 APPLYING MODELS OF ALTERED TRANSPORTER FUNCTION TO MECHANISMS OF TOXICITY AND DRUG INTERACTIONS.
L. Lehman-McKeeman, Bristol-Myers Squibb, Princeton, NJ.
Xenobiotic transporters influence the disposition of many xenobiotics and endoge- nous compounds. Consequently, alterations in expression or function of influx/ efflux transporters may affect target organ concentrations, the likelihood of toxicity and increase the potential for drug interactions. Several mutant models such as Mdr2-deficient mice have been widely used to evaluate transporter function, and more recently, a variety of transporter deficient mouse models have been genetically engineered. The organic anion transporting polypeptides (Oatps) are a superfamily of solute carriers that play a major role in the hepatic uptake of compounds. Null mouse models for Oatp1a1 (Oat1a1), Oatp1a4 (Oat2a4) and Oatp1b2 (Oat3) have been developed. In Oatp1a1 and Oatp1b2 knockouts, no compensatory changes in trans- porter expression have been observed. Clinically, young mice (3-4 mo) show small increases in total bilirubin that become more severe as the animals age (1 yr). In vitro functional assessments using primary hepatocytes from these mice demon- strate reduced capacity to transport substrates such as estradiol 17β-D-glucuronide, and in vivo experiments reveal alterations in plasma clearance and tissue concentra- tions of numerous substrates, thereby suggesting a role for these transporters in he- patic and renal uptake. In a manner consistent with sex-dependent differences in constitutive expression observed in this family of carriers, male mice lacking Oatp1a1 show the most significant changes in kinetic evaluations and tissue uptake. Additional phenotypic characterization using urinary or serum metabolic eval- uations provide more global evidence of transport deficiencies and potential endo- genous substrates. Collectively, the development and characterization of null or mutant models of transporter deficiencies can be applied to determining trans- porter function in xenobiotic disposition and toxicity.

S 606 CLINICAL DRUG AND TOXICOLOGICAL INTERACTIONS INVOLVING DRUG TRANSPORTERS.
Detailed elucidation of the role of drug transporters on the absorption, distribu- tion, metabolism and excretion (ADME) of a drug candidate are often completed after the selection of the compound for initial clinical testing. Transporters can be effective barriers to drug exposure, be the rate determining step in the uptake and/or excretion of a compound or metabolite, and be a cause of drug-drug inter- actions or toxicity. The recognition of the influence of drug transporters on disposi- tion and toxicity is driving a surge in transport-related research activities within the drug metabolism and pharmaceutical sciences. A case study for lapatinib, a tyro- sine kinase inhibitor, where preclinical and clinical data support a role of drug transporters in clinically relevant drug interactions will be given to highlight the in- tegrated preclinical development approach used to identify drug transporters in- volved in its disposition. Integration of data from pharmacokinetic, mass balance, whole-body autoradiography, in situ perfusion experiments and in vitro studies are utilized to determine when and which transporter is involved in the disposition of lapatinib.

W 607 AGGLOMERATION VERSUS DISPERSION: HOW NANOPARTICLE BEHAVIOR AFFECTS EXPOSURE AND TOXICITY IN VITRO, IN VIVO, AND IN THE REAL WORLD.
J. Tulp and C. Saves, 1 Exponent Inc., Bellevue, WA and 2 Texas A&M University, College Station, TX.
Many studies of nanoparticles have noted a tendency of these particles to agglom- erate and form larger particles in air, solution, or suspension. Consequently, disper- sion of nanoparticles has been a challenge for toxicity studies and pharmaceutical or medical applications, which have used various means, both chemical and physical to deliver nanoparticles to cells, tissues, or organisms. Such methods are important in evaluating free nanoparticles. However, clumping of nanoparticles, or particle agglomerates, is a real world phenomenon that is relevant to understanding risks posed by nanomaterials. In some systems, agglomeration of particles in air or sus- pension/suspension appears to increase with particle concentration and decreasing size. The solution’s ionic strength and electrolyte concentration can affect agglomeration and surface characteristics. Particle characteristics can in turn affect agglomeration. Other factors, such as dispersants used in sunscreens or dissolved organic matter in aquatic environments may prevent clumping. Consequences of agglomeration in- clude exclusion by biological barriers. Agglomerated carbon nanotubes have also been shown to exhibit different effects in the lungs than their more dispersed coun- terparts. Studies with aquatic organisms indicate less toxicity with increasing size of particle agglomerates, although agglomerated nanoparticles are not necessarily sim- ilar in toxicity to micron-sized particles. Therefore, it is important to explore deter- minants of nanosized particle-to-particle interactions, including particle proper- ties, environmental conditions, consequences of change in size on fate in the environment and within organisms, its effects on toxicity, and the real world conse- quences of such particle behavior on health and environmental risks. Our increase in the understanding of nanoparticle exposure and toxicology in recent years is en- abling inferences on certain aspects of nanoparticles that may aid us in design and in- terpret toxicity studies to better assess the health risks and beneficial applications of nanomaterials.

W 608 PHYSICAL & CHEMICAL CHARACTERISTICS AFFECTING NANOPARTICLE BEHAVIOR IN TOXICOLOGY AND ECO-TOXICOLOGY STUDIES.
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Relevant nanomaterial characterization profiles is essential for the successful develop- ment of various nanotechnologies, including pharmaceutical & medical re- search, toxicology, environmental sciences and engineering, and regulatory and pol- icy decisions. In this talk, we will discuss some of the most common physical and chemical properties often reported in both scientific literature and industrial speci- fication sheets. Further, we will identify some of the nano-properties most relevant to toxicological assessments in cell cultures, whole animals, and ecological systems. Particles such as metal nano-colloids (silver), metal oxide nanoparticles (aluminum oxide), and carbon-based nanostructures (C60). By varying concentration (0.001 - 1000 mg/L), changing solvent conditions in various aqueous media, and assessing nano- particle characteristics at different time points after suspension (e.g., hours, weeks, and months), we have studied changes in the aggregation potential of each nanoparticle system. Results showed that, depending on the nanoparticle sys- tem and its concentration, the aggregation potential for each of material changes over time. Some systems show evidence of de-aggregation as well as change in sur- face charge, pH, and iso-electric point depending on solvent (medium) and time duration in suspension. Further, for some systems, the particle sizing data demon- strated that the nano-sized particles aggregate to a greater extent than the fine-sized particles. This study presents our results regarding material characterization of nano and fine-sized particles in aqueous media, a review of the current literature, and suggestions for relevant and adequate characterization defining the “nano” propri- eties in reference to eco-toxicity assessments of particle systems.

W 609 EFFECTS OF PARTICLE AGGLOMERATION ON PULMONARY TOXICITY.
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Nanoparticles tend to agglomerate into um-sized structures upon suspension in bi- ologically compatible media such as phosphate-buffered saline (PBS), due to the high ionic strength and near neutral pH of the media. Dispersion of nanoparticles in chemical surfactants is effective but is problematic because of bio-incompatibil- ity and alteration of the nanoparticle surface. The effectiveness of albumin, phospho- lipid, dilute alveolar lining fluid, and an “artificial dispersion fluid” containing a phospholipid/albumin/PBS mixture in suspending ultrafine carbon black, ultrafine titanium dioxide, and multi-walled carbon nanotubes has been evaluated with di- lute alveolar lining fluid and the “artificial dispersion fluid”, proven to significantly enhance dispersion. Evidence indicates that at the low concentration used disper- sion fluid does not mask the activity of the nanoparticle surface. Improved disper- sion significantly increases the inflammatory potential of ultrafine carbon black in an intratracheal instilled rat model. Evaluation of the pulmonary responses to poorly dispersed vs well dispersed preparations of single-walled carbon nanotubes (SWCNT) in a pharyngeal aspiration mouse model indicates that granulomas form at deposition sites of agglomerates, while more dispersed SWCNT structures avoid phagocytosis by alveolar macrophages, rapidly cross the alveolar epithelial lining
and enter the interstitium where a persistent fibrotic response is initiated. Inhalation studies with SWCNT indicate that pharyngeal aspiration of dispersed SWCNT more closely mimics inhalation a dry aerosol of SWCNT than aspiration of a poorly dispersed suspension.

**W 610 STABILITY OF NANOPARTICLES FOR BIOMEDICAL APPLICATIONS.**

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A great deal of attention has been paid to agglomeration and aggregation of nanomaterials due to the exponential increase in surface area to volume ratios at radii below 50nm. Aggregation of nanoparticles may be enhanced by surface modifications intended for enhancing targeting and/or functionality or the incorporation of ferromagnetic moieties for MR imaging. The resulting increase in particle size alone may hamper the ability of the nanoparticle to reach its intended target, to be sequestered by immune cells or accumulate in the interstitial compartment. Charged polymer chains have been successfully used to reduce such particle-to-particle interactions. Modulation of surface charge, however, may alter the final disposition and fate of nanoparticles independent of their intrinsic chemical identities. Other potential complications include disintegration of composite nanoparticles with unintended and premature release of active components. Depending on composition, release of active components prior to administration results in reduction/loss of efficacy and increases the possibility of collateral toxicity. Formulations incorporating photoactive components must be stable with respect to minimal quenching of reactive oxygen species, inactivation of the photodynamic moiety and retention in the body of the nanoparticle. While many of the available analytical techniques provide adequate determination of particle integrity in vitro, new measurement technologies will have to be developed for in vivo and in vivo quantification of stability. Acknowledgements: NIEHS-ES08854; NCI-N01-CO-37123, Air Force-FA9550-06-1-0098.

**W 611 NANOPARTICLE-MACROMOLECULE INTERACTIONS AND THEIR IMPACT ON THE RATE AND EXTENT OF PARTICLE AGGLOMERATION.**


Most nanoparticles rapidly aggregate or agglomerate in air and water. The rate and extent of aggregation/agglomeration is typically greater in biological fluids (e.g. physiological buffer) due to the high salt concentration and presence of divalent cations (e.g. Ca²⁺). This physical transformation of nanoparticles can affect their reactivity (e.g. ROS generation), mobility in the environment, translocation in organisms, and their overall toxicity. A fundamental understanding of the behavior of nanoparticles in fluids, and the relationship between the particle surface properties and the rate and extent of agglomeration of those particles is needed to determine the mechanisms of nanoparticle toxicity. Medium and high molecular weight macromolecules such as proteins and DNA adsorb to nanoparticle surfaces with high affinity so even very low concentrations of these compounds in solution can lead to significant adsorbed mass. Both the adsorbed mass and the layer conformation of the adsorbed macromolecule affect the magnitude of the attractive and repulsive forces afforded by the adsorbed macromolecule, and therefore the aggregation and agglomeration behavior of the particles. These molecular scale interaction forces can be measured and correlated with the macroscopic behavior of nanoparticle suspensions (e.g. agglomeration and sedimentation). Even for highly attractive particles expected to aggregate into a primary minimum, adsorbed macromolecules lead to agglomeration in a secondary minimum at separation distances of ~100nm. These agglomerates are more readily detached and resuspended as individual nanoparticles than aggregated particles. Exposure studies conducted with highly stabilized particles can decrease contact of those particles with plated cells, and alter the properties of the particles contacting the cells. Measurements of the agglomeration and sedimentation rate are therefore a required step toward identifying the mechanisms of toxicity.

**W 612 APPLICATION OF TOXICOLOGY STUDIES FOR RISK ASSESSMENT IN THE REAL WORLD.**

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Risk assessment of nanotechnology applications must consider the relevance of laboratory studies for worker, consumer, or environmental exposures. Studies on nanomaterials typically use high doses delivered in vitro to cells or tissue cultures, short-term in vivo by injection or bolus dosing (e.g., intratracheal instillation, gavage), or in small test chambers for aquatic or environmental studies. Particle behavior under these conditions can differ dramatically from behavioral in the real world, thereby complicating dose-response assessments and evaluation of effects. Nanoparticles readily agglomerate in air particularly at higher concentrations, posing challenges for inhalation studies, but indicating limits to higher "real-life" nanoscale particle exposure. Studies of more well dispersed carbon nanoparticles in the lungs of rats via pharyngeal aspiration indicate that fibrotic or systemic effects (e.g., cardiovascular) may be more relevant than granuloma formation in risk assessment of chronic exposure to nanoparticles in the workplace or in the environment. High exposure to nanoparticles in aquatic environments may be limited by increased agglomeration with higher concentration and smaller particle size, as shown for fullerenes and titanium dioxide. Although agglomerated nanoparticles may have greater reactivity than similar-sized solid particles, larger size may restrict translocation of agglomerated nanoparticles from nose to brain via axon transport or through cell membranes, according to research indicating a 200 nm upper limit for such transport. Conversely, surface coatings, dispersants (e.g., in sunscreens, organic matter in water), and functional groups on nanoparticles can reduce or alter agglomeration behavior. Dose-response assessments incorporate the complexity of nanoparticle behavior for specific compounds and conditions at relevant dose ranges for nanomaterials in consumer products, pharmaceuticals, and other applications.

**PL 613 IDENTIFICATION OF SELENOCYSTEINE ADDUCTS IN THIOREDOXIN REDUCTASE BY 2-CHLOROETHYL ETHYL SULFIDE (CEES), A MODEL SULFUR MUSTARD VESICANT.**

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Thioether reductase (TrXR), a selenocysteine-containing flavoprotein that catalyzes the reduction of oxidized thioredoxin, is a critical target for CEES. Previously, we reported that CEES effectively inhibited purified TrXR and TrxR in vitro in human epithelial cells. CEES was significantly less effective in inhibiting mutant TrXR in which selenocysteine was replaced by cysteine indicating that the vesicant reacts with the C-terminal redox center of TrXR. In the present studies alkyla- tion sites in TrXR modified by CEES were identified. Rat TrXR was reacted with CEES, purified by SDS-PAGE, cleaved by in gel lysyl cleavage, and subjected to LC-MS/MS. Based on distinct isotope patterns of selenium-containing peptides and searching for critical b and y ions in MS/MS, several specific alkylated selenium-containing peptides were identified in CEES-treated enzyme. One of the alkylated selenium-containing peptides, a doubly charged ion at m/z 723.22, corre- sponded to a mass addition of 145 Da as compared with the mass of unmodified peptide. MS/MS sequence analysis revealed that a cysteine residue is alkylated by a single ethylthioethyl group (Cys58). No peaks with mass increases corre- sponding to two ethylthioethyl-modifications were detected, suggesting that CEES specifically alkylated the selenocysteine, but not cysteine residues, in the C-terminal redox motif of TrXR. Taken together, these data demonstrate that mustard vesicants target TrXR by selectively forming selenocysteine adducts. Inhibiting adduct forma- tion may be an important mechanism to counter vesicant toxicity. Supported by CA100994, CA059378, ES004738, ES050522, GM034310 and AR055073.

**PL 614 CALCIUM SIGNALING IN NEURONAL CELLS EXPOSED TO THE MUNITIONS COMPOUND CYCLOTROMETHYLENETRINITRAMINE (RDX).**

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Cyclotromethylenetritramine (RDX) has use as an explosive. Past studies in animals have demonstrated spontaneous seizures as an adverse effect of oral administration. Mechanisms for seizure production, however, have not been described. In the present study we examined the ability of RDX applied to human neuroblas- toma SH-SY5Y cells to increase cellular calcium, as increased calcium levels are a possible contributing mechanism for excitoctrosis. For these experiments, cells were loaded with calcium-indicating fluorescent dye one hour before application of RDX in concentrations between 1.5 μM and 7.5 mM. Fluorescence was immedi- ately measured on a kinetic fluorescent microplate reader with autoinjest, and recordings were made for a total of 30 cycles of 11 seconds each. A concentration response was noted, with even the lowest concentration of RDX tested capable of
ranging calcium fluxes above baseline. A return toward baseline was noted by the end of the observation period. The rise in calcium levels were similar to that noted with 200 mM carbactal, and could be prevented by pretreatment with 10 mM of the calcium chelator tetrasodium EGTA. Partial attenuation of the carbactal effects by the RDX-induced increase in calcium were evident after exposure to imidazoline calcium blocker verapamil (20 μM), the sarcoplasmic reticulum CaATPase inhibitor thapsigargin (5 μM), and the general membrane stabilizer lidocaine (10 mM), although the latter was only effective in medium containing serum. Results suggest that RDX has capability to transiently increase intracellular calcium levels and that multiple mechanisms may be involved.

**PL 615 MODIFICATION OF NADPH CYTOCHROME P450 REDUCTASE BY 2-CHLOROETHYL ETHYL SULFIDE (CEES) STIMULATES PRODUCTION OF REACTIVE OXYGEN SPECIES.**

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NADPH-cytochrome P450 reductase, a flavin-containing electron donor protein for cytochrome P450s, is a crucial enzyme in xenobiotic metabolism. Disruption of xenobiotic metabolism is known to lead to oxidative stress and toxicity. Sulfur mustard and related vesicants are known to target the cytochrome P450 system and inhibit drug metabolism. In previous studies we demonstrated that the sulfur mustard analog 2-chloroethyl ethyl sulfide (CEES) causes a time- and concentration-dependent inhibition of NADPH-cytochrome P450 reductase activity in MLE-15 lung epithelial cells. Effects were maximal after 1 hr with concentrations of CEES ranging from 0.1-10 μM. In the present studies, we analyzed the effects of CEES on a recombinant human cytochrome P450 system. We found that CEES inhibited the reduction of cytochrome c as well as cytochrome P450 1A1 activity. Despite this inhibition, NADPH consumption and electron flow through the flavin cofactors of the reductase were not affected as measured by NADPH utilization and the reduction of 2,6-dichlorophenolindophenol (DCPIP), respectively. NADPH-cytochrome P450 reductase also generates reactive oxygen intermediates (ROI) via oxidation of NADPH. We quantified ROI production by the reductase by measuring hydrogen peroxide production in the Amplex-Red assay. Surprisingly, CEES markedly enhanced the generation of ROI by the reductase. These data demonstrate that CEES can selectively target multiple sites within cytochrome P450 reductase. Enhanced formation of ROI by the enzyme following CEES treatment can lead to ‘uncoupling’ of xenobiotic metabolism and increased oxidative stress, and may contribute to the toxicity of sulfur mustard. Supported by CA100994, CA093798, ES004738, ES005022, GM034310 and AR055073.

**PL 616 INHALED SULFUR MUSTARD PRODUCES SEVERE LEUKOCYTE DEPLETION IN AN ANIMAL MODEL: COMPARISON WITH HALF-MUSTARD.**


Sulfur mustard (SM) is a chemical threat vesicant which causes skin blistering and long-term pulmonary dysfunction. Severe leukopenia has been reported in humans believed to have been exposed to SM aerosols during warfare. However, exposure levels and confounding effects of exposure to other agents are unknown for these subjects. This study evaluated this effect in an animal model where the exposures were well defined, and compared the effects of SM with chloroethyllylethil sulfide (CEES, half-mustard). Briefly, F344 rats were conditioned to nose-only exposure tubes, then exposed to aerosols generated using a Swirler nebulizer from solutions of SM or CEES in ethanol. Aerosol concentrations were determined by gas chromatography. Exposures to SM were for 5, 10 or 15 min at 190 μg/m3 for 30 or 60 min at 197 μg/m3. Exposures to CEES were 254 or 1200 μg/m3 for 5, 10 or 15 min. At intervals following the exposure, the rats were humanely killed. Blood was collected and analyzed for hematological parameters using an Advia automated system. SM exposure caused dose-dependent suppression of most types of leukocytes and reticulocytes 24 h later, without substantial alterations in the erythrocytes. Recovery studies indicated that lymphocytes recovered by 3d after exposure to 10 μg/m3 SM for 30 min. Depletion of other leukocytes and reticulocytes was more sustained but recovered by 10d. CEES at 254 μg/m3 for up to 15 min had little effect at 24 hr, but exposure to 1200 μg/m3 CEES suppressed lymphocytes at 24 hr similarly to exposure to 190 μg/m3 HD. However, in contrast to HD, CEES exposure did not affect monocytes; and neutrophils, reticulocytes, and erythrocytes were elevated. These results suggest differences between the effects of CEES and HD. The mechanisms for these effects and the differences between the two agents are not known, but there are clear implications for protection of humans exposed through acts of war or terrorism from secondary infections. Funded by Cooperative Agreement NIH NINDS U54NS058185-05.

**PL 617 SULFUR MUSTARD-INDUCED ENDOPLASMIC RETICULUM STRESS RESPONSE IN THE MOUSE EAR VESICANT MODEL.**

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Topical exposure to the chemical agent, bis(2-chloroethyl)sulfide (SM) in the mouse ear vesicant model (MEVM) resulted in time-related changes in the histopathology of the skin. Changes included increased edema over time, detachment of the epidermis from the dermis, and upregulation of laminin-332, a skin glycoprotein required for both basal keratinocyte adhesion in undamaged skin, and keratinocyte migration after wounding. Homeostatic dysfunction caused endoplasmic reticulum stress response (ERS), as observed by confocal microscopy. ESR is activated to increase the capacity of the ER to fold and process proteins. These perturbations occur when the lumen either accumulates misfolded proteins or when it has lipid imbalances. ESR can also occur after excessive internal ionic changes. Confocal microscopy experiments and microarray data suggested that ERS stress occurs very quickly in the MEVM. Microarray analysis accompanied by confirmaory RT-PCR experiments indicated there was upregulation of several late-acting stress proteins and folding chaperones in the ER. Some of these included calreticulin, immunoglobulin-binding protein, and protein disulfide isomerase. The accumulation of laminin-2 protein, one of the chains of laminin-332, in the ER was visualized by confocal microscopy and colocalized with several of the ER chaperones. This accumulation appeared specifically in the migrating, but not proliferating cells. These observations are consistent with recent reports that laminin-332 is found in migrating, transformed epithelial cells that have left the cell cycle, but not in proliferating cells. Taken together, these results suggest that laminin-2 is misfolded after SM treatment, resulting in decreased secretion and reduced overall amounts of laminin-332 in the extracellular matrix. This would explain the observation that there is a delayed wound healing response evident in this wound model. Supported by ES005022, ES004738, EY09056, and the NIH CounterACT Program through NIAMS U54AR055073.

**PL 618 EXPOSURE TO CEES INDUCES OXIDATIVE STRESS AND PRODUCES AN INFLAMMATORY RESPONSE IN SKH-1 HAIRLESS MOUSE SKIN.**

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CEES, 2-chloroethyl ethyl sulfide, is an analog of sulfur mustard (HD) that induces tissue damage similar to HD. Our recent findings have shown that topical application of CEES on to the dorsal skin of female SKH-1 hairless mice causes skin inflammation as measured bi-fold thickness, edema, hyperplasia, together with a strong activation of transcription factors AP1 and NF-κB via upstream signaling pathways including MAPKs and Akt. Based on these outcomes, the major objective of present study was to assess: 1) whether CEES exposure of skin causes oxidative stress that could be responsible for the activation of signaling pathways and transcription factors, and 2) what are the effector inflammatory molecules which are induced, possibly through CEES-caused activation of transcription factors, and play a role in overall inflammatory and blistering effects of CEES in skin. CEES exposure of SKH1 hairless mice at different doses and treatment times resulted in oxidative stress as measured by immunoblotting of skin tissue samples, which showed HNE- and DMPO-adducted protein modifications. Parallel to γ-HNE and DMPO adduct formation, we also observed by immunoblotting that protein oxidation is significantly increased following CEES exposure in a dose- and time-dependent manner. Our results also showed that topical CEES exposure of mice induces both COX-2 and iNOS expression at protein level in skin, providing a convincing link with well known inflammatory activity of CEES. We also studied the expression of MMP-2 (gelatinase A) and -9 (gelatinase B) in CEES-exposed mice to determine their role in tissue blistering injury. A dose- and time-dependent increase in overall gelatinase activity was observed in CEES-treated skin epidermis. Collectively, our results suggest that topical application of CEES to SKH-1 hairless mouse skin induces inflammatory responses through COX-2 and iNOS and skin blistering through MMP-2 & -9 via CEES-induced oxidative stress.
619 MODULATION OF GENE EXPRESSION BY MMP INHIBITORS IN SULFUR MUSTARD EXPOSED MOUSE SKIN.
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Skin exposure to sulfur mustard (SM) causes detachment of the epidermis from the dermis within hours. Damage may be enhanced by matrix metalloproteinases (MMPs), zinc-dependent proteinases involved in the degradation and remodeling of extracellular matrix proteins. Several MMP inhibitors were selected for evaluation as potential therapeutic countermeasures to SM-induced injury. MMP-2/MMP-9 Inhibitor I, Iomastat, and GM4189 were used as pretreatments in time course studies (24h, 72h, and 168h post-SM treatment) to evaluate their therapeutic effectiveness. Histological examination was used to assess the gross pathology and Draize scores were assigned to the samples. Microarray analysis was performed to identify subtle gene changes. Only the Iomastat pretreatment in conjunction with SM exposure coincided with a slightly improved Draize score. All three inhibitors showed improvement in overall tissue structure and a reduction in edema by 72 hours post-treatment as determined by histological examination. Affymetrix microarray and ANOVA analysis (false discovery rate of 0.05) was used to create gene profile lists for each of the compounds tested. KEGG library pathways analysis for each time period was performed and the data assigned Fisher p values. The most common pathways affected by inhibitor treatments included: apoptosis, immune system and signal transduction pathways for 24h, cytokine-cytokine receptor interaction for 72h, and innate immune system and wound repair pathways for 168h. Results were verified by RT-PCR. *This work is supported by ES05022, ES07148, EY09056, and the NIH CounterAct Program NIAMS U54AR055073. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the federal government.

620 TRPA1 MEDIATES THE ACUTE NOXIOUS EFFECTS OF INDUSTRIAL ISOCYANATES AND TEAR GASES.
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The release of the methyl isocyanate in Bhopal, India, caused the worst industrial accident in history. Exposures to industrial isocyanates induce lacrimation, pain, airway irritation and edema. Similar responses are elicited by chemicals used as tear gases. Despite of frequent exposures the biological targets of isocyanates and tear gases in vivo have not been identified, precluding the development of effective countermeasures. We use Ca2+ imaging and electrophysiology to show that the noxious effects of isocyanates and all major tear gas agents are caused by activation of Ca2+ influx and membrane currents in mustard oil-sensitive sensory neurons. These responses are mediated by Transient Receptor Potential Ankyrin 1 (TRPA1), an ion channel serving as a detector for reactive chemicals. We identify 2-chloroethylidenemalonitrile (CS tear gas agent) as the most potent TRPA1 agonists known to date (EC50≈741 nM). In mice, generic ablation or pharmacological inhibition of TRPA1 dramatically reduces isocyanate- and tear gas-induced nocifensive behavior following both ocular and cutaneous exposures. We conclude that isocyanates and tear gas agents target the same neuronal receptor, TRPA1. TRPA1 antagonists can prevent and alleviate the adverse health effects of exposures to a wide range of toxic noxious chemicals.

621 MAP KINASES REGULATE CHANGES IN ANTIOXIDANT AND INFLAMMATORY MEDIATOR EXPRESSION IN MOUSE KERATOCYTES INDUCED BY THE VESICANT 2-CHLOROETHYL ETHYL SULFIDE.
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The vesicant 2-chloroethyl ethyl sulfide (CEES), a model sulfur mustard vesicant. CEES was found to cause marked increases in Cu/Zn superoxide dismutase, catalase, thioroeductase and the glutathione-S-transferases, GSTA1-2, GSTP1 and mGST2. CEES also upregulated TGF-β and IL-1α, as well as several enzymes important in the synthesis of prostaglandins, including COX-2, microsomal prostaglandin E synthase-2 (mPGES-2) and prostaglandin D synthase (PGDS). CEES readily activated keratinocyte JNK and p38 MAP kinase signaling pathways which are known to regulate expression of antioxidants and prostaglandin synthases. Inhibition of p38 MAP kinase suppressed expression of GSTA1-2, COX-2, mPGES-2 and PGDS while JNK inhibition suppressed PGDS and GSTP1. CEES also induced caverol-1, a membrane protein known to interact with MAP kinases and regulate their activity. These data indicate that CEES modulates antioxidants and inflammatory mediators by distinct mechanisms. Alterations in expression of these mediators are likely to be important mechanisms regulating inflammation and/or protection of the skin from CEES oxidative stress. Supported by CA100994, CA093798, ES047438, ES05022, GM034310 and AR055073.

622 DEVELOPMENTAL EFFECTS OF PM DURING POSTNATAL LUNG GROWTH.
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Early childhood exposures to environmental contaminants may contribute to disease, either during childhood or later in life, both because children’s exposures may be higher and because they may be more vulnerable to the toxic effects of the contaminants. We hypothesize that early life exposure to ambient ultrafine particles alters lung response and structural development; furthermore re-exposure as adults will lead to increased sensitivity. These studies will (1) Identify critical time points in the differential response of newborn vs. adult lung to inhaled environmental particles, (2) Compare effects of age on uptake efficiency and particle distribution and after exposure. C57BL/6 mice at 2 and 56 days old were exposed to concentrated ambient “Real World” particles from ultraclean particle concentrator (HUCAP) for a duration of 4 hours a day for 4 consecutive days and compared to sham controls for either 1 week or 2 weeks and examined at the end of exposure or allowed to recover until 8 weeks of age. At that time mice were either sacrificed or exposed to ambient concentrated particles for 4 days and examined at the end of exposure. Additional studies compared particle distribution in the lung and whole-body translocation in HUCAP exposed mice exposed to ~ 10μm and ultrafine particles from 4 days and examined 24 hours later. Lungs harvested from mice exposed starting at 2 days of age to HUCAPS for 4 hours a day for 4 consecutive days showed increased mRNAs encoding MnSOD. Mice exposed to HUCAPs for 2 consecutive weeks early in development and re-exposed as adults to HUCAPs for 4 hours a day on 4 consecutive days demonstrated decreased in mRNAs encoding TNFα and RANTES compared to mice that were exposed only as adults. Early life exposures to ambient ultrafine particles induce antioxidant defenses and activate cell cycle and inflammatory systems. Re-exposure as adults demonstrated decreases in inflammatory genes which may suggest a suppression of the immune system in response to subsequent particle exposure. Supported by EPA Star PM Center R-827354 and P30 ES-01247.

623 THE EFFECTS OF HIGH FAT DIET INDUCED GESTATIONAL DIABETES ON MOUSE METABOLIC DISORDER AND SKELETAL MALFORMATION.
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Adult diets rich in saturated fat augment LDL cholesterol, elevate oxidative stress (OS), and increase risk of cardiovascular disease, obesity, cancer, and osteoporosis. However, the contribution of gestational high saturated fat diet (HFD) and OS to metabolic disease and skeletal dysfunction in perinates and adults offspring is not known. Animal models show deleterious effects of maternal HFD during pregnancy, including excessive OS, placental and vascular dysfunction, insulin resistance, and metabolic syndrome. Pathophysiologic mechanisms linking gestational HFD, OS, and insulin resistance to perinatal development and adult-onset skeletal and metabolic dysfunction are explored in the present study. Female C57BL/6 mice were fed cafeteria-style rodent diet HFD which including 32.1% saturated fat for one month prior to conception, and throughout gestation. Dam body weight increase from gestation day 0 (G0) to G19 by 41% with HFD, as compared to 23% in control dams; G19 placental OS was 87% higher in HFD vs. control mice. HFD dams also developed hyperglycemia and insulin resistance. Results of this study also demonstrate for the first time a marked reduction in G19 perinatal bone mineral density (BMD; 20%), total bone volume (45%), and crown-to-7ump length (12%) in progeny of dams who consumed HFD, as compared to controls. Additionally, offspring that were fed conventional rodent chow exhibited lifetime-long effects of gestational HFD, including hyperglycemia, insulin resistance, obesity, lower femoral epiphyseal BMD (HFD offspring were 87% of control), and...
dysregulation of distal femoral trabecular architecture at 6 months of age, as measured by micro-computed tomographic image analysis. Collectively, these data suggest that offspring of dams who consume HFD during pregnancy are at increased risk of permanent skeletal malformation and adult-onset fragility fractures, as well as metabolic dysfunction.

**PL 624**

**DIOXIN SUPPRESSION OF DUCT FORMATION AND BRANCHING IN AN ENGINEERED 3D IN VITRO MODEL OF THE HUMAN MAMMARY GLAND.**

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Mammary gland development is a carefully orchestrated, chronological process that is influenced by a variety of environmental agents including 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin). Exposure of pregnant rodents to dioxin late in gestation profoundly suppresses mammary duct development and branching in female progeny. The mechanisms by which dioxin suppresses mammary duct development and branching, let alone the signaling processes involved in normal duct development, are not known. To address these issues, we have developed a Cultrex™-based 3-dimensional in vitro model in which the co-culturing of single cell suspensions of human mammary epithelial and myoepithelial cell lines, plus/minus human myofibroblasts, give rise to structures (>1 mm) within 10 days, consisting of primary and secondary ducts with attached alveoli-like lobular units. Structure development was monitored by differential interference contrast microscopy; and 3D reconstruction of confocal image stacks of the phalloidin stained actin cytoskeleton, and immunostained laminin-5. Duct branching, but not alveoli development, was impaired when epithelial and myoepithelial cells were cultured in the presence of 10 nM dioxin. The co-culturing of mammary and myoepithelial cells on a layer of myofibroblasts (embedded in a solidified matrix of type I collagen) did not affect gland development. Inclusion of 10 nM dioxin in the tricell co-culture model did not affect alveoli development, but severely suppressed duct development and eliminated ductal branching. These effects of dioxin on duct development and branching in the co-culture model mimics what occurs in vivo following fetal exposure. These data suggest that the Cultrex™-based 3D mammary gland culture model may be useful for the screening of putative modifiers of mammary gland development, elucidation of cell types responsible for toxicant effects, and unraveling mechanisms of action. Supported by CA56586 and a pilot award from ES06659.

**PL 625**

**PERSISTENT MODULATION OF T CELL AND B CELL MATURATION AND FUNCTION IN 48-WEEK-OLD C57BL/6 MICE DEVELOPMENTALLY EXPOSED TO TCDD.**

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The extent to which prenatal TCDD exposure can permanently alter immunity and affect disease progression during aging is unknown. Preliminary experiments showed altered immune development, enhanced immune complex formation, and lupus-like lesions in 24-week-old C57BL/6 mice after a single prenatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). In this study, 48 week-old offspring of high affinity Akr C57BL/6 dams prenatally exposed to oral TCDD (0.25 or 5.0 μg/kg) were evaluated for changes in T cell and B cell maturation and function as well as the progression of immune-mediated renal disease. As at 24 weeks, TCDD-treated females continued to display decreased thymic weight and cellularity. TCDD-treated male offspring had increased percentages of auto-reactive CD3+Vβ3 TcRs T cells in axillary and inguinal lymph nodes. Con-A stimulated splenocytes from prenatal TCDD-treated female mice produced increased IL-12 and INF-γ and diminished IL-4, while males showed increased IL-10 and IL-12. Alterations in bone marrow lymphopoiesis were seen in both sexes. Splenic transitional 2B cells (CD21sinCD24hi) were increased in males while transitional 1B cells (CD23negCD1neg) were increased in females. Autoantibodies to cardiopin were significantly increased in the TCDD-treated males. Anti-IgG and anti-C3 immune complex renal deposition was significantly increased in TCDD-treated males, and enhanced in the TCDD-treated females. These collective findings suggest that developmental TCDD exposure permanently and differently alters the immune system function based on sex. This immune dysregulation appears to include a sustained type III hypersensitivity, lupus-like autoimmune-mediated disease in this genetically non-predisposed murine strain.

**PL 626**

**TCDD DECREASES β-CATENIN DEPENDENT SOX9 EXPRESSION DURING PROSTATIC BUD INHIBITION IN MOUSE UROGENITAL SINUS.**

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The developing mouse prostate is a model for androgen-dependent prostate growth. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) interferes with the earliest stage of prostate morphogenesis by impairing formation of prostatic buds from the fetal prostate anlage, the urogenital sinus (UGS). In utero TCDD exposure completely prevents buds from forming on the ventral UGS surface and causes buds on the lateral UGS surface to form in an inappropriate dorsal position. In search of a mechanism, we considered SOX9, a transcription factor required for prostatic bud formation and reduced in abundance by TCDD in the developing zebrasfish jaw and regenerating fin. In utero TCDD exposure (5 μg/kg, maternal dose) on mouse embryonic day (E) 15.5 reduced SOX9 protein levels on E16.5 in the same UGS epithelial cell layer where Cyp1a1 was induced. To identify a mechanism, we tested the hypothesis that SOX9 is regulated by β-catenin-dependent transcription. The β-catenin null UGS did not form prostatic buds and did not express detectable levels of SOX9 protein. However, in utero TCDD exposure did not alter β-catenin abundance or distribution in the UGS epithelium of wild type mice. Therefore, it appears that TCDD acts downstream of β-catenin to repress SOX9, and this may be the mechanism by which TCDD impairs prostatic bud formation in mice. (Supported by NIH grants F32 ES014284, R37 ES01332, and ES 10820).

**PL 627**

**ASSESSING THE METHYLATION STATUS OF EMBRYOS TREATED WITH VALPROIC ACID AS A POSSIBLE MECHANISM OF TERATOGENESIS.**

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Exposure to the antiepileptic drug valproic acid (VPA) in utero is associated with a 1-2% increase in neural tube defects (NTDs), however the molecular mechanisms by which VPA induces NTDs are not known. VPA is a histone deacetylase (HDAC) inhibitor and has been shown to increase histone acetylation in embryos 1 hour after maternal administration of the drug. Chromatin structure and DNA methylation are tightly correlated. Open chromatin is associated with unmethylated DNA, and DNMTs and HDACs can trigger demethylation of hyperacetylated genes. Previously in our laboratory, we demonstrated that folic acid pre- and co-administrated with VPA was protective against the teratogenic effects caused by VPA, indicating that one-carbon metabolism may be disrupted. The purpose of this study was to evaluate the methylation status of exencephalic embryos exposed to VPA during gestation using the cytosine extension assay. On gestational day (GD) 9.0, pregnant dams were injected with 400 mg/kg VPA or saline subcutaneously. On GD 10.5, embryos were harvested and categorized by treatment groups and neural tube closure status. The cytosine extension assay was then used to assess the degree of hypomethylation in these embryos. Results indicate that methylation is unchanged in VPA treated embryos with closed neural tubes (n=6) and VPA treated embryos with exencephaly (n=6) when compared with saline treated embryos (n=5). In addition, western blotting did not show any changes in the expression of DNA methyltransferase (DNMT) 1 or hyperacetylated histone H4 on GD 10.5. Currently, we are evaluating methylation changes 1 hour and 6 hours after exposure to VPA. Western blotting will also be completed for DNMTs 3a and 3b, the methylated DNA binding protein McCP2, and acetylated histone H3 (Support: CIHR).

**PL 628**

**PRENATAL EXPOSURE TO B(A)P IMPAIRS LATER LIFE NEURONAL FUNCTION AND BEHAVIOR IN CPR MICE.**

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Exposure to toxicants during the critical window of corticogenesis may contribute to alterations in the developing brain with respect to the neuropathology of autism spectrum disorders. To characterize the impact of prenatal B(A)P exposure on the development of a metabolite burden that degrades cortical neuronal activity and function; pregnant Cpr+/+ mice were exposed to B(A)P (0, 150, 300 and 1500 mg/kg) were evaluated for changes in T cell and B cell maturation and function as well as the progression of immune-mediated renal disease. As at 24 weeks, TCDD-treated females continued to display decreased thymic weight and cellularity. TCDD-treated male offspring had increased percentages of auto-reactive CD3+Vβ3 TcRs T cells in axillary and inguinal lymph nodes. Con-A stimulated splenocytes from prenatal TCDD-treated female mice produced increased IL-12 and INF-γ and diminished IL-4, while males showed increased IL-10 and IL-12. Alterations in bone marrow lymphopoiesis were seen in both sexes. Splenic transitional 2B cells (CD21sinCD24hi) were increased in males while transitional 1B cells (CD23negCD1neg) were increased in females. Autoantibodies to cardiopin were significantly increased in the TCDD-treated males. Anti-IgG and anti-C3 immune complex renal deposition was significantly increased in TCDD-treated males, and enhanced in the TCDD-treated females. These collective findings suggest that developmental TCDD exposure permanently and differently alters the immune system function based on sex. This immune dysregulation appears to include a sustained type III hypersensitivity, lupus-like autoimmune-mediated disease in this genetically non-predisposed murine strain.

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and neural tube closure. Normal formation of the placenta, and increased risk for NTDs. Saison-1-P and sphingoid base-1-P in maternal and fetal tissue, altered migration of uNK cells, absence of the ectoplacental cone, fewer uNK at the implantation site, and uterine natural killer (uNK) cell markers were labeled with cytokeratin, and uterine natural killer (uNK) cells were identified.

Previously in Phase III human clinical trials. Phosphorylated FTY720 acts as an adenosine receptor antagonist. FB1 (20mg/kg/day ip, E7.5-8.5) or FTY720 (10mg/kg/day po, E6.5-7.5) were administered to pregnant inbred SWV and SannP rats. These treatments results in elevated sphingoid bases. FTY720 is a sphingoid base analog currently in preclinical development.

Increased neural tube defect (NTD) risk is observed in human populations. The study of maize. Increased neural tube defect (NTD) risk is observed in human populations. The study of maize.

A number of human diseases, mammalian developmental abnormalities and cancer. Loss of genomic imprinting (LOI) has been associated with environmental factors. Loss of genomic imprinting (LOI) has been associated with environmental factors. Loss of genomic imprinting (LOI) has been associated with environmental factors.

Imprinting is an epigenetic modification of the genome that results in differential expression of maternal or paternal alleles in somatic cells. Since imprinting involves precise allele expression controlled by DNA methylation and histone modifications, these epigenetic machineries may be especially sensitive to dysregulation by environmental factors. Loss of genomic imprinting (LOI) has been associated with a number of human diseases, mammalian developmental abnormalities and cancer. The goal of this study was to determine whether LOI could serve as a useful biomarker of rodent carcinogenesis. Liver from male Big Blue® Fischer 344 rats treated for 28 days with the genotoxicant 2-acetylaminofluorene (AAF; 3 mg/kg/day) or non-genotoxicant phenobarbital (PB; 100 mg/kg/day) was examined. Robust changes in gene expression were observed as a result of treatment (Seidel et al. Int J Toxicol, 2006). DNA isolated from four representative animals per group was modified with bisulfite to edit site specific methylated and unmethylated cytosines within CpGs. Bisulfite sequencing (BS) and methylation specific PCR (MSP) were used to characterize DNA methylation of selected genes. Lac I was confirmed to be extensively methylated in controls and was unchanged after 28-day treatment with the carcinogen. Rat H-Ras promoter was hypomethylated in controls and unchanged by AAF or PB treatment. Differentially methylated regions (DMRs) of the insulin-like growth factor 2 gene (Igf2) and the non-coding RNA gene H19, two imprinted genes often associated with cancer, were investigated and compared to controls. Treatment with AAF or PB did not alter methylation status within Igf2 DMRs. The extent of methylation within the H19 promoter decreased (AAF>>PB) after treatment with both carcinogens. Taken together, this study demonstrates that assaying DNA methylation within CpG islands of imprinted genes could be a useful biomarker of liver tumorigenesis.

600μg/kg BW) by oral gavage on gestational days 14 through 17. Metabolite disposition was quantified during the preweaning period. Primary cortical neuronal cultures were prepared from PND 0 control and B(a)P-exposed Cpr+/+ pups, patch clamp analysis was performed and revealed significant decreases in the magnitude of inward current in neuronal cultures from exposed Cpr+/+ mice. Immunohistochemical analysis of primary cortical neuronal cultures also revealed significant shifts in peak mRNA and protein expression period for MET receptor tyrosine kinase and NR2B derived from B(a)P-exposed Cpr+/+ offspring as compared to cultures from control Cpr+/+ offspring. These shifts occur at a time when synapses are forming for the first time in sensory pathways.
using this method. In addition, phenobarbital induces specific alterations in promoter methylation patterns in liver (target tissue) as compared to kidney (non-target tissue). The application of this approach for identifying early mechanism-based markers of nongenotoxic carcinogenicity may ultimately increase the quality of cancer risk assessments for candidate drugs and ensure a lower attrition rate during late-phase development.

### 634 ONTOGENIC EXPRESSION OF HEPATIC MICRORNAs CORRELATES WITH HISTONE H3K4 DIACETYLATION DURING MOUSE LIVER DEVELOPMENT.

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MicroRNAs are short, endogenously-expressed non-coding RNAs that down-regulate gene translation by base-pairing to corresponding target mRNAs. Most studies have focused on the role of microRNAs in carcinogenesis. Recent evidence suggests that microRNAs also have critical functions in regulating normal development, as well as seno- and endobiostatic metabolism in liver. Although many microRNA targets have been identified, the in vivo regulation of microRNAs themselves is poorly understood. The purpose of the present study was to characterize the ontogenic expression patterns of microRNAs in mouse liver, and determine the epigenetic mechanisms for their expression during postnatal development. C57BL/6 mouse livers were collected from 2 days prenatal to 45 days of age. The ontogenic profiles of microRNAs were characterized by microarray, and confirmed by the Multiplex Suspension Array. Of the 574 verified mouse microRNAs, 79 microRNAs are expressed in liver, falling into three distinct patterns: fetal, neonatal, and adult-specific. In addition, the microRNAs sharing a similar expression pattern are usually closely located as a cluster on a chromosome (c10kb), suggesting a common regulatory mechanism may exist. ChIP-on-chip analysis performed on mouse chromosomes 5, 12, and 15 demonstrated that DNA methylation and histone H3K27 trimethylation, the two major epigenetic marks for gene suppression, were consistently low in regions encoding microRNAs expressed in liver. In contrast, enrichment of histone H3K4 di-methylation, a hallmark for gene activation, occurred in the vicinity of microRNA clusters at ages when microRNAs were highly expressed. Regression analysis demonstrated a strong correlation between enrichment of histone H3K4 di-methylation and microRNA ontogenic expression. Collectively, this study revealed that specific microRNA ontogenic expression patterns of microRNAs correlate with histone H3K4 dimethylation during mouse liver development (Supported by NIH grants ES09716, ES07079, ES013714, ES09649, and RR021940).

### 633 EVALUATION OF TOXIC POTENTIAL BASED ON CYTOTOXICITY AND EPIGENETIC TOXICITY OF CHEMICALS IN MOUSE HEPATOMA CELLS.


Epigenetic mechanism is understood to determine gene expression with DNA base sequence. This study was performed to estimate toxic potential of chemicals based on cytotoxicity and global DNA methylation. Phenylbutazone, biphenol A and chloramphenicol which are carcinogens or suspected to link with cancer are assessed with positive controls, 5-aza-2-deoxycytidine (5aza) and sodium butyrate, which are inducers of hypo- or hyper-global DNA methylation in mouse hepatoma cells (Hepa1c1c7). The toxic potential of phenylbutazone, chloramphenicol, and biphenol A was estimated by employing the results of global DNA methylation combined with cytotoxicity data (MTT, ATP and Cell proliferation assay). In vitro cytotoxicity assessments showed cytotoxic and non-cytotoxic concentrations (CL, NCL) of phenylbutazone at 0.55mM and 0.065mM. CL and NCL of chloramphenicol were determined as 0.3mM and 0.03mM. The CL and NCL of biphenol A were 0.08 and 0.045mM. The CL and NCL of 5aza and sodium butyrate were 0.25 and 0.0025mM and 6.5 and 1.5mM, respectively. Global methylation status in hepatoma cell treated with CL and NCL of these chemicals were measured. Hypermethylation at CL but hypomethylation at NCL were observed for phenylbutazone. Choramphenicol did not change global DNA methylation at both doses of CL and NCL. Biphenol A led to reduction of global methylation levels as CL. 5aza at CL led to hypomethylation but sodium butyrate induced hypermethylation at NCL. Finally, we estimated toxic potential rankings following to cytotoxicity and global DNA methylation. Phenylbutazone and Biphenol A ranked the highest position for they presented changed DNA methylation at NCL or high cytotoxicity with change of DNA methylation at CL. 5aza and sodium butyrate ranked second one for they showed moderate cytotoxicity with changed DNA methylation at CL or NCL. Chloramphenicol was allotted at the lowest toxic position for moderate cytotoxicity with un-changed DNA methylation.

### 635 THE BENZENE METABOLITE, HYDROQUINONE ALTERS GLOBAL DNA METHYLATION AND SPECIFIC GENE PROMOTER METHYLATION IN HUMAN TK6 CELLS.

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DNA methylation, the addition of a methyl group at the C-5 position of cytosine in CpG dinucleotides, plays a critical role in maintaining genome stability and in controlling gene expression. aberrant DNA methylation patterns have been frequently observed in human leukemia and other cancers. Benzene is an established human carcinogen, to which chronic exposure causes leukemia and other hematological cancers. In one recent study (Cancer Res, 67:876, 2007), global DNA hypomethylation and hyper- and hypomethylation of gene-specific promoters was detected in healthy individuals exposed to low levels of benzene slightly above background. In order to further explore the effects of benzene exposure on DNA methylation, we conducted a study in vitro to examine whether or not hydroquinone (HQ), a major metabolite of benzene, altered global DNA methylation and gene-specific promoter methylation in the human lymphoblastoid TK6 cell line. TK6 cells were treated with HQ at 0 - 20 μM for 48 hours. The DNA methylation inhibitor 5-aza-2-deoxycytidine, served as positive control. Global DNA methylation was determined by immunocytochemistry using flow cytometry. Methylation of specific gene promoters was detected by Illumina’s GoldenGate Methylation Cancer Panel I assay, in which 1505 loci on 807 genes were analyzed. As expected, 5-aza-2-deoxycytidine reduced global DNA methylation and had a demethylating effect on many genes. After HQ treatment, global DNA methylation was reduced in a dose-dependent manner. Methylation of specific gene promoters was also altered, with certain genes hypermethylated and other genes hypomethylated. In conclusion, HQ reduced global DNA methylation and induced hyper- and hypomethylation of multiple gene promoters in TK6 cells. These DNA methylation alterations might lead to genomic instability, the abnormal expression of certain genes and may be one of the mechanisms producing leukemogenesis from benzene exposure.

### 636 EPIGENETIC CONTROL OF MAMMALIAN LINE-1 RETROTRANSPOSON BY RETINOBLASTOMA PROTEINS.

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Long interspersed nuclear elements (LINEs or L1 elements) are targeted for epigenetic silencing during early embryonic development and remain inactive in most cells and tissues. Although DNA methyl transferases (DNMTs) contribute to L1 silencing via CpG-rich regions hypermethylation, the exact epigenetic mechanisms involved in L1 silencing remain largely unknown. Since E2F/Retinoblastoma protein complexes localize to highly repetitive pericentromeric regions we sought to determine if L1 epigenetic regulation is E2F/Retinoblastoma-mediated. Here we show that E2F/Retinoblastoma family complexes participate in L1 epigenetic regulation via nucleosome and histone modifications and recruitment of histone deacetylases (HDACs) HDAC1 and HDAC2. ChIP experiments demonstrated that (i) Retinoblastoma protein interacts with human and mouse L1 elements, (ii) L1 elements are deficient in both heterochromatin-associated histone marks H3 tri methyl K9 and H4 tri methyl K20 in Retinoblastoma family triple knock out fibroblasts (TKO), (iii) L1 promoter enhances isolated histone H3 acetyltransferase in the absence of HDAC1 and HDAC2 recruitment. (iv) L1 expression in TKO fibroblasts is upregulated compared to wild type counterparts, (v) treatment of TKO cells with L1 also alters epigenetic profiles in the vicinity of microRNA clusters at ages when microRNAs were highly expressed. Regression analysis demonstrated a strong correlation between enrichment of histone H3K4 di-methylation and microRNA ontogenic expression. Collectively, this study revealed that specific microRNA ontogenic expression patterns of microRNAs correlate with histone H3K4 dimethylation during mouse liver development (Supported by NIH grants ES09716, ES07079, ES013714, ES09649, and RR021940).
Assessment of allergic potency of low molecular weight compounds is generally performed using animal models, such as the murine local lymph node assay. Progress in understanding the mechanisms of skin sensitization, including effects on the production of cytokines by the different cell types within the skin, provides us with the opportunity to develop in vitro tests as an alternative to in vivo sensitization testing. The aim of the present study was to evaluate the possibility to use cytokine production, namely interleukin-18 (IL-18), as indicators of cutaneous contact sensitization potential of low molecular weight chemicals. In the study, the human keratinocyte cell line NCTC 2415 was used. IL-18 has been demonstrated to favor Th-1 type immune response by enhancing the secretion of pro-inflammatory mediators such as TNF-α, IL-8 and IFN-γ, and to play a key proximal role in the induction of allergic contact dermatitis. Cells were exposed to contact allergens (dinitrochlorobenzene, cinnamaldehyde, tetramethylthiuram disulfide, eugenol, isoeugenol, paraphenylenediamine, resorcinal) to respiratory allergens (diphénylmethane disocyanate, trimellitic anhydride, ammonium hexachloroplatinate) and to irritants (sodium lauryl sulphate, salicylic acid, phenol). Cell-associated IL-18 was evaluated 24 h later by a commercially available ELISA kit. At non-toxic concentrations (cell viability higher than 90% as assessed by MTT reduction assay), all contact sensitizers induced a dose-related increase in IL-18, whereas both irritants and respiratory failed. Results obtained indicated that cell-associated IL-18 may provide an in vitro tool for identification and discrimination of contact versus respiratory allergens and/or irritants. Acknowledgements: This research was supported by the EU grant SENSIT-IV #018681.
tion were obtained and an addition of skin-relevant metabolic activation system was investigated. We present reactivity profiles of forty chemicals of known sensitizing potential. The data show that peptide depletion above 10% is likely to be due to covalent modification. However, peptide depletion below this value does not always correlate with observation of adduct(s) and is often inconclusive. It is therefore necessary to additionally confirm the reactivity by identifying the adduct(s). The approach enables investigation of reactivity that may arise from the presence of impurities or a combination of chemicals. By optimizing the incubation conditions we achieved high levels of confidence in detection of non-reactive chemicals. The data is analysed using mathematical and statistical approaches to obtain a rank order of reactivity based on specificity, reaction rate, mechanism(s) and complexity. This is analysed using mathematical and statistical approaches to obtain a rank order of reactivity based on specificity, reaction rate, mechanism(s) and complexity. This is achieved using a rank order of reactivity based on specificity, reaction rate, mechanism(s) and complexity. This in vivo dataset will be integrated with outputs from other predictive in vitro assays in the future, to help make decisions about the safe use of chemicals without using animal tests.

**PL 642 MOUSE MESENTERIC LYMPH NODE GENE EXPRESSION PROFILES DURING SENSITIZATION, AND EAR-SWELLING, HISTAMINE, AND IMMUNOGLOBULIN RESPONSE DURING ELICITATION IN RESPONSE TO COMMON FOOD ALLERGENS.**

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The development of novel foods, including genetically modified (GM) foods, has amplified the risk of food allergy in certain individuals. Therefore, there is a need to evaluate the potential allergenicity of novel foods before they reach consumers. In the following study, Balb/c mice were sensitized and then challenged with common food allergens (0.2% peanut agglutinin (PNA), 2% egg ovalbumin (OVA) or 2% beta lactoglobulin (BLG)) to test the hypothesis that gene expression profiling during sensitization can be used to predict allergenicity during elicitation. For the elicitation phase, female Balb/c mice were sensitized i.p. with allergen or saline for 2 d, and 2 wk later challenged with the respective allergen on the dorsal side of the ear to measure the ear swelling response 0, 0.5, 1, 2, 6 and 24 hr post-challenge. Blood was collected from one group of mice 20 min after challenge to measure histamine levels, and 24 hr after challenge in another group to measure the IgG1 and IgE. For the sensitization phase, mice were sensitized as described above and then euthanized 24 and 48 hr later to measure mesenteric lymph node (MLN) gene expression profiles using a mouse oligoarray (Mouse 22.4K ESTs). During elicitation, PNA (38% increase) and OVA (58% increase) induced a stronger ear swelling response than BLG (29% increase). Histamine levels were 2 to 17 times higher in the allergen-challenged mice compared to the control mice. Lastly, levels of IgG1 and IgE in the allergen-challenged mice were 2 to 6 times higher than the control mice. During sensitization, a number of genes were induced in the MLN in response to the various allergens, and the expression of these genes is being correlated with endpoints measured during the elicitation phase.

**PL 643 COMPARISON OF CONTACT ALLERGEN-INDUCED GENE EXPRESSION CHANGES IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELL- DERIVED DENDRITIC CELLS AND DC-SURROGATE CELL LINES.**

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To help identify predictive endpoint measures for use in an in vitro cell based assay(s) for assessing the skin sensitization potential of chemicals, we focused on a genomics approach to identify genes that are regulated in human peripheral blood derived dendritic cells (PBMC-DC) following chemical allergen exposure. A list of 29 potentially predictive genes was derived using PBMC-DC which has been evaluated with 26 chemicals using a multiplexed bead assay for measuring gene expression. With PBMC-DC, 14 out of 29 genes are consistently positive in response to 5 mM DNBS with very little donor-to-donor variability. Excluding DNBS, no genes were consistently positive with all allergens tested and some allergens induced changes in only a few genes. These same genes have been evaluated in two DC-surrogate cell lines, THP1, U937 with a smaller number of chemicals. These cell lines show few changes in the expression of the 29 genes following allergen treatment. In U937 cells the few positive responses observed were minimal with only 2-6 fold changes over control except for a maximal response of 8.5-fold increase in one gene, ISG15. A few more gene changes are observed in the THP1 cell lines. Only one gene was positive in THP1 cells with all of the allergens tested, AKR1C2. There are clear differences between PBMC-DC and the U937 and THP1 cell lines in the allergen-induced expression changes of these 29 genes. PBMC-DC appear to be the cells of choice for use in this gene expression-based method.

**PL 644 DISTINGUISHING CONTACT SENSITIZERS, RESPIRATORY SENSITIZERS, AND IRRITANTS IN THE LOCAL LYMPH NODE ASSAY (LLNA): A TOXICOGENOMIC APPROACH.**


Genomic approaches have the potential to enhance existing toxicology endpoints, including those for chemical sensitization. The present study was conducted to identify gene expression responses with the ability to distinguish between irritants, contact sensitizers and respiratory sensitizers. The evaluated chemicals included the contact sensitizers dintrichlorobenzene (DNCB) and heptyl cinnamic aldehyde (HCA), the respiratory sensitizers ortho-phthalaldehyde (OPA) and trimellitic anhydride (TMA), and the non-sensitizing irritants methyl salicylate (MS) and nonanonic acid (NA). Female Balb/C mice received three doses of each chemical per the standard LLNA dosing regimen. Auricular lymph nodes were analyzed for both 3HTdR incorporation and gene expression responses. All chemicals induced dose-dependent increases in stimulation index values with EC50 values of 0.09%, 8.6%, 0.01%, 0.15%, 52%, and 18.9% for DNBC, HCA, OPA, TMA, MA and NA, respectively. Analysis of the gene expression data for the chemicals induced a dose-dependent increase in the number of active responses with a strong correlation to the corresponding stimulation indices. Genes such as IL-4, IL-21 and IFNgamma were similarly modulated by all chemicals and appear to lack any discriminating potential. The majority of the genes modulated by the irritants were similarly regulated by the sensitizers, consistent with the irritant effects of the sensitizers at high doses. A select number of responses were unique to the sensitizers and therefore offer the ability to distinguish sensitizers from irritants. In addition, a small subset of genes was identified that appeared to be unique to the respiratory sensitizers. Collectively, the data suggest that gene expression responses observed in the mouse LLNA may serve as useful biomarkers to distinguish irritants from sensitizers and respiratory sensitizers from contact sensitizers.

**PL 645 THE CHEMICAL SENSITIZER NICKEL SULFATE INDUCES THE PRODUCTION OF INTERLEUKIN-12: COOPERATION AMONG P38MAPK, NF-kB AND IRF-1 PATHWAYS.**


Allergic contact dermatitis, caused by metallic ions such as nickel, is a T cell-mediated inflammatory skin disease. Interleukin-12 (IL-12) is considered to be important in the generation of the allergen-specific T cell response. IL-12p70, the bioactive form of IL-12, is a heterodimeric protein composed of two subunits IL-12p40 and IL-12p35. Dendritic cells (DC) are professional antigen presenting cells that are involved in the induction of primary immune responses as they possess the ability to stimulate naïve T cells. In this study we address the question whether NOS4a by itself or in synergy with other signals can induce the secretion of IL-12p70 by human monocytes derived DC (Mo-DCs). Mo-DCs were obtained from human monocytes purified from peripheral blood and cultured in the presence of GM-CSF and IL-4 for 5 days. Nickel sulfate by itself induced the production of IL-12p40 in a dose dependent manner but was unable to induce the production of IL-12p70. The concomitant presence of IFNγ and nickel was necessary to induce the production of IL-12p70. Both signals were required for the expression of the p35 subunit of IL-12 as shown by RT-PCR. Interestingly, we found that in addition to the activation of MAPK (Mitogen-activated protein kinase) and NF-kB (Nuclear factor kappa B) pathways, nickel sulfate by itself activated the induction of IRF-1 (a member of the family of transcription factors IRF (Interferon Regulatory Factors)). To test the impact of these signaling pathways in IL-12 production, we used well known pharmacological inhibitors of MAPK and NF-kB pathways and RNA interference-mediated silencing of IRF-1. Results suggested that p38MAPK and NF-kB but not JNK (Jun N-terminal kinase) are involved in IL-12p40 production induced by nickel. Moreover, specific IRF-1 silencing nearly totally abrogated IL-12p40 and IL-12p70 production provoked by nickel or nickel plus interferon-gamma. Finally, our results indicate that p38MAPK, NF-kB and IRF-1 cooperate in IL-12p70 induced by nickel sulfate and interferon-γ.
MULTIPLEXED QUANTITATIVE HIGH CONTENT SCREENING ASSAYS REVEAL THAT CIGARETTE SMOKE CONDENSATE ACTIVATES NUCLEAR FACTOR KAPPA B AND INDUCES CELL DEATH IN HUMAN BRONCHIAL EPITHELIAL CELLS.


Human bronchial cells are one of the first cells types exposed to inhalable environmental toxins. Nuclear transcription factor KB (NF-KB) responds to environmental stress as a ubiquitous transcription factor that regulates the inflammatory response, cell proliferation, apoptosis and disrupts the F-actin cytoskeleton. The effects of cigarette smoke condensate (CSC) on NF-KB, F-actin, and apoptosis were evaluated in BEAS-2B immortalized human bronchial epithelial cells. We hypothesized that CSC would activate NF-KB, disrupt the F-actin cytoskeleton and induce apoptosis. NF-KB translocates from the cytoplasm to the nucleus upon activation. This translocation is observed when BEAS-2B cells are treated with doses above 20μg/ml CSC for 24 h. As NF-KB is activated, cell number and nuclear size decrease. Cell structural alterations often accompany toxic insults to cells. CSC treatment of BEAS-2B cells induces a rearrangement of F-actin such that stress fibers are no longer prominent at the cell periphery and throughout the cells, but relocalize to perinuclear regions. Because toxins induce F-actin relocalization to perinuclear areas and perinuclear F-actin is a nucleus marker, we evaluated the effects of CSC on apoptosis. CSC induces apoptosis in BEAS-2B cells evidenced by an increase in caspase 3. CSC doses above 10μg/ml induce a significant increase in caspase 3 when cells are treated for 1- or 24 hrs compared to DMSO controls. Caspase 3 levels are twice that of DMSO controls when cells are treated with 40μg/ml CSC for 1- or 24 hrs. As caspase 3 increases, cell number decreases. After treatment for 24 hrs, a dose-dependent decrease in cell number with an average of 85% remaining when cells are treated with 10μg/ml CSC while only 46% remain when treated with 40μg/ml CSC. CSC activates NF-KB in BEAS-2B cells and induces cell death with a concomitant disruption of the F-actin cytoskeleton.

UP-REGULATION AND NUCLEAR ACCUMULATION OF P53 PROTEIN IN HUMAN BRONCHIAL EPITHELIAL CELLS EXPOSED TO 4-(METHYLNITRO-SAMINO)-1-(3-PYRIDYL)-1-BUTANONE.

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Our previous study showed that 150 μM of 4-(methylnitro-saminio)-1-(3-pyridyl)-1-butanone (NNK), a tobacco specific lung carcinogen, inhibited cell proliferation and down-regulated the expression of chromosomal region maintenance 1 (CRM1) in human bronchial epithelial cells (BEAS-2B). CRM1 is an exporter for leucine-rich nuclear export signal proteins including p53, a critical tumor suppressor gene product that plays an important role in lung carcinogenesis. In the present study, we hypothesize that NNK exposure not only changes the expression level of p53 protein, but also alters p53 subcellular localization due to CRM1 down-regulation. p53 export from nucleus. Further studies will focus on evaluating the role of CRM1 in modulating the p53 shuttling between nucleus and cytoplasm.

BENZO[α]PYRENE INDUCES DEDIFFERENTIATION OF HUMAN BRONCHIAL EPITHELIAL BEAS-2B CELLS.

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Although extensive studies have shown that benzo[a]pyrene (BaP), a ubiquitous environmental carcinogen, is closely associated with multiple human pulmonary diseases, molecular mechanisms of BaP toxicity are still not fully understood. Given that epithelial to mesenchymal transition (EMT) in the lung has been observed in response to environmental injury, and this type of transition has been linked to the onset and progression of pulmonary diseases, we hypothesized that BaP induces pulmonary disease via EMT. To examine the possible role of BaP in EMT, we examined a reliable epithelial marker, E-cadherin, in human bronchial epithelial BEAS-2B cells following BaP challenge. Our results showed that BaP (0.3 and 30 μM) decreased protein levels of E-cadherin in a concentration dependent manner. The loss of E-cadherin was specific since other cellular markers remained unchanged. BaP at 30 μM significantly altered the normal structure of cytokeratin, another marker of epithelial functional integrity, as assessed by immunostaining. The molecular basis for the shift toward mesenchymal phenotype is currently under investigation.

EFFECTS OF SIDE STREAM TOBACCO SMOKE IN DNA DAMAGE REPAIR AND GSH-DEFICIENT MICE.

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Side stream tobacco smoke (SSS), the main component of secondhand smoke, is classified as a human carcinogen, but has been difficult to study in animal models. Cigarette smoke causes free radicals and inflammation, both lead to DNA damage. Thus, animals with inadequate oxidative DNA damage repair, with the propensity to develop spontaneous lung tumors, may be more sensitive to the biological activity of SSS. Likewise, animals with a decreased capacity to combat oxidative stress may also be susceptible to SSS-induced damage. We used mice lacking the gene Ogg1, Myh, or double mutant mice, both of which are involved in base excision repair, removing oxidatively damaged DNA bases. We also used Gclm-deficient mice, which have decreased levels of glutathione (GSH). We have found that DNA strand breaks are increased immediately after a 4-hour exposure to 55.8 mg/m3 total particulate matter (TPM) of SSS in wildtype, Ogg1, and Myh single mutant mice. Moreover, micronucleated cells in the peripheral blood are increased in Ogg1-deficient but not in wildtype mice indicating that while strand breaks are repaired in wildtype mice, they seem less easily repaired in Ogg1-deficient mice. Our results may clarify the roles of oxidative stress and oxidative DNA damage-repair in SSS-induced cancers as well as elucidate the role of genetic polymorphisms in SSS disease susceptibility. Understanding gene-environment interactions in SSS as well as the interactions of oxidative stress and DNA damage will aid in the development of chemoprevention and treatment of lung cancer.
GLUTATHIONE S-TRANSFERASE P PROTECTS AGAINST ENDOTHELIAL DYSFUNCTION INDUCED BY EXPOSURE TO TOBACCO SMOKE AND ACROLEIN.

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Exposure to tobacco smoke impairs endothelium-dependent arterial dilation. Although the mechanism by which tobacco smoke induces endothelial dysfunction is unclear, reactive constituents of cigarette smoke are metabolized and detoxified by glutathione S-transferases (GSTs). Polymorphisms in GST genes are associated with the risk of cancer in smokers, yet the role of these enzymes in regulating the cardiovascular effects of smoking has not been studied. Because short-chain, unsaturated aldehydes, such as acrolein or crotonaldehyde, are excellent substrates of the GSTP1 enzyme, we tested if GSTP1 mice were more sensitive than GSTP2 mice to inhaled tobacco smoke and acrolein. The GSTP1 protein and activity were present in high abundance in mouse lung and aorta. Because GSTP1 also affects cell stress signaling, we determined MAPK activation in lungs of tobacco smoke- or acrolein-exposed mice. JNK activation was apparent after 6h exposure although no change in the level of ERK or p38 phosphorylation was detected. Aortic rings isolated from tobacco smoke-exposed GSTP1 mice (3 days) showed greater attenuation of acetylcholine-evoked relaxation than those from similarly exposed GSTP2 mice although no change in the sodium nitroprusside-induced relaxation was observed in either GSTP1 or GSTP2 mice. Similarly, aortic rings prepared from GSTP2 mice inhaling acrolein (1 ppm, 6h/day, 3 days) or those exposed to acrolein (10 μM) in the organ bath showed diminished acetylcholine-induced relaxation more strongly than GSTP1 mice. Endothelial dysfunction due to acrolein was prevented by pretreating the isolated aorta with N-acetylcycteine. These results indicate that GSTP1 protects against the endothelial dysfunction induced by exposure to tobacco smoke and that this protection could be related to the detoxification of acrolein or other related electrophilic cigarette smoke constituents. This work supported by ES11860 (AB), HL89380 (DJC), AHAF/NHF (DJC), and a Phillip Morris grant (AB).

THIOREDOXIN MEDIATES TGF-β1 AGAINST OXIDATIVE STRESS OF CIGARETTE SMOKE IN HUMAN AIRWAY EPITHELIUM.

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Cigarette smoke (CS) contains reactive oxygen species (ROS) to cause oxidative damage, inflammation, and dysfunction of repair process in airway cells. Thioredoxin (TRX) has anti-oxidation capability against oxidative stress, and involves in cell growth and differentiation. Transforming growth factor (TGF-β1) is found to regulate the growth of normal epithelial cells and initiate a signaling pathway that suppresses the early development of cancer cells. As airway epithelium provides a protective barrier against external environment, this study was to investigate how TRX mediates oxidative stress of CS in human bronchial epithelial cells and its following signaling transduction of stress-activated mitogen-activated protein kinases (MAPKs) in the regulation of TGF-β1. Results showed that CS caused intracellular ROS generation and apoptosis, and induced TRX but attenuated TGF-β1 expression in human bronchial epithelial (BEAS-2B) cells. Additionally, the constructed TRX-overexpressing BEAS-2B cells could repress CS-induced apoptosis and enhance TGF-β1 expression. After neutralized with anti-TGF-β1 antibody, TRX-overexpressing BEAS-2B cells exposed to CS decreased TRX expression. These represented TRX regulated TGF-β1 expression in CS exposure. TRX suppressed CS-induced c-Jun-N-terminal kinase (JNK) to prevent from apoptosis. This study demonstrated that oxidative stress of CS induced MAPK signaling, and TRX mediated TGF-β1 against oxidative stress of CS in human airway epithelial cells.

ENHANCEMENT OF NNK METABOLISM BY ARSENIC AS THE MECHANISM OF INCREASED CIGARETTE SMOKE LUNG CARCINOGENICITY BY ARSENIC.

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Mounting epidemiological evidences indicated that interaction between cigarette smoke and arsenic increased lung and liver cancer risks among cigarette smokers in arseniasis areas. We believe that the metabolism of NNK, a specific and known carcinogenic component in cigarette smoke, can be enhanced by arsenic leading to increased NNK metabolites, harmful DNA adducts and eventual carcinogenic risk. Young male ICr mice were exposed to single injection of NNK (1 mg/kg). These animals were also co-exposed to arsenite (10 mg/kg) or saline via gavage for 10 days. All animals, in groups of 5, were housed in metabolic cages for urine collections. Changes in hepatic P450 enzyme, Cyp2a5/6, a specific P450 enzyme for NNK metabolism, and urinary NNK metabolites were assayed with LC/MS/MS analytical method. Significant increased hepatic Cyp2a enzyme expression and activity were found in all arsenic treated animals. All NNK metabolites in the urine were also found to be elevated. More importantly, O6- and N7-MeG DNA-adducts in the liver were also found to be highly elevated in all arsenic treated animals. Our findings affirmed that arsenic can increase NNK metabolism. The increased NNK metabolites and the formations of DNA damaging O6- and N7-MeG adducts would enhance carcinogenic risk. Our findings also provide the needed scientific base for the epidemiological observations.

ENVIRONMENTAL TOBACCO SMOKE-INDUCED ENDOTHELIAL DYSFUNCTION: ROLE OF ACROLEIN AND PROTEIN-ACROLEIN ADDUCTS.

P. Haberzettl, D. J. Conklin and A. Bhatnagar, 1. Inst. Mol. Card., University of Louisville, Louisville, KY.

This study delineates the contribution of unsaturated aldehydes, such as acrolein to endothelial dysfunction after environmental tobacco smoke (ETS) exposure. We exposed 12-week old male C57BL/6 mice to air or ETS (average TSP - .91 mg/m3 over 2 separate experiments) for three consecutive days (6h/day). To evaluate the effects of scavenger, mice were treated with N-acetylcysteine (NAC; 200 mg/kg, i.p.) at 0.5h before each day. Animals were sacrificed 24h after the 3rd exposure following an 8h fast. ETS exposure decreased endothelial function in isolated mouse aorta and NAC pretreatment prevented the loss of endothelium-dependent acetylcholine-induced (ACh) relaxation supporting the role of ETS-derived unsaturated aldehydes as mediators of endothelial injury. NAC pretreatment did not enhance endogenous endothelium function in air-exposed mice because ACh-induced relaxation of phenylephrine-precontracted (PE) thoracic aortic rings was similar in air-exposed mice with or without NAC pretreatment (ACh EC50 Air = 243 ± 98nM; Air+NAC = 413 ± 98nM; n=3; ACh % relaxation, Air = 83±1; Air+NAC = 96±5; n=3). ACh relaxation of NAC-pretreated ETS-exposed mice (~78±6%) was significantly greater than in ETS-exposed mice (~50±5%). Aortic sensitivity to PE was greater in ETS-exposed mice compared with all other groups perhaps due to underlying endothelium dysfunction. To elucidate the role of potential reactive electrophiles in ETS responsible for endothelial dysfunction, the presence of acrolein-protein adducts was determined by Western blotting in lysates of lung tissue and plasma. In ETS-exposed mice the quantity of acrolein-protein adducts in lung (~700±75 and ~150 kDa) and plasma (~150 kDa) was significantly increased compared with air-exposed animals. These data support the hypothesis that acrolein in ETS is delivered systemically and could directly cause endothelial dysfunction, a condition prevented by addition of a thiol aldehyde scavenger. This work supported by PO1 ES11860 (AB) and a Phillip Morris grant (AB).

LEVERAGING NONCLINICAL DISEASE MODELS FOR EARLY PERSPECTIVE ON SAFETY AND RISK DURING DRUG DISCOVERY.


Preclinical toxicology contributes to ~70% of compound failure during the drug discovery process, suggesting that approaches that reduce attrition due to pharmacology or chemistry can lead to successful selection of candidate drugs. The use of animal disease models to test for toxicity presents a unique opportunity for toxicologists to explore liabilities early in the drug discovery process. Rodent models, including tumor xenograft, metabolic, and inflammation models are especially attractive for combined efficacy/toxicology testing due to their repeat-dose testing paradigm and study duration. Thus, this forum will highlight the successes and challenges in the use of nonclinical disease models to evaluate safety endpoints. Thorough parallel testing in xenograft tumor models that includes acute and sub-chronic dosing paradigms, MTD determination, histological evaluation of tumors and normal tissue, and DMPK profiling can inform on both safety and efficacy of lead compounds. In addition, information obtained from such studies can often be applied toward a biomarker strategy or dose-scheduling plan for dose-prediction and clinical development. Metabolic and chronic inflammatory disease models have altered physiology needs to be considered, as this may modulate the toxicological response. A robust analysis of the impact of disease phenotype on toxicity should be based on a reductive mechanistic model of an aspect of the human disease that has
common mechanisms with nonclinical models. Creative ways to generate early tol-
erability data for CNS molecules include an understanding of target pharmacology and interactions with other receptors, ion channels, and transporters. Behavioral or physiological changes (e.g., altered activity, stereotype, body temperature) can be evaluated in early pharmacokinetic studies. Combinations from pharmacology models, behavioral, and pharmacokinetic data can provide an inte-
grated assessment for potential CNS side effects.

**656 ROLE OF REGULATORY COOPERATIVE EFFORTS IN FOOD PROTECTION.**

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Chemical and microbial risk assessments are widely used in food safety decision making, in identifying data needs, and in implementing the Hazard Analysis and Critical Control Point (HACCP) Program. The HACCP Program is an excellent example of a collaborative effort between the U.S. Department of Agriculture and the Food and Drug Administration. Collaboration among all food safety bodies, including state, federal and global partners provides a robust system for continued protection and preparedness of the food protection system. The chemical and mi-
crobial risk assessment communities benefit from this on-going collaboration and cooperation. Collaboration has led to advancements in the food safety information infrastructure, data mining, data sharing and the development of sophisticated risk assessment models (risk assessments, vulnerability assessments, etc.) to guide the creation of preventive measures as part of food protection efforts. Leaders from state, federal and international bodies will discuss cooperative and innovative ap-
proaches for chemical and microbiological risk assessments in order to provide the safest food supply to the consumer.

**657 WEIGHT OF EVIDENCE ADVANCES IN RISK ASSESSMENT: CONCEPTUAL FRAMEWORKS AND CASE STUDIES ILLUSTRATING FUNDAMENTALS OF APPLICATION.**

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Chemicals interact with biological targets ultimately at the molecular level produc-
ing key, necessary and causative events. Identification and quantification of these events leads to understanding of a toxicant’s mode of action (MOA). Weight of evi-
dence (WOE) approaches consider relevant scientific data, underlying assump-
tions and correlations to characterize the overall support for a hypothesized MOA.

Frameworks for WOE analyses for MOA in animals and their associated human relevance (HR) have been developed by the International Life Sciences Institute (ILSI) and International Programme on Chemical Safety (IPCS). Information re-
viewed as a basis for consideration of the WOE of MOA and its HR can be applied to refine the dose-response relationship and with sufficient data may be used in the derivatio

d of Chemical Specific Attention Factors (CSAF), as outlined in the guidance developed by the IPCS. This includes using PBPK modeling to develop quantitative data to replace default assumptions for inter- and intra-species differ-

ces in tissue dosimetry. The principles for WOE considerations embodied in the HRF can potentially be extended to other areas to refine human health risk assess-

cm. Characterizing the MOA and determining its relevance to the human sys-
tem provides the foundation for developing non-default, CSAFs for application in health risk assessment. Therefore, it is important to provide a forum for discussion on how one might extend the framework into other areas with chemical specific case studies that use the MOA-HRF for analyzing cancer mode of action in ani-
mals, characterizing human dose-response and development of a CSAF.

**658 GRANTSMANSHIP FORUM: TOOLS AND SKILLS NEEDED TO NAVIGATE TOXICOLOGY RESEARCH FUNDING.**

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Toxicology research at academic institutions is supported by various extramural re-
search funding mechanisms, of which the most common are research grants and fellowships. These research grants can be obtained either by investigator-initiated, generally unsolicited, or in response to research funding announcements by various funding agencies. Traditionally, the major research support for understanding the impact of toxic substances on public health is supported by the National Institute of Health (NIH) and its 26 different Institutes or Centers. While the National Institute of Environmental Health Sciences (NIEHS) supports toxicology research efforts to understand the impact of environmental pollutants, the National Institute of General Medical Sciences (NIGMS) supports research grants for a wide variety of agents including pharmaceuticals. Some of the federal agencies, such as the National Science Foundation, support research in the areas of environmental biology. Numerous non-profit organizations including the Pharmaceutical Research and Manufacturers of America (PhRMA) Foundation also provide re-
search grant support, starting from pre-doctoral to sabbatical opportunities in phar-
macology, toxicology and informatics. A representative Program Director from NIGMS, NIEHS, NSF and PhRMA Foundation will present the opportunities, tools, and skills needed for successful research funding. In highlighting this impor-
tant funding opportunity available, one presentation will focus exclusively on suc-
cessful grant writing noting specific requirements such as the correct mix of scien-
tific knowledge and salesmanship to enable your to navigate NIH funding.

**659 BIOMARKERS OF CARDIAC HYPERTROPHY AND SKELETAL MUSCLE TOXICITY – SUCCESSES AND CHALLENGES RELATED TO THEIR IMPLEMENTATION IN DRUG DEVELOPMENT.**

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Drug-related injury to cardiac and/or skeletal muscle is a common cause of safety-related attrition in drug development, and has resulted in the withdrawal of several efficacious pharmaceutical agents from the market. Improving our ability to detect muscle injuries should improve patient safety. Case studies are presented on suc-
ces and challenges related to the implementation in drug development of soro-
logical biomarkers of skeletal muscle necrosis, including differentiation of injury to Type I versus Type II muscle fibers; cardiac myocyte injury, including comparisons of the performance of cardiac troponins with other serological biomarkers and histopathology; and cardiac hypertrophy, including serological concentrations of natriuretic peptides, and their relationship to hemodynamic and structural changes in the heart. Scientific challenges that limit the broader application of these bio-
markers are also addressed in a presentation on the changes in muscle structure and biochemistry that are driven by physiological and pathological processes, including those related to exercise, muscle atrophy, and drug toxicity in human muscle.

**660 THE REGULATORY FRONTIER: ADDRESSING PRODUCTS OF NANOTECHNOLOGY.**


Nanomaterials, typically defined as manufactured materials that have at least 1 dimen-
sion <100 nanometers, are being increasingly produced worldwide. Nanomaterials have been used or proposed for use in a variety of products, ranging from computers, clothing, cosmetics, medical devices, coatings and fuel cells, to new technologies for environmental clean up. Use is expected to increase and it is estimated that by 2015 about 10% of output from the chemicals sector will have some influence from nanotechnology, greatly increasing opportunities for human exposures. There has been some evaluation of potential health risks from nanoma-
terials, but to date, these have not been pursued in a systematic way. Nanomaterials pose new challenges and opportunities to the regulatory and policy framework prior to widespread use and adoption. This symposium will present an overview of nanomaterials, the current state of regulations and policies for addressing nanomaterials and a discus-
sion of how various entities propose the government move forward to address nanomaterials.
III 661 DIOXIN, FORTY YEARS OF SCIENCE: ARE WE ANY CLOSER TO ASSESSING POTENTIAL RISK?
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The word dioxin conjures up fear whenever another article appears in the media. Since the 1970s, there have been scientific publications reporting the potential environmental and human health hazards of exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; dioxin) and possible mechanisms of toxicity. Numerous epidemiological studies have reported on Vietnam veterans, residents of towns in Italy and Missouri as well as workers in plants that produce the substances containing TCDD. Identification of possible health hazards has been the basis of toxicological research with an examination of possible reproductive effects, immunotoxicity, hepatic effects, cancer and a number of endpoints. Early mechanistic work identified the Ah receptor (AhR) and the role of this receptor in toxicity and changes in metabolic processes. AhR receptor research and the awareness that there is likely to be endogenous and naturally occurring AhR ligands that govern normal physiological processes has shed new light on the significance of the AhR. The EPA report of the 1980s examined the extent of environmental contamination leading to greater interest in environmental analysis of dioxin and the potential for accumulation into the food chain. However, because of regulatory and voluntary efforts subsequently introduced, the levels of dioxin in the environment and food chain have significantly declined. The margin of exposures should continue to increase as additional controls on dioxin emissions are enacted and environmental levels dissipate. The available scientific data have improved our understanding of the strengths and weaknesses of toxicity equivalency factors used in dioxin risk assessment. The NAS- and EPA-established dioxin reassessment identified a number of important uncertainties and errors of how dioxin risks were managed. This session will focus on the weaknesses of toxicity equivalency factors used in dioxin risk assessment. The NAS and EPA will discuss the environmental and human health and environmental effects of this particularly toxic compound.

D. Shaughnessy, DERTIS/PHB, NIEHS, Research Triangle Park, NC. Sponsor: M. Smith.

The NIH established the Genes, Environment, and Health Initiative (GEI) in 2006 with a goal of establishing a foundation for large-scale gene-environment interaction studies. A central component of this initiative is the Exposure Biology Program, led by the National Institute of Environmental Health Sciences in collaboration with the National Cancer Institute, National Heart, Lung, and Blood Institute, National Institute for Drug Abuse, and other NIH Institutes and Centers. The Exposure Biology Program aims to develop a new generation of tools for comprehensive exposure assessment. These tools stem from the efforts of four complementary program areas focused on (1) the detection of individual exposures to traditional environmental toxicants, assessing psychosocial stress and addictive substances, assessing diet and physical activity, and measuring biological responses to these factors. This activity is focused on the development and validation of new tools, approaches, and biomarkers that will enable fundamentally new directions in environmental epidemiology and the exploration of gene-environment interactions. An overview of the GEI Exposure Biology Program will be presented by grantees from the Biological Response Indicators Program. The evolution of these new biomarkers and biosensors and how they may be used to assess early but persistent changes in key physiological pathways known to be involved in disease pathogenesis will be the primary focus. In addition, the application of new technologies and methodologies for improved detection of patterns of response to different chemical and lifestyle stressors in pathways that include oxidative stress, inflammation, and DNA damage will be highlighted. Presentations will cover transcriptomic and DNA adduct markers of tobacco smoke exposure, epigenetic alterations in breast stem/progenitor cells from endocrine disrupting chemicals, gene expression patterns from specific PCB congeners, and biosensors for detecting protein adducts and genetic damage resulting from chemical exposures.

N. H. Zawia, Biomedical, University of Rhode Island, Kingston, RI.

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder whose clinical manifestations appear with advancing age. One of the pathological hallmarks found in brains of AD patients is a buildup of extracellular amyloid plaques that are rich in beta amyloid which is derived from the amyloid precursor protein (APP). Studies have shown that beta amyloid is a metalloprotein which binds zinc (Zn), copper (Cu) and iron (Fe). Exposure to low levels of lead (Pb) in early life has been linked to abnormal regulation and expression of APP, possibly by the reprogramming of APP expression. An epigenetic study of Pb-exposed subjects and work on beta-amyloid clearance from the brain following Pb exposure also provides evidence for a possible role for Pb in the etiology of AD. Manganese (Mn) exposure in primates has been recently shown to result in diffuse beta-amyloid plaques in the frontal cortex of young non-human primates. Experts in metal toxicology, neurotoxicology, and environmental epidemiology, who have performed pioneering work to address this newly emerging research area in metal neurotoxicology will address these issues.

H. Hu, School of Public Health, University of Michigan, Ann Arbor, MI.

Lead is well-known to toxicologists and the public health community as a potent neurobehavioral toxicant in children. More recently, a critical mass of studies has developed using molecular and biomarker epidemiology to demonstrate that, if the dose received is chronic over many years, lead is both a potent neurobehavioral toxicant in community-exposed adults as well as a trans-generational neurotoxicant resulting from the mobilization of maternal skeletal lead stores during pregnancy. This presentation will review this recent work, emphasizing the multi-disciplinary study conducted by the Michigan-Harvard Metals Epidemiology Research Group on the Normative Aging Study (NAS) and the Early Life Exposures in Mexico to Environmental Toxictants (ELEMENT) Study. Attention will be particularly focused on studies demonstrating that (A) cumulative lead exposure assessed by K- or X-ray fluorescence (KXRF) measures of lead in bone are associated with functional deficits in cognition that are (i) modified by genetic polymorphisms related to the regulation of iron; and (ii) associated with evidence of gliosis in the hippocampus as reflected by in-vivo-injected to create rats measured by proton magnetic resonance spectroscopy (MRS); (B) prenatal lead exposure results in neurobehavioral deficits that are long-lasting; (C) preliminary epidemiologic analyses demonstrating that lead exposure is inversely associated with global DNA methylation within ALU elements, reducing DNA methylation and underscoring the potential for lead exposure to result in epigenetic changes that may contribute towards Alzheimer’s pathology.

N. H. Zawia, BPS, University of Rhode Island, Kingston, RI.

The sporadic nature of most AD cases strongly argues for an environmental link that may drive AD pathogenesis; however, it is not clear when this may occur. This presentation will present evidence that exposure to Pb in early life promotes AD-like pathogenesis in old age and will argue for both an environmental link and a developmental-basis for AD. Furthermore, the epigenetic mechanisms involved as well as potential novel therapeutic approaches will be discussed.

W. Zheng, Purdue University, West Lafayette, IN.

Accumulation of beta-amyloid peptides (Abeta) in brain extracellular fluids is the key event in the amyloid cascade leading to neuronal cell damage in the etiology of Alzheimer’s disease (AD). Excess amounts of extracellular Abeta may result from an overproduction, inadequate metabolic clearance, and/or imbalanced import and export of Abeta by brain barrier systems. The choroid plexus (CP) constitutes a barrier between the blood and cerebrospinal fluid (CSF). Clinical evidences have shown elevated Abeta levels in the CSF of AD patients, a marked deposition of Abeta in the CP of postmortem AD brains, and an age-related buildup of lead (Pb) in human CP. To understand relations between CSF Abeta, CP functions and Pb exposure, we examined (1) kinetics of Abeta uptake and transport by rat CP (2) proteins that are critical to Abeta degradation and transport in the CP; and (3) Abeta accumulation in the CP as the consequence of acute Pb poisoning. Results show that the CP accumulates Abeta with 3 distinct characteristics: (a) CP takes up small fragments; (b) the uptake occurs rapidly and by a non-diffusive process; and (c) the CP has a large capacity in accumulating Abeta. In Transwell transport studies with primary CP cells, the efflux transport of Abeta is a non-diffusive process; and c) the CP has a large capacity in accumulating Abeta. This newly emerging research area in metal neurotoxicology will address these issues.

S 664 EVIDENCE FOR LEAD AS AN ENVIRONMENTAL STRESSOR OF ALZHEIMER’S DISEASE AND THE ROLE OF EPIGENETICS.

H. Hu, School of Public Health, University of Michigan, Ann Arbor, MI.

Lit is well-known to toxicologists and the public health community as a potent neurobehavioral toxicant in children. More recently, a critical mass of studies has developed using molecular and biomarker epidemiology to demonstrate that, if the dose received is chronic over many years, lead is both a potent neurobehavioral toxicant in community-exposed adults as well as a trans-generational neurotoxicant resulting from the mobilization of maternal skeletal lead stores during pregnancy. This presentation will review this recent work, emphasizing the multi-disciplinary study conducted by the Michigan-Harvard Metals Epidemiology Research Group on the Normative Aging Study (NAS) and the Early Life Exposures in Mexico to Environmental Toxictants (ELEMENT) Study. Attention will be particularly focused on studies demonstrating that (A) cumulative lead exposure assessed by K- or X-ray fluorescence (KXRF) measures of lead in bone are associated with functional deficits in cognition that are (i) modified by genetic polymorphisms related to the regulation of iron; and (ii) associated with evidence of gliosis in the hippocampus as reflected by in-vivo-injected to create rats measured by proton magnetic resonance spectroscopy (MRS); (B) prenatal lead exposure results in neurobehavioral deficits that are long-lasting; (C) preliminary epidemiologic analyses demonstrating that lead exposure is inversely associated with global DNA methylation within ALU elements, reducing DNA methylation and underscoring the potential for lead exposure to result in epigenetic changes that may contribute towards Alzheimer’s pathology.

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protein and beta-secretase, and in Abeta degradation, i.e., insulin degrading enzyme (IDE1), endothelin-converting enzyme-1 (ECE1), and nephrin. After rats were injected ip with 50 mg Pb/kg, an evident increase in Abeta stains in the CP tissues was observed at 24 hrs. Acute Pb exposure also mobilized the low density lipoprotein receptor protein-1 (LRP1) from CP cytosol to the apical surface facing the CSF. The data suggest that the CP selectively cleanses Abeta from the CSF, potentially reducing Abeta from the brain. Pb exposure may alter these functions, leading to a halted clearance of Abeta by the CP and a subsequent elevation of Abeta in the CSF.

**S 667 INCREASED BRAIN AMYLOID STAINS IN NON-HUMAN PRIMATES FOLLOWING CHRONIC MANGANESE EXPOSURE: A POSSIBLE RELATIONSHIP TO AD**

T. R. Guilarte, School of Public Health, Johns Hopkins University, Baltimore, MD.

Recent studies from our laboratory have shown that chronic exposure to manganese (Mn) produces increased levels of amyloid-beta precursor-like protein-1 (APLP1), a member of the APP family, and diffuse amyloid-beta plaques in the frontal cortex of non-human primates. These changes in APLP1 and amyloid-beta were associated with neuronal degeneration including hypertrophic nuclei, intracytoplasmic vacuolization, increased silver staining and apoptosis. These novel findings suggest that chronic Mn exposure dysregulates the APP processing leading to increased vacuolization, increased silver staining and apoptosis. These novel findings suggest that chronic Mn exposure dysregulates the APP processing leading to increased vacuolization, increased silver staining and apoptosis.

**S 668 EARLY-LIFE EVENTS MAY TRIGGER BIOCHEMICAL PATHWAYS FOR ALZHEIMER’S DISEASE: THE “LEARN” MODEL**

D. K. Lahiri, Indiana University School of Medicine, Indianapolis, IN. Sponsor: N. Zawia.

Alzheimer’s disease (AD), the most common form of dementia among the elderly. In addition to genes, environmental factors, including diet, metals and lifestyle, play an important role in the development of disease phenotype. To explain the etiology of AD, we have recently proposed and tested the “Latent Early Associated Regulation” (LEAR) model, which postulates latent expression of specific genes triggered at the developmental stage. This model integrates both the neuropathological features and environmental factors associated with the disease. The LEARn model operates through the regulatory region (promoter) of the gene and by affecting the methylation status within the promoter of specific genes.

**S 669 EPIGENETIC IMPLICATIONS TO TOXICOLOGY.**

R. Rasoulpour1, K. Gabrielson2, J. Goodman3, M. Costa4, R. Jirtle4, J. Herman5, S. Ho6, and C. Harris7. 1The Dow Chemical Company, Midland, MI, 2Michigan State University, East Lansing, MI, 3New York University School of Medicine, New York, NY, 4Duke University, Durham, NC, 5Johns Hopkins University, Baltimore, MD, 6University of Cincinnati, Cincinnati, OH and 7National Institutes of Health, Bethesda, MD.

The emerging field of epigenetics may profoundly impact the future of toxicology. Epigenetics can be defined as heritable changes in gene expression that do not involve genetic mutations and are propagated without continued stimulus. Discrete chemical modifications of the chromatin can regulate gene expression or repression and can be transmitted to daughter cells or future generations due to epigenetic memory. Although potentially reversible, these heritable changes may be classified as transgenerational, mitotic, or meiotic, implicating the wide-ranging impact of epigenetic control in cellular function. These epigenetic processes play fundamental roles in cell proliferation, differentiation, cancer development and toxicities. Epigenetic processes that occur in the cell include DNA methylation/demethylation at CpG islands, small nuclear RNA processes and protein acetylation/deacetylation. Understanding how these modifications are inherited from mother cells to daughter cells or from an organism to its progeny remains a major scientific challenge. Recently, there has been a growing concern that epigenetic events may play a role in chemo- and/nutritionally driven adverse health effects, with particular focus toward reproductive toxicity and non-genotoxic carcinogenesis. For example, changes in DNA methylation which target tumor suppressor DNA and repair genes for silencing is a well established and validated step in cancer etiology. Overall, although the current literature consists of relatively few studies, there has been considerable interest by the popular press, government agencies, and the scientific community. Therefore, it is important to provide an introduction to epigenetic mechanisms and to highlight the current state-of-the-science in epigenetic toxicology.

**S 670 EPIGENETIC MECHANISMS OF NICKEL ION CARCINOGENESIS.**

M. Costa and H. Chen, NYU School of Medicine, New York, NY.

Water insoluble nickel (Ni) compounds are carcinogenic due to delivery of high concentrations of Ni ions into cells by phagocytosis and dissolution. The major target of Ni ions in cells is the iron–, ascorbate–, oxoglutarate-dependent dioxygenase enzymes. Examples of these enzymes include the HIF prolyl hydroxylases, the AlkB DNA repair enzyme homolog included the FTO and the newly-discovered histone H3 demethylase. The specificity in Ni inhibition is because all these enzymes contain loosely bound Fe within ±2-His-1-carboxylate triad termed in some enzymes the [M][C] domain. Since Fe binding to this domain is required for catalytic activity and Ni ions readily displace Fe from all of these enzymes, Ni exposure results in essentially inactive enzyme. Ni ions were found to induce global DNA and H3K9 methylation by inhibiting the H3K9 histone demethylase of which JHDM2A is an example. Preincubation of recombinant JHDM2A with 5 μM Ni ions essentially inactivated the enzyme since even 2000 μM Fe ions were not able to displace the Ni ions from the active site of the enzyme. A ratio of Ni ions to enzyme molecules of >1:1 was required for complete inhibition. Since Ni ions are one of the least toxic metal ions, this ratio was easily achievable at non-toxic levels. Transfection of flag-tagged JHDM2A resulted in a loss of H3K9 Dimethylation which was prevented when cells were treated with Ni ions, indicating that this enzyme was a target of Ni ions in intact cells. Ni ion exposure as well as knockdown of JHDM2A resulted in the down-regulation of a number of genes which remained low in expression for as much as 25 days after Ni ions were removed from the media, suggesting that there is epigenetic inheritance of gene expression changes brought about by Ni ion exposure. The genes most strongly downregulated by Ni ion exposure had greater H3K9 Dimethylation changes in their promoter based upon Chip assays. Chip on chip using H3K9 di me and H3K4 tri me antibodies mapped the coordinates on the promoter of genes that were up or down regulated and these changes were correlated with down regulation (H3K9 di me) or up regulation (H3K4 tri me) marks in their promoters.

**S 671 EPIGENETICS: THE NEW GENETICS OF TOXICOLOGY.**

R. Jirtle, Department of Radiation Oncology, Duke University, Durham, NC.

This presentation will be in two parts: 1) present data on a recently developed computer-learning algorithm that predicts 600 imprinted genes in mice versus 156 in humans with a mere 30% overlap between the two; and, 2) show data on how in utero internal dietary supplements with various compounds cause an alteration in expression of the metastable Aty allele as a sensor for epigenetic changes.

**S 672 EPIGENETIC REGULATION IN DEVELOPMENT: IMPLICATIONS FOR STEM CELL BIOLOGY AND TOXICITIES.**

J. Herman, Department of Oncology, Johns Hopkins University, Baltimore, MD. Sponsor: K. Gabrielson.

Epigenetic mechanisms are important in cancer stem cell biology. Environmental toxins such as NNI have been shown to alter DNA methylation patterns. This presentation will review the epigenetic mechanisms involved in gene expression modulation of cancer stem cells by alkylating agents including NNI, a nitrismine constituent of cigarette smoke.

**S 673 IS THERE A COMMON “E-EPIGENOME”?**

S. Ho, Department of Environmental Health, University of Cincinnati, Cincinnati, OH.

Estrogen and estrogen mimics are now known to have distinct epigenetic effects on various reproductive end organs. Focusing on DNA methylation changes induced by the natural estrogen or its mimics in the environment, we asked the question of whether there is a common set of genes whose methylation status is affected by estrogens/estrogen mimics in a convergent manner that transcends tissue specificity. The effects of estradiol-17β, bisphenol A, diethylstilbestrol, soy-estrogens were investigated for their epigenetic effects on the prostate, uterus, and breast to determine if an “e-epigenome” could be identified and/or constructed.
674 MICRORNA EPIGENETIC REGULATION


Infection and chronic inflammatory response contribute to about 1 in 4 of all cancer cases. Mediators of the inflammatory response, e.g., cytokines, free radicals, prostaglandins and growth factors, can induce epigenetic changes (1) influencing tumor suppressor gene expression. Recent discovery of an interaction between microRNAs and innate immunity during inflammation has further strengthened the association between inflammation and cancer. This presentation will review microRNA molecules as they regulate gene expression important in the pathogenesis of toxicities and carcinogenesis.

675 IMMUNOMODULATION DURING COMPLEMENTARY AND ALTERNATIVE MEDICINE (CAM) THERAPY: RISKS AND BENEFITS

P. S. Nagarkatti. Pathology, Microbiology and Immunology, University of South Carolina School of Medicine, Columbia, SC.

There are over 40 million Americans who suffer from some form of degenerative disease and it is estimated that approximately one third of them will attempt using complementary and alternative medicine (CAM) to alleviate pain and suffering. Of the many different CAM therapies, the use of plant products remains popular. However, there is lack of sufficient experimental and clinical proof that they are safe and effective. In 1998, the National Center for Complementary and Alternative Medicine (NCCAM) was established by the U.S. Congress as one of the NIH Institutes to provide funds to investigate if the popular CAM modalities are truly beneficial. In the United States, while drugs must be approved by the FDA as being safe and effective before they can be sold, the FDA is not authorized to evaluate the safety or efficacy of dietary supplements. However, the FDA can ban the sale of supplements that are shown to be unsafe. On the other hand, the immunosuppressive properties of plant products, if found safe, can also be used to develop new therapeutic modality against inflammatory and autoimmune diseases. Herbal and plant-derived compounds are widely available in the market that claim to sustain, restore or enhance immunity. To begin addressing this issue an overview of CAM therapy with an emphasis on herbal and plant-derived compounds and their potential risks/benefits will be highlighted for their immunomodulatory properties. Benefits versus the risks of using certain plant-derived products that constitute CAM will be discussed including Cat’s Claw, Echinacea, Ginseng, Thunder God Vine, Aristolochia, Kava, Ephedra and St. John’s Wort. (Supported in part by NIH grants R01-DA016545, R01-ES09098, R01-AI053703, R01-AI058300, R01-HL058641, and P01-AT003961).

676 IMMUNE MODULATION BY PLANT-DERIVED CANNABINOID COMPOUNDS

B. L. Kaplan, 1, 2 and N. E. Kamindu, 1, 2. 1Center for Integrative Toxicology, Michigan State University, East Lansing, MI and 2Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI.

Cannabinoids are a group of more than 60 plant-derived compounds from the plant Cannabis sativa, more commonly known as marijuana. Use of marijuana for medicinal purposes is an ongoing debate in the United States, although the mechanisms by which it exerts any therapeutic benefits are not fully elucidated. Further complicating our understanding of the risk/benefit ratio of medical marijuana use is the demonstration that many plant-derived cannabinoid compounds, such as Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD), also modulate immune function. For example, CBD either stimulated or suppressed interleukin-2 (IL-2) or interferon-γ (IFN-γ) production from primary mouse splenocytes depending on the magnitude with which the leukocytes were stimulated. The objective of these studies was to characterize the molecular mechanisms by which plant-derived cannabinoid compounds modulate immune function. Using phorbol ester plus calcium ionophore (PMA/IO) to stimulate the splenocytes, bacterial lipopolysaccharides (LPS) and medium (no LPS) alone, CBD suppressed cytokine release. In contrast, CBD enhanced IL-2 and IFN-γ steady state mRNA levels in splenocytes stimulated “suboptimally” (4 nM PMA/0.05 μM LPS). Concordant with this, NFAT transcriptional activation was either suppressed or enhanced by CBD when human Jurkat T cells were stimulated optimally or suboptimally, respectively. Interestingly, CBD enhanced intracellular calcium in resting cells, which was further enhanced with PMA/IO regardless of the magnitude of stimulation. Overall, these results suggest that the plant-derived cannabinoid, CBD, might affect immune function differentially depending on the magnitude with which the immune system is stimulated.

677 MECHANISMS OF RESVERATROL-INDUCED IMMUNOMODULATION AND ITS POTENTIAL USE IN THE TREATMENT OF INFLAMMATORY AND AUTOIMMUNE DISEASES

P. S. Nagarkatti and M. Nagarkatti. Pathology, Microbiology and Immunology, University of South Carolina School of Medicine, Columbia, SC.

Resveratrol (3,4′-dihydroxystilbene), which is found in many plants, including mulberries, red grapes and peanuts, possesses anti-cancer and anti-inflammatory properties. Recent studies from our laboratory have demonstrated that resveratrol activates Akt and ERK thereby triggering apoptosis in activated T cells. In vitro studies on the mechanism of action revealed that resveratrol triggered high levels of apoptosis in activated T cells and to a lesser extent in unactivated T cells. Moreover, resveratrol-induced apoptosis was mediated through up-regulation of AβR, Fas, and FasL expression. In addition, resveratrol-induced apoptosis in primary T cells correlated with cleavage of caspase-8, caspase-9, caspase-3, poly(ADP-ribose) polymerase, and release of cytochrome c. In vivo, resveratrol was also found to affect the induction of Th17 cells while increasing the generation of FoxP3+ Treg. We have shown that resveratrol can suppress a wide range of inflammatory disorders including the murine model of multiple sclerosis, known as experimental autoimmune encephalomyelitis (EAE). Resveratrol administration also led to significant down-regulation of certain cytokines and chemokines in EAE-induced mice including tumor necrosis factor-α, interferon-γ, interleukin (IL)-2, IL-9, IL-12, IL-17, macrophage inflammatory protein-1alpha (MIP-1α), monococyte chemoattractant protein-1 (MCP-1), RANTES, and Eotaxin. Resveratrol was also effective on a wide range of inflammatory disorders including bacterial super-antigen-induced lung injury and graft-versus-host disease. These studies demonstrate, for the first time, the ability of resveratrol to trigger apoptosis in activated T cells and its potential use in the treatment of inflammatory and autoimmune diseases including MS (Supported in part by NIH grants R01-DA016545, R01-ES09098, R01-AI053703, R01-AI058300, R01-HL058641, and P01-AT003961).

678 IMMUNOMODULATION BY N-3 POLYUNSATURATED FATTY ACIDS

J. Poku1, 2, 1Center for Integrative Toxicology, Michigan State University, East Lansing, MI and 2Microbiology and Molecular Biology, Michigan State University, East Lansing, MI.

Clinical studies suggest that consumption of n-3 polyunsaturated fatty acids (PUFAs) derived from marine sources and algal fermentation might be efficacious for both prophylaxis and treatment of inflammatory diseases. Although nearly 26 million Americans over 18 years of age currently consume n-3 PUFAs, mechanisms of action of these supplements remain incompletely understood. Critical gaps exist in our knowledge of how n-3 PUFAs attenuate immune gene expression and impact responses to pathogens. This gap impacts: (1) prediction of which inflammatory diseases n-3 PUFAs would be most effective; (2) knowledge of required n-3 PUFA therapeutic concentrations in tissues and corresponding dosages to achieve these; and (3) mechanism-based safety assessment of n-3 PUFAs. Recent studies in our laboratory have focused on the mechanisms by which n-3 PUFAs modulate abnormal pathologic and normal requisite IgA production in the mouse. n-3 ameliorative effects on pathologic IgA were addressed using an experimental IgA nephropathy model induced by feeding the mycoxin, deoxynivalenol (DON). Responses to gut mucosal infection by reovirus were used to gauge n-3 suppression of the normal IgA response. The results of these studies provide insight into therapeutic ratios for n-3 PUFA suppression of pathologic effects relative to impairment of normal immune response.

679 IMMUNOMODULATION BY 3,3′-DIINDOYL METHANETHANE

J. Biedlmann1 and J. Poku2, 1Department of Nutritional Sciences and Toxicology, University of California, Berkeley, Berkeley, CA and 2Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI.

3,3′-Diindolylmethane (DIM) is a natural isoflavonoid product present in food plants of the Brassica genus. The anticarcinogenic effects of DIM are well established in rodent models and the compound is currently used in several clinical trials. In a series of studies of the effects of DIM on immune function, we observed that DIM activated the interferon gamma (IFNγ) signal transduction pathway in human breast cancer cells. MCF-7 cells were found to express IFNγ mRNA and to secrete IFNγ protein into the culture medium, in response to DIM treatment. DIM treatment also activated the expression of the IFNγ receptor (IFNγR1) and the IFNγ responsive gene glutathione peroxidase 1 (GPX1). DIM also produced a synergistic activation of IFNγ of interferon-inducible-reporter constructs in transfected cells. Combined treatment with DIM and IFNγ produced a synergistic increase in level of MHC-I expressed...
on the cytosolic membrane, as well as increased levels of mRNAs of associated pro-
teins and transporters. In a subsequent study we used promoter deletion constructs
to determine that DIM can activate IFNγ expression by a mechanism that involves
stimulation of JNK and p38 stress signaling pathways and displacement of the neg-
ative regulatory factor, CREB, from a specific IFNγ promoter regulatory element.
Finally, in rodent studies we found that DIM induced proliferation of splenic lymp-
phocytes, augmented mitogen- and IL-2-induced splenic lymphocyte proliferation.
DIM also stimulated the production of ROS by murine peritoneal macrophage cul-
tures. Of note, some inhibition of DIM, but not intraperitoneal injection, induced ele-
vation of serum cytokines in mice, including interleukin (IL)-6, granulocyte-colony
stimulating factor (G-CSF), IL-12 and IFN-γ, enhanced both clearance of reovirus
from the GI tract and the subsequent mucosal IgA response. Thus, DIM is a potent
stimulator of immune function in both tumor cells and in the response of rodents
to immune challenge.

S 680 EOTAXIN-1 INHIBITION BY 7, 4-DIHYDROXY FLAVONE ISOLATED FROM GLYCRRHIZA URALENSIS.

X. Li. Department of Pediatrics and Immunobiology, Mt. Sinai School of Medicine,
Eosinophilic inflammation plays an important role in pathological mechanisms of
asthma. Eotaxin, a CC chemokine, stimulates the migration of eosinophils from
blood into the lungs by acting on the CC chemokine receptor CCR3. The human lung
fibroblast cell line (HFL-1 cell), which produces eotaxin, has been widely used to
investigate of mechanisms of inflammation and anti-inflammatory therapy.
There is an increasing interest in investigating botanical products for treatment of
allergy and asthma. In a controlled clinical study, we showed that an anti-
asthma herbal medicine intervention (ASHMI) significantly improved lung function,
reduced symptom scores and reduced beta-2 agonist use. We recently showed that
ASHMI suppressed eotaxin production by HFL-1 cells. Glycyrrhiza uralensis (G.
uralensis) Chinese licorice (Gan-Cao), is one of the most commonly used herbs in
Traditional Chinese Medicine and is a constituent in ASHMI. Previous study showed that
glycyrrhizin, a compound isolated from G. uralensis had an inhibitory effect on eotaxin production. Here we report the structure and biological activities of
flavonoids isolated from G. uralensis, and compare the potency of these
flavonoids with glycyrrhizin. We demonstrated that 7, 4-dihydroxy flavone from G.
uralensis suppressed eotaxin production in vitro and in vivo. Eosinophilic inflammation
in a murine model of allergic asthma. Studies on pure compounds will enhance
our understanding of the pharmacological mechanisms G. uralensis and may lead
to a novel therapy for asthma and perhaps other inflammatory diseases.

S 681 NANOTOXICOLOGY AND DRUG DELIVERY.

C. Samps and R. Chapin. Drug Safety, Pfizer Global R&D, Groton, CT.
The biopharmaceutical industry is looking at the rapidly developing, multifaceted
field of nanotechnology as an opportunity for improved approaches to drug
development. A good example is the ongoing effort to exploit the unique physical and
chemical properties of nanoscale materials for the purpose of improved drug deliv-
ery. Using nanoparticles with targeting ligands to precisely deliver a drug payload to
a specific diseased tissue, while by-passing all other parts of the body, would clearly
represent a game changing approach to drug development. However, before that fu-
ture vision can be realized, significant unknowns and gaps in our understanding of
the toxicology of nanoscale drug delivery platforms will need to be addressed.
Academic and industry researchers, as well as government regulators, interested in
the unique safety issues confronting drug developers will explore the use of nano-
materials for improved drug delivery. The program will consider the design and de-
velopment of nanomaterials compatible with the unique requirements for drug
delivery and therapy, focusing on material distribution and safety when intentionally
delivered into physiologic systems. Special emphasis will be placed on properties
that influence absorption, distribution, metabolism and excretion of nanomaterials,
including immune system interactions, and properties that influence the toxicity of
nanomaterials and their degradation products. Finally, we will explore the FDA's
consideration of these novel materials and their impact on the safety evaluation of
candidate drugs or drug carriers. A nanoparticle's interaction with immune cells
and-receptor dependent targeting mechanisms. Nanotechnology can also eliminate
the hurdles for the toxic vehicles required for administration of hydrophobic drug
terpenes. Platforms presently undergoing evaluation include nanoemulsions, nanolipo-
somes, and metal, polymeric and carbon-based nanoparticles. Critical parameters
that can influence the success of nanotechnology platforms include stability, clear-
antage, and targeted toxicity. This presentation will specifically address the impor-
tance of the unique safety issues confronting drug developers and the unique challenges of monitoring the disposition and in vivo integrity of nan-
tenotechnology platforms, using preclinical and clinical case studies to demonstrate
the analytical methodologies and the pharmacokinetic models employed. Examples
will include radiolabel, elemental analysis, ELISA, I.C. and imaging techniques,
with resulting data analyzed by both compartmental and noncompartmental meth-
ods. Funded by NCI Contract N01-CP-12400.

S 682 DESIGNING FAVORABLE ELIMINATION FEATURES INTO NANOSCALE DRUG DELIVERY SYSTEMS.

P. J. Sinko, Rutgers University, Piscataway, NJ. Sponsor: C. Samps.
Our lab has been involved in the design, synthesis, characterization and evaluation of
targeted PEG-based nanocarriers for the treatment of AIDS and certain other
types of cancers (e.g., DCIS breast cancer). Nanoscale drug delivery allows for the
precise control of drug concentrations and exposure times in target organs, tissues
and in specific cell types. This is a major advantage since therapy is maximized (i.e.,
high local concentrations of drug can be achieved) while drug toxicity is minimized
(i.e., healthy tissues are not being exposed unnecessarily to drug). However, unlike
conventional dosage forms (e.g., capsules, tablets, injections where excipients are
absorbed into the body or are readily eliminated), the biological fate of the materials comprising
nanoscale delivery systems is not fully understood. The physical, chemical and bio-
chemical properties of biocompatible materials that are known or suspected to influ-
ence elimination from the body will be briefly reviewed and several case studies will
be discussed. Following this, our efforts to design biodegradable dimeric nanocarri-
ers will be described. These flexible nanocarriers have features that are counterintu-
itive to currently used PEGylation approaches (i.e., they have been designed to enter
into cells not remain in the blood, deliver their cargo then selectively degrade
into components that can be readily eliminated via the kidneys). Several features
will be discussed including target cell uptake, blood and target cell persistence, tox-
icity and effectiveness in in vitro and in vivo models. Finally, a detailed analysis of the
‘enhanced elimination’ design elements relating to the selective intracellular
degradation of the dimeric nanocarriers will be presented followed by results sup-
porting our hypothesis.

S 683 EVALUATION OF CANCER NANTHERAPEUTICS’ STABILITY AND DISPOSITION.

S. T. Stern. Nanotechnology Characterization Laboratory, SAIC-Frederick, Inc., NCI-
Frederick, Frederick, MD.
Nanotechnology is finding increasing application in cancer therapy. Nanotechnology-based drug delivery platforms offer the potential to rejuvenate tradi-
tional chemotherapeutics by improving safety and efficacy profiles. For example,
nanotechnology platforms can target drugs to tumor sites via passive mechanisms,
resulting from the inherent leakiness of the tumor vasculature, and via active lig-
and-receptor dependent targeting mechanisms. Nanotechnology can also eliminate
the hurdles for the toxic vehicles required for administration of hydrophobic drug
terpenes. Platforms presently undergoing evaluation include nanoemulsions, nanolipo-
somes, and metal, polymeric and carbon-based nanoparticles. Critical parameters
that can influence the success of nanotechnology platforms include stability, clear-
antance and tissue permeability. This presentation will specifically address the impor-
tance and challenges of monitoring the disposition and in vivo integrity of nan-
tenotechnology platforms, using preclinical and clinical case studies to demonstrate
the analytical methodologies and the pharmacokinetic models employed. Examples
will include radiolabel, elemental analysis, ELISA, I.C. and imaging techniques,
with resulting data analyzed by both compartmental and noncompartmental meth-
ods. Funded by NCI Contract N01-CP-12400.
SAFETY CONSIDERATIONS FOR THE REGULATION OF NANOMATERIAL-CONTAINING THERAPEUTICS.

N. Sadrieh, FDA, SilverSpring, MD.

Products containing nanomaterials are being investigated for potential applications as therapeutics. While some of these nanomaterials are nanoscale versions of larger materials used in approved products, other nanomaterials are novel and have never been used in drug products. Meanwhile, the regulatory requirements to ensure preclinical safety for products containing such novel materials, remain identical to those required for products that do not contain nanoparticles. There are various reasons why there are no "nano-specific" requirements at this time. One reason is that the preclinical studies currently required of sponsors are very comprehensive, and include many studies that measure endpoints that should pick up injury in most organs. Another reason is that, while some have suggested that the current preclinical screening tests might need to be tailored for nanomaterial-containing products, no one has clearly communicated in what respect the current requirements might be lacking in their capacity to assess safety concerns from nanomaterial-containing products, and what are other available tests that might improve the predictive value of the current regulatory preclinical requirements. Nevertheless, it is widely accepted that there are unique features associated with nanotherapeutic products, and these unique features may lead to future challenges for the development, manufacturing, safety evaluation and review of these products. These challenges are both for the sponsor of nanotechnology-based products, as well as for regulatory agencies, who must regulate these products. The FDA must ensure the safety and efficacy of nanomaterial containing products, yet, it must also promote innovation in order provide the public with access to the best medical products. With this goal in mind, the Agency is working on future guidance documents to help industry bring to fruition the promise of nanotechnology in drug applications.

LOW-DOSE NON-LINEARITY: WHAT CAN EMERGING TECHNOLOGIES TELL U.S.?

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Characterization of dose-response, as captured in the Paracelsus phrase, "The dose makes the poison", is a central tenant to the field of toxicology and risk assessment. Until recently, methodological limitations have prevented comprehensive examination of many of the fundamental biological phenomena underlying both toxicological responses and risk assessment assumptions at or below the low end of traditionally-accepted dose-response curves. Recently, a number of rapid advances in cellular, biochemical, toxicogenomic, and analytical technologies, however, are now offering new opportunities to more accurately and comprehensively characterize the nature of dose-response curves, including its shape in low-dose ranges that are more relevant to actual, real-world human exposures. To this end, and pursuant to the recent recommendations proposed by the National Academy/National Research Council Report and a joint SETAC-SOT sponsored Pellston Conference (NAS, "Applications of Toxicogenomic Technologies to Predictive Toxicology and Risk Assessment", 2007; DiGiulio and Benson, "Genomic Approaches to Cross-Species Extrapolation in Toxicology," 2007), the practice of applying emerging technologies to characterize toxicant-induced responses in the low-dose range and shape of the dose-response are now being realized. Importantly, application of these molecular-level technologies may allow a more complete and predictive analysis of dose responses to toxicants and/or associated risk assessment assumptions that are key to understanding human relevance of responses observed at the low end of the dose-response curve, i.e., decisions of whether to apply linear versus non-linear risk assessment approaches. Low-end dose-response analysis using emerging technologies on a number of divergent-acting compounds such as direct genotoxicants, cytotoxicants, and receptor-mediated- and undefined-acting toxicants, and will discuss the evidence for the existence, or lack thereof, of thresholds and non-linearity for genomic and other biological responses to xenobiotics will be addressed.

THE POTENTIAL OF GENOMIC DOSE-RESPONSE DATA TO DEFINE MODE-OF-ACTION AND LOW-DOSE BEHAVIOR OF CHEMICAL TOXICANTS.

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There is increasing acceptance within the toxicology community that high dose animal studies are not predictive of low dose risks in humans or even in the test animals themselves. New genomic technologies now provide a unique opportunity to evaluate the relevance of current low dose default assumptions used in chemical risk assessment and identify dose-dependent transitions in modes of action. In this presentation, we describe the application of benchmark dose analysis to gene expression microarray data collected following ninety day exposures to five different lung and liver carcinogens. The benchmark dose methods were used to estimate doses at which different cellular processes are altered and showed that benchmark dose values for certain processes mirrored the tumor response in a two-year rodent bioassay. The results show that dose-response changes in gene expression, when related to higher-order biological processes and pathways, reflect the dose dependent changes in key events in the carcinogenic process and can support nonlinear modeling of the events in the low dose region within the framework of a mode of action risk assessment.
adduct levels (NODAL) correspond closely to the doses that had no observable effect on the cells gene expression profile (NÖTEL). Analyses of similar in vivo experiments using Fischer 344 rats exposed to deceasing, non-tumorigenic doses of N-nitroso-N-methylurea (NNU) are in progress. Our findings have significant implications for the use of linear low dose extrapolations in risk assessment for genotoxic compounds and carcinogens, and suggest that toxicogenomic assays may provide useful endpoints for benchmark dose modeling.

W 691 LOW-DOSE GENOTOXICITY ASSESSMENT USING BIOMARKERS OF INTERNAL DOSE AND FLOW CYTOMETRY-BASED MICRONUCLEUS ASSAY. LOW-DOSE ACRYLAMIDE STUDY IN MICE.


Low-dose genotoxicity studies require robust measures of dose and response. Measures of internal dose based on PB-PK dosimetry, hemoglobin adducts or DNA adducts and a 'high content' flow cytometry-based micronucleus (MN) assay are useful for low-dose genotoxicity assessments. The MN response in mouse bone marrow, and the shape of the dose-response curve was evaluated in mice administered low doses of acrylamide. B6CF1 mice were dosed orally with acrylamide for 28 days using logarithmically spaced doses from 0.125-24.0 mg/kg/day, and micronuclei were assessed in peripheral blood reticulocytes and erythrocytes by flow cytometry. Liver glycidamide DNA adducts, and acrylamide and glycidamide N-terminal valine hemoglobin adducts were also determined. Acrylamide produced a weak MN response, with statistical significance at 6.0 mg/kg/day, or greater, in micronuclei were assessed in peripheral blood reticulocytes and erythrocytes by flow cytometry. Liver glycidamide DNA adducts, and acrylamide and glycidamide N-terminal valine hemoglobin adducts were also determined. Acrylamide produced a weak MN response, with statistical significance at 6.0 mg/kg/day, or greater, in micronuclei were assessed in peripheral blood reticulocytes and erythrocytes by flow cytometry. Liver glycidamide DNA adducts, and acrylamide and glycidamide N-terminal valine hemoglobin adducts were also determined. Acrylamide produced a weak MN response, with statistical significance at 6.0 mg/kg/day, or greater, in micronuclei were assessed in peripheral blood reticulocytes and erythrocytes by flow cytometry.

W 692 BIOLOGICAL THRESHOLDS AND NONLINEAR DOSE-RESPONSES FOR MULTIPLE ENDPOINTS IN METHYL METHANESULFONATE (MMS)-TREATED RATS.


The existence of thresholds for DNA reactive agents is a contentious topic. Using the well-characterized alkylating agent MMS, we sought to evaluate the relationship between dose (administered, systemic, and target), changes in liver transcriptome profile, and genotoxicity (peripheral blood micronuclei) in male Fischer 344 rats. Six animals per group were dosed orally for 4 consecutive days at 0 (DMSO vehicle), 0.5, 1, 5, 25, or 50 mg MMS/kg bw/day (naled). Characterization of systemic and liver doses was based on hemoglobin (HbP) and hepatic DNA adduct levels, respectively. N-terminal methyl (Me)-Hb adducts, quantified as signature peptides by HPLC/EIS-ESI/MS/MS, demonstrated linear dose-response. Analysis of hepatic DNA for O6- and N7-methylguanine (MeG) adducts as performed by LC/ESI-MS/MS identified N7-MeG adducts as NQ (not quantifiable; >6.8/109 nt and ≥2.0/109 nt), NQ, 2.9, 8.9, 30.9, 31.8, and 67.9/109 nt for control, 0.5, 1, 5, 25, and 50 mld, respectively. O-MeG adducts, however, were NQ (<2.0/109 nt) at any dose. Gene expression from hepatic RNA was evaluated, and under the experimental conditions used, there were no significantly altered genes in the 0.5, 1, or 5 mld dose groups when compared to vehicle control, whereas this number was 20 and 121 at 25 and 50 mld, respectively. Interestingly, the ratio of altered genes compared to systemic or liver adducts was nearly identical, suggesting optimal sensitivity to maternal toxicity apparently influenced fetal findings, as well as studies with severe maternal toxicity and few or no obvious effects on the offspring. Current regulatory views of the impact study design and interpretation and discussion of these areas in which more knowledge is needed will be addressed.

W 693 MATERNAL TOXICITY AND ITS IMPACT ON STUDY DESIGN AND DATA INTERPRETATION.

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Assessing maternal toxicity in DART studies is important because it can potentially influence the study’s outcome, thus impacting risk assessment and regulatory decisions. Some degree of maternal/parental toxicity is required in developmental and reproductive toxicity (DART) studies by regulatory agencies. However, excessive maternal/parental toxicity is a confounding factor in study design and data interpretation. There is no clear consensus on levels of toxicity that are high enough to meet regulatory requirements but low enough to avoid confounding data interpretation. In addition, there is a need to distinguish true toxicity from exaggerated pharmacology. It also appears that there may be some differences in species susceptibility to maternal toxicity, with the rabbit being more sensitive than the rat in certain cases. Finally, there are conflicting reports in the literature about the relationship between maternal toxicity and fetal abnormalities. Current views of the issues as they impact study design and interpretation and discussion of these areas in which more knowledge is needed will be addressed.

W 694 OVERVIEW AND BACKGROUND.

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Adverse effects on the offspring seen only at maternally toxic dosages may be caused by direct effects, indirect (maternally-mediated) effects, or a combination of the two. Effects on developing offspring can also result from maternal stress, as well as (or perhaps in addition to) maternal toxicity. Determining which, if any, of these alternatives is operative in a given study is difficult, typically requiring additional studies to even attempt to make a supportable determination. The issue is complex, and its interpretation can be subjective to bias. Thus it remains an area of controversy. Background information will be presented, together with examples of studies where maternal toxicity apparently influenced fetal findings, as well as studies with severe maternal toxicity and few or no obvious effects on the offspring. Current regulatory views of how fetal findings seen at maternally toxic dosages should be interpreted will also be addressed.

W 695 EXAGGERATED PHARMACOLOGY VERSUS TRUE TOXICITY.

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Maternal toxicity is important in setting doses and interpreting results from embryofetal toxicity studies. This is a particular challenge for therapeutic biologics that have a very targeted mechanism of action. Maternal effects may range from no effect at any dose, to effects at all doses, to maternal toxicity due to on-target exaggerated pharmacology. Examples including study designs, dose selection, and results will be presented.

Oxygen is essential for development, and periods of interrupted oxygen supply result in stage-specific malformations and embryonic death. Several drugs are believed to produce embryonic hypoxia and malformations due to vasoconstriction of uterine vessels on the maternal side. Antihypertensives, such as nifedine and felodipine, are associated with hypoxia-related teratogenesis via dilation of peripheral vessels and a diversion of blood flow from central (i.e., uterine) to peripheral vessels in the mother, resulting in a decrease in utero-placental blood. A large number of drugs (>100) have the potential to cause QT changes on the ECG and cardiac arrhythmia in adults of many species (e.g., humans, rabbits, and dogs, but not rodents) via blockade of the cardiac repolarisation ion channel, IKr. IKr seems to be very important for cardiac rhythm regulation in all species in embryonic life, including the mouse, rat, rabbit, and human. Reviewing scientific and regulatory teratology studies allows the following conclusions to be made: 1) IKr blockers cause embryotoxicity/ teratogenesis as a result of embryonic cardiac arrhythmia and hypoxia, 2) toxicity signs due to maternal cardiac arrhythmia occur in the rabbit at lower dosages than in rats because of species differences in IKr expression, 3) embryotoxicity by IKr blockers is often explained by observed maternal toxicity (e.g.,
Slight weight changes even when a direct effect on the embryonic heart is more likely, and 4) high incidences of embryonic death may mask the teratogenic potential of IKr blockers.

**W 696 RELATIONSHIPS OF MATERNAL AND FETAL WEIGHT CHANGES IN DEVELOPMENTAL TOXICOLOGY BIOASSAYS.**


Standard developmental toxicology bioassays are designed to identify agents with the potential to induce adverse effects in the embryo/fetus. Guidelines require the inclusion of a dose level(s) that induces "overt maternal toxicity". The common occurrence of dose levels at which both maternal and fetal toxicity has been observed has led to a number of attempts to characterize possible toxicological relationships between these endpoints. An analysis of 125 developmental toxicity bioassays in the mouse, rat, and rabbit conducted by the National Toxicology Program from 1982 to 2004 allows a number of general conclusions to be made: 1) most Lowest Observable Adverse Effect Levels (LOAELs) were determined by reduced maternal gestational weight gain or fetal weight at term, 2) maternal weight reductions are associated with reduced food intake for a variety of dissimilar test agents, 3) lower fetal weights were associated with reduced maternal weight gains late in gestation, and 4) the degree of fetal weight reduction is correlated with the extent of the maternal weight loss. In a substantial number of the studies, reduced fetal weight may, therefore, be due to maternal undernutrition caused by general toxicity rather than direct developmental insult. Consequently, such test agents may be erroneously classified as primary developmental toxicants. There are experimental approaches that can test the hypothesis that maternal undernutrition in standard developmental toxicology bioassays may be responsible for significant term fetal weight decrements.

**W 697 POSTNATAL CONSEQUENCES OF MATERNAL TOXICITY.**

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Exposures that cause human developmental toxicity in the presence of maternal toxicity include maternal alcoholism and therapy with methotrexate and some anti-convulsant medications. There is little evidence that the developmental toxicity is secondary to the maternal toxicity in these cases, but in some cases, the developmental effects appear to be exaggerations of the pharmacological effects of the medication. There are also examples of maternal illnesses that may be associated with developmental toxicity, including diabetes mellitus, hypertension, autoimmune disorders, and nutritional deficiencies. These illnesses may offer an opportunity to study the role of maternal toxicity in developmental toxicity.

**W 698 REGULATORY PERSPECTIVES AND CASE STUDIES.**

K. L. Davis-Bruno1 and W. A. Harrouk2. 1Division of Metabolism and Endocrinology Products, U.S. FDA, Silver Spring, MD and 2Division of Nonprescription Clinical Evaluation, Office of Nonprescription Products, U.S. FDA, Silver Spring, MD.

Maternal toxicity can be a confounding factor in DART study design and interpretation. Conflicting published literature exists in describing the relationship between maternal toxicity and fetal abnormalities in DART studies. The regulatory perspective, based on illustrative case studies will be presented, and interpretation of such findings relative to potential clinical relevance and risk will be discussed.

**W 699 PESTICIDE MIXTURES: EXPERIMENTAL EVALUATION AND COMPUTATIONAL MODELING.**

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Pesticides are applied in the environment to kill insects, weeds, fungi, or other pests. They may be applied in mixtures, or, because of overlapping pest pressures, may result in environmental mixtures because of the proximity of times and spaces over which they are applied. Exposure of humans and other non-target organisms to currently registered pesticides and/or persistent legacy pesticides will, therefore, likely be to pesticide mixtures. Because of the vast number of potential mixtures, predicting the effects of pesticide mixtures for risk assessment purposes is ultimately best accomplished through the use of computational models that are based upon experimental results with defined mixtures. Recent research conducted on the neurotoxic or immunotoxic effects of pesticide mixtures and the mathematical approaches to predictive modeling of the resultant data will be reviewed. Modeling approaches include the use of biomarker data predicted for some of these pesticides. We will address exposures to the organophosphorus insecticides, i.e., the organophosphorus and N-methyl carbamate classes of insecticides and the results in serine esterase inhibition. The levels of inhibition of some of these serine esterases, such as blood cholinesterase, is routinely used, in laboratory animal experiments and in worker exposure monitoring, to assess the level of exposure and potential toxicity. To fully understand these experimental results, biomarker data in terms of experimental results and the use of biomarker data in constructing the computational models will be highlighted.

**W 700 CELLULAR SIGNALING PATHWAYS AS A TARGET OF A PESTICIDE MIXTURE.**

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Dieldrin, a persistent organochlorine insecticide, which is now banned, is still present in the food supply, and a majority of people in the U.S. are exposed at levels greater than the reference dose. Atrazine is the most abundantly used conventional pesticide in the U.S. Risk assessments for these pesticides have been done without considering the probability of concomitant exposure to both compounds. These risk assessments would be quite different if this was considered and these (or any) compounds acted additively or synergistically. In the present study, interactions between these compounds with regard to activation of TLR4 signaling and resultant cytokine production were evaluated. The results demonstrate a greater than additive suppression of IL-6 production and inhibition of NF-kappaB activation in peripheral macrophages from mice treated with the TLR4 ligand, bacterial lipopolysaccharide (LPS). Inhibition of AP-1 by these agents was essentially additive. Another pro-inflammatory cytokine, IL-12, was affected in a manner similar to IL-6. These changes in cellular activation and cytokine production were not caused by cell death, as indicated by an MTT cell viability/proliferation assay. Initial results indicate that LPS induces a complex between NF-kappaB and AP-1 in this experimental system, and other investigators report that this complex induces much higher levels of transcription that either transcription factor alone. Mathematical modeling with ordinary differential equations was done with the idea that a complex of NF-kappaB and AP-1 binds to the response element for NF-kappaB and increases transcription much more than NF-kappaB alone. A key assumption is that the formation of the complex is reversible and driven by mass action. Modeling based on these concepts predicted levels of IL-6 production very similar to those measured experimentally, indicating that the overall structure of the model is acceptable. Future studies will allow refinement of hard data become available for more parameters. This work was funded in part by grant ES0913708 from NIEHS.
**702 IN VITRO AND IN VIVO EFFECTS OF SEVERAL LOW-DOSE BINARY MIXTURES OF ORGANOPHOSPHORUS INSECTICIDES.**

I. E. Chambers, College of Veterinary Medicine, Mississippi State University, Mississippi State, MS.

The primary neurochemical target of the organophosphorus (OP) insecticides, acetylcholinesterase (AChE), varies in its sensitivity to inhibition by the various insecticides or their oxon metabolites. The sensitivity to inhibition of protective non-target esterases, such as carboxylesterases, and the efficiency of catalytic detoxifying enzymes, such as paraoxonase (A-esterase), also vary among the various compounds. The response of an organism’s AChE (i.e., level and time course of inhibition and recovery) following exposure of any given OP insecticide cannot be predicted by the anticholinesterase potency alone, since the efficiencies of both these protective mechanisms and any required bioactivation will also influence the magnitude and time course of the responses. Prior inhibition of target and non-target esterases from one OP compound will influence the level of response to a second OP compound. Experiments in vitro with preparations of rat brain, where OP detoxication potential is minimal, have indicated that inhibition of AChE to binary or ternary mixtures of low concentrations of OP compounds can be predicted by non-linear mass action models using ordinary differential equations. However, these models were not always effective at predicting inhibition of the non-target esterases from binary mixtures in vitro in liver and serum where substantial detoxication mechanisms were present for some OP compounds. Patterns of inhibition of brain AChE following in vivo treatment of rats with low and moderate levels of binary mixtures of OP insecticides could frequently be predicted based upon the potencies of the oxon as an inhibitor of AChE or carboxylesterases, lipophilicity, and likelihood of detoxification by paraaxon. Thus the in vitro information can be used in developing predictive models for responses of AChE following exposures to mixtures of OP insecticides. Supported by American Chemical Society grant 0161 00080636 and US Environmental Protection Agency grant RD 83345101.

**703 OP INSECTICIDE MIXTURE EXPOSURE: INTEGRATING COMPUTATIONAL APPROACHES AND BIOMARKERS TO RECONSTRUCT DOSE.**

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Although various biomarkers have been used to assess exposure to and poisoning from organophosphorus (OP) pesticides/insecticides, the complexity of OP absorption, distribution, metabolism, and elimination, especially for mixtures of these chemicals, warrants integration of computational modeling tools with the biomarker data for more accurate quantitation and assessment of actual whole body exposures and target tissue dosimetry. In this presentation, we detail the development of a computer-assisted framework to aid in the identification, characterization, and understanding of biomarkers resulting from human exposure to mixtures of OP insecticides, using chlorpyrifos and diazinon as the initial test compounds. The framework uses existing human biomarker data and data from our own intermittent-dose studies, along with information about population and exposure variability and uncertainty, to reconstruct absorbed dose and exposure scenarios. The software may also be used to predict levels of biomarkers resulting from known exposures to these chemicals. Ultimately, we anticipate that the software tool developed, and targeted data acquired, will be useful in informing the cumulative risk assessment process for mixtures of organophosphorus insecticides.

**704 IN VITRO TO IN VIVO CONCORDANCE OF A HIGH THROUGHPUT ASSAY OF BONE MARROW TOXICITY ACROSS A DIVERSE SET OF DRUG CANDIDATES.**

A. J. Olahalski1, D. Uppal2, D. Goldstein1, M. Cooper2, S. Priest2, T. Zabka1, J. Jin1 and K. Kohli1, Roche Palo Alto, Palo Alto, CA and Medicinal Chemistry, Roche Palo Alto LLC, Palo Alto, CA.

Drug-induced bone marrow toxicity can hinder or delay drug candidate development. The colony forming assay (CFA) has been the most commonly used in vitro method to address this toxicity prior to running in vivo studies, but the associated cost, technical difficulty, and requirement for highly-trained personnel have limited its broad usage. New platforms that assess hematotoxicity via luciferase readout in C. elegans are an alternative approach by which the CFA can be run in a high-throughput manner without the requirement for highly trained personnel. This novel assay, the CFC-GEHM HALO® assay was used to identify bone marrow toxicity in vitro across a structurally diverse set of 56 compounds that have known in vivo toxicological properties. Concordance of the in vitro assay with in vivo toxicity data, depends upon how the in vitro toxicity limits are set (i.e. should an IC50 value of 5 or 30 μM be considered toxic?). Utilizing various in vitro toxicity classification thresholds, it was identified that a 20 μM in vitro classification provided the highest combination of sensitivity and specificity to in vivo bone marrow toxicity (76% and 73%, respectively). Interestingly, a 1 μM toxicity limit provided the highest specificity (100%) with a moderate level of sensitivity (45%), indicating that if a compound tested below 1 μM in the HALO® assay then there was a perfect prediction of in vivo bone marrow toxicity. The information obtained by the high-throughput CFC-GEHM HALO® assay can be used to rank compounds at earlier stages of development in order to reduce the likelihood of failure at later stages due to bone marrow toxicity.

**705 INTERACTIVE EFFECTS OF SEX HORMONES AND PRO-INFLAMMATORY CYTOKINES ON A CULTURED HUMAN HEPATOCYTE CELL LINE.**

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Women are more susceptible than men to acute liver injury from hepatotoxic drugs, dietary supplements and other xenobiotics. The underlying biological mechanisms for this gender difference are unknown, but known sex differences in steroid hormone levels and immune response could play a role. A human hepatocyte cell line, HepG2/C3A, was cultured for 8 days in either a male hormone, female hormone, or sex-hormone-free medium. The cells were then exposed to a mixture of pro-inflammatory cytokines (IL-1β, IL-6, TNFα) for 72 hours to simulate acute inflammation. Various metabolic functions (e.g., reactive oxygen species, neutral and polar lipid accumulation, CYP activities) were measured fluorometrically, and acute phase proteins were measured in culture medium by ELISA. The effects of cytokines on cell viability (4-8% decrease), generation of reactive oxygen species (8-12% increase) cytokrome P450-1A1/2 activity (20-30% decrease) and acute phase protein production (albumin 10% decrease, IL-1α 500% increase) were comparable regardless of hormonal environment. However, there were marked gender hormone-related differences in the cellular accumulation of neutral lipids. In the absence of cytokines, relative to the hormone-free group male hepatocytes significantly reduced neutral lipid storage while female hepatocytes significantly increased neutral lipid storage. In the presence of cytokines, relative to the hormone-free group male hepatocytes significantly increased neutral lipid storage while female hepatocytes had no significant effect. These findings suggest that sex hormones and pro-inflammatory cytokines can interact to alter normal liver metabolism in ways that may contribute to the marked gender difference in susceptibility to chemical-induced acute liver injury.
**PL 707** TOXICITY SCREENING OF THE TOXCAST CHEMICAL LIBRARY USING A ZEBRAFISH DEVELOPMENTAL ASSAY.

As part of the chemical screening and prioritization research program of the U.S. Environmental Protection Agency, the toxicity of the 320 ToxCast1,2 Phase I chemicals were assessed using a vertebrate screen of developmental toxicity. Zebrafish embryos/larvae (Danio rerio) were exposed from late blastula stage (approximately 6 hr post fertilization, pf) to day 5 pf (1 day post hatch). The 320 chemicals tested here included 309 unique chemicals, most of which are pesticide actives and metabolites, and several replicates for quality control. All exposures were by immersion, continuously, at a single concentration of 80 μM. On day 5 pf, fish were removed from the exposure solution to a control solution without chemicals, and on day 6 pf were assessed for death and structural defects (n = 4 independent embryos per chemical). Gross structural abnormalities or death was observed with 61% of the 309 chemicals. The developmental toxicity of the chemicals varied by chemical class. Classes in which all members caused either structural defects or lethality included: antibiotics (neomycin and streptomycin, 7 tested); gonzoines (imidazoles and triazoles, 16 tested); organochlorines (4 tested); dithiocarbamates (11 tested); and pyrethroids (12 tested). Other chemical classes showed a range of positive and negative results: 4 of 10 carboxamides showed no toxicity; 5 of 36 organophosphates showed no toxicity; and 2 of 6 phthalates showed no toxicity. The 6 neo-nicotinoids tested did not produce any toxicity in the developing zebrafish embryo. In conclusion, the early development of zebrafish is sensitive to many pesticides following exposure at 80 μM, and that sensitivity seems to vary by chemical class. Efforts are underway to compare the response in zebrafish embryos with rat/clinical developmental toxicity in mammalian systems captured within the U.S. EPA ToxRefDB database. This abstract may not necessarily reflect official Agency policy.

**PL 708** ASSESSMENT OF OTOTOXICITY AND OTOPROTECTION IN A ZEBRAFISH HAIR CELL MODEL.

Drug-induced ototoxicity from antibiotics and chemotherapeutics can cause hearing loss and vestibular disorders. Conventional histological and auditory functional methods are costly and labor intensive. The structure and function of zebrafish neuromasts, which are arranged in a stereotaxic pattern along the head, body and tail, have been shown to be similar to inner ear hair cells in mammals. To facilitate identification of ototoxic and otoprotective agents, we developed a quantitative in vivo assay using a vertebrate screen of structural toxicity. Zebrafish were exposed to test compound and gentamicin (75 μM) at 5 days post fertilization (pf), then maintained continuously, at a single concentration of 80 μM. On day 6 pf, fish were mounted on a de-attachment device (Microsource Discovery Systems). Zebrafish were exposed to test compound and screened ~300 potential protectants from the Natural Product Collection (MicroSource Discovery Systems). Zebrafish were exposed to test compound and screened ~300 potential protectants from the Natural Product Collection (MicroSource Discovery Systems). Developmental toxicity in zebrafish correlated with results in mammals, underscoring the high degree of conservation among species and supporting the use of zebrafish for preclinical drug screening.

**PL 709** CARDIAC CHANNEL PANELTM: A CRITICAL PATH TOOL FOR UNDERSTANDING CARDIAC RISK.

Drug-induced Torsades de Pointes (TdP) is always associated with delayed repolarization (DR: prolonged QT/APD) but DR may not be associated with TdP. Likewise, drug-induced DR may be associated with block of hERG, but hERG block may not be associated with DR. We surveyed 118 drugs that we tested with the hERG assay and for DR using the Purkinje fiber APD assay and the QT in paced, isolated rabbit hearts. We used an IC50 for hERG block of 0.1 μM, an increase of APD90 ≥ 10% and an increase of paced QT ≥ 10% as positives. We found 24% discordance, 16% with positive hERG and negative DR (Type 1 discordance) and 8% with negative hERG and positive DR (Type 2 discordance). The discordances may be resolved by assuming that many drugs act on cardiac channels in addition to or in the absence of effects on hERG. To test this hypothesis, we have assembled a Cardiac Channel PanelTM whose members may include cell lines over-expressing hERG, Ko14T1/miK, Ko15, Ko4, Koz1, Nav1.5, Cav1.2, Cav3.1, HCN2 and HCN4. These cell lines have been validated using manual patch clamp, PatchXpress, QPatch and Ion Works Qstarro. We have screened the Cardiac Channel PanelTM using these instruments and have identified effects that explain concordance and both types of discordance. Examples for each case are presented. With automation, the Cardiac Channel PanelTM can be screened accurately, quickly and inexpensively. We propose that screening the Cardiac Channel PanelTM is a Critical Path Tool for understanding and predicting cardiac risk.

**PL 710** INCREASED PREDICTIVE VALUE OF STUDIES IN RABBITS WITH VENTRICULAR HYPERTROPHY AND HEART FAILURE FOR PREDICTING ARRHYTHMOGENICITY OF DRUGS IN MAN.
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It is well-known that arrhythmias occur more prevalently in humans with ventricular hypertrophy (VH) and/or heart failure (HF), therefore this study was conducted to demonstrate that rabbits with VH and/or HF serve as models to predict arrhythmia in man. VH was produced by banding of the pulmonary trunk and/or aorta and HF was produced by ligating 1 major and 2 minor coronary arteries. These rabbits and normals were challenged with a drug (dofetilide) which produces tor-sades de pointes or with programmed electrical stimulation (PES) that produces reentrant arrhythmia. Increased arrhythmogenicity was demonstrated in the following order: HF > (VH+LV) > VH + RVH-control. Thus, in man, preexisting heart disease increases likelihood of drug-induced arrhythmia, and rabbits with iatrogentic heart disease may increase the predictive value for arrhythmogenicity in man. These studies must be extended to include more arrhythmogens and non-arrhythmogens to demonstrate both sensitivity and specificity.

**PL 711** ANALYSIS OF GENE EXPRESSION DATA USING A SYSTEMS BIOLOGY APPROACH: CONCORDANCE OF MODEL PREDICTIONS AND CLINICAL OBSERVATIONS.
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A systems model of the rat hepatocyte was created using enzyme and transporter kinet-ics of proteins belonging to several essential pathways, such as energy metabolism, antioxidant metabolism, bile and lipid homeostasis and cytokoskeletal organization, which are known to suffer derangement in the liver during drug-induced toxicity. This approach was focused on accurately reproduce liver-like behavior under several physiological and drug-treated situations. The model was tested for its ability to interface with "omics" data. Towards this end, microarray gene expression data from the livers of rats treated with two compounds, BAY 16-4749 from Bayer Healthcare AG, a compound having been dropped out from development, and 2NHEERL, U.S. EPA, RTP, NC.

**PL 712** V-LIVER: SIMULATING HEPATIC TISSUE LESIONS AS VIRTUAL CELLULAR SYSTEMS.
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The US EPA Virtual Liver (v-Liver) project is aimed at reducing uncertainty in esti-mating the risk of toxic outcomes in humans by simulating the dose-dependent effects of environmental chemicals in silico. The v-Liver embodies an emerging field
of research in computational tissue modeling that integrates molecular and cellular scales to simulate adverse outcomes. At its current phase of development v-Liver is a cellular systems model of a complex liver acinus: parenchymal and non-parenchymal cells represented as autonomous biological entities (“agents”) connected and spatially organized according to lobular morphotype. The tissue microenvironment is dynamically modeled with portal to centrilobular blood flow. Each agent autonomously processes inputs from the microenvironment using biological rules to generate a response. The biological rules and agent responses are derived from prior knowledge and experimental data on hepatocyte (HC) and Kupffer cell (KC) physiology. This includes a range of cellular inputs leading to HC adaptation, HC injury, KC migration, HC necrosis, apoptosis and proliferation. The v-Liver system enables in silico investigation of liver lesions as emergent tissue-level outcomes. We demonstrate the use of the v-Liver to analyze the formation of pre-neoplastic and neoplastic lesions by: (a) selecting non-genotoxic carcinogens, (b) encoding knowledge about carcinogenic MoA as biological rules, and (c) calibrating quantitative agent responses using cell-level in vitro data from ToxCast and ex vivo tissue samples. In the long-term the v-Liver will aid decision support through in silico experiments on new environmental chemicals to qualitatively and quantitatively explore the risk of chemicals and assess critical periods of susceptibility.

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Uncertainty still remains in regards to the association between postnatal exposure to persistent organic pollutants (POPs) and adverse effects in infants. This may be partly due to exposure assessment in environmentally based epidemiologic studies. We report in this study measurements of breast milk and/or infant blood POP levels, yielding only partial insights into infant lifetime toxicokinetic profiles and hence preventing the determination of critical windows of susceptibility. Our goals were to i) build a generic physiologically-based pharmacokinetic (PBPK) model for POPs, and ii) validate the model using data on POP levels from longitudinal study on Inuit mothers and infants. A previously published PBPK model for mothers was modified to better assess mother-infant transfer. Using the model and measured maternal and newborn blood POP levels, exposure to p,p’-DDE, p,p’-DDT, ODDA, DDE, p,p’-DDE, p,p’-DDT, HCB, β-HCH, PCB138, PCB153 and PCB180 in mothers was estimated to subsequently simulate infant plasma, breast milk and cord blood concentration. Simulations were compared to corresponding measured levels through Spearman correlation analyses. Model predictions were highly correlated with measured concentrations for PCB153, PCB180, PCB138, HCB and p,p’-DDE (r=0.83 to 0.96). Weaker correlations were observed for p,p’-DDT and β-HCH for which levels were close to the limits of detection. This is the first PBPK model for POPs to be validated on such a large scale. An approach using the maximum concentration (and the time when this concentration is reached) as well as the area under the curve for different time frames might provide crucial information on critical windows of exposure as well as dose-response relationships.
717 AN IMPROVED MODEL OF HUMAN RESPONSE TO AEROSOL CHEMICAL AND BIOLOGICAL AGENT HAZARDS.

An improved mathematical model for predicting the probability of infection or injury from exposure to aerosol chemical and biological (CB) agent hazards is being developed for the CB defense community. Many models currently assume that only particles reaching the lung are of concern and therefore estimating the inhalation of 1.5 micron ‘respirable’ particles is adequate; however, coarse particle deposition must be included. Coarse particle deposition in the nose, mouth and throat may pose a substantial health risk, particularly for infectious agents. The Multiple Path Particle Dosimetry (MPPD) model (The Hammer Institutes For Health Sciences, RTP NC, V2.01) is used to estimate the respiratory dose associated with the inhalation hazard. Several modifications were made to MPPD to extend the range of applicability for predicting the deposition pattern for aerosol particle size distributions of interest. Changes included extending the upper bound of particle size to 100 micrometers and implementing new inhalability curves to include outdoor environments with non-zero wind speeds. Other changes were made to the dosimetry model to allow for better integration with existing atmospheric transport and dispersion models. An uncertainty analysis based on the stochastic lung models in MPPD was performed to provide a level of confidence in the deposition results. These changes to MPPD improved its applicability to particle size distributions of interest to the CB defense community and provided a solid foundation for improving the human response model.

718 UPTAKE OF C60 FULLERENE 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. While research concerning their applications is extensive, studies necessary to evaluate their potential toxicity are limited. In particular, information on nanoparticle disposition will be necessary for human health risk assessments of exposure to the materials. Tissue distribution, one component of pharmacokinetics, can be assessed by quantifying arterial extraction of materials by isolated perfused porcine skin flaps (IPPSF). The purpose of this study was to determine the extent of tissue uptake of C60 fullerene nanoparticles (C60) by IPPSF. IPPSF were infused first for 4 hr with regular or IgG-free media that contained 1-2 ug/ml C60 and then for 4 hr with media only during a washout phase. Arterial and venous concentrations of C60 were measured in the media by HPLC/UV-vis chromatography. The size distribution of the C60 nanoparticles used for the experiments in regular and IgG-free media was analyzed by a Zetasizer. Steady-state differences in the arterial and venous C60 concentrations were compared to determine tissue extraction from the vascular space of the IPPSF and the venous C60 washout concentrations were used to calculate compartmental pharmacokinetic parameters. The steady-state differences in the arterial and venous concentrations in the IPPSF were small; extraction ratios ranged from 0 to 15.8% with a mean of 6.3%. During the washout phase greater than 95% of C60 was eliminated with a mean half life of 1.2 min. Although the Zetasizer analysis indicated agglomeration of C60 nanoparticles in media containing IgG, there was no statistically significant difference in the extraction ratio or pharmacokinetic parameters between the IPPSF and the venous C60 washout concentrations were used to simulate compartmental pharmacokinetic parameters.

719 COMPARISON OF QUANTUM DOT BIODISTRIBUTION WITH BLOOD FLOW-LIMITED PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL.
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Physiologically based pharmacokinetic (PBPK) modeling is a widely used tool to simulate chemical biodistribution in complex biological systems and for dose-response modeling in human health risk assessments. Their applicability to nanomaterial biodistribution has not been assessed. The biodistribution of nanoparticles such as quantum dots (QD) will be important since systemic disposition of the nanoparticle following absorption may influence the site and magnitude of toxicity. The purpose of this study was to compare a general blood flow-limited PBPK model with QD distribution to determine the model’s predictive ability across QD types, species, and exposure routes. The distribution of QD from blood to tissue was flow-limited, and all compartments (blood, kidney, liver, muscle and skin) were non-eliminating (QD > 10min). Tissue-to-blood partition coefficients were estimated from a study of mice administered QD705 (Yang et al., 2007, Environ, Health Perpect. 115, 1359-1364). The model with estimated partition coefficients was used to simulate QD biodistribution in the liver and kidney from published studies. Although model simulations were consistent with constant QD concentrations in tissues at later time points, the predicted elimination from the blood and accumulation in the tissue compartments was too rapid. In addition, the percentage of initial dose per g of tissue varied among the studies used for the comparison; therefore, the estimated partition coefficients were not predictive of liver and kidney concentrations for the various QD types, species or exposure routes. Since the partition coefficients represent the movement of the QD from the vascular to extracellular space or uptake by cells, more complex models that incorporate physiological differences among tissues and physicochemical information for particular QD will be needed to account for pharmacokinetic differences among QD types and animal species.

720 MICRONA BIOMARKERS FOR CARCINOGEN ARISTOLOCHIC ACID EXPOSURE IN RATS.
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MicroRNAs (miRNAs) are small non-coding RNAs that negatively regulate gene expression and control cellular mechanisms. However, the functional significance of each miRNA is mostly unknown. Despite the postulated involvement of miRNAs in carcinogenesis, there are only a few experimental examples that support an oncogene or a tumor suppressor as a miRNA target. Aristolochic acid (AA) is a model human carcinogen and has been associated with the development of urothelial cancer in humans, and kidney tumors in rodents. To investigate the potential miRNA biomarkers for chemical carcinogens, we determined miRNA expression profiles by microarray from the kidneys of rats treated with a similar dose protocol that produce kidney tumors. In addition, the genomic gene expression data generated from the same samples were analyzed to explore whether miRNA alterations were related to carcinogenesis of AA. A number of miRNAs were significantly altered after AA treatment. Among them, miR-34a and miR-21, were further analyzed and confirmed by QPCR. Many miR-34a and miR-21 target genes were significantly altered by AA treatment concurrently. Most of these genes are involved in cancer-related functions like DNA repair, cell apoptosis and cell growth. Our results suggest that miR-34a and miR-21 are oncogenic miRNAs and increase their expressions may be early indicators of carcinogenic insult. Thus, these miRNAs can become potential biomarkers for exposure of carcinogens.

721 METABOLOMIC APPROACHES TO CHARACTERIZING CHANGES IN TRANSFER RNA SECONDARY MODIFICATIONS IN CELLULAR RESPONSES TO TOXINS.
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It has been known for several decades that there are ~90 different non-canonical nucleobase structures in tRNA and tRNA, yet the precise physiological functions of most of these modifications have not been established. Recent evidence suggests that the modifications play a role in cellular responses to toxins and other stimuli, with changes contributing to translational regulation of gene expression. To test this hypothesis, we have developed a coupled liquid chromatography-triple quadrupole mass spectrometry (LC/MS-MS) approach to characterize and quantify changes in the spectrum of the secondary modifications of tRNA in S. cerevisiae exposed to different toxic agents such as hydrogen peroxide and methyl methane sulfonate. This analytical method should be generally applicable to characterize modifications in all RNA species, including rRNA, mRNA and micro-RNAs. Results from our analysis revealed that exposure of cells to hydrogen peroxide leads to an increase in levels of several tRNA modifications, including 5-methylcytidine (m5C), 2′-O-methyluridine (Cm), and N2,N2-dimethylguanosine (m22G). In parallel, we also showed that cell strains deficient in methyltransferase enzymes responsible for these modifications were more sensitive to hydrogen peroxide exposure than the wild-type strain. These results suggest that these modifications are involved in modulating the toxicity of hydrogen peroxide. Also, they demonstrated changes in the spectrum of tRNA modifications have the potential to serve as biomarkers of cellular exposures to toxins.


**PL 722 IMMUNOGLOBULIN SWITCH TRANSCRIPTS ARE NON-INVASIVE SURROGATE BIOMARKERS OF ADAPTIVE IMMUNE FUNCTION AND BACTERIAL INFECTION.**


Effective therapeutic intervention in autoimmune disease requires inhibiting innate and adaptive immunity, but not to an extent that predisposes subjects to infection. In developing an anti-inflammatory therapeutic for autoimmune disease, we repeatedly encountered atrophy of follicular germinal centers in secondary lymphoid organs, impaired T cell-dependent antibody responses (TDAR) and bacterial infection in a subset of Cynomolgus macaques in the high dose groups of one and three month chronic toxicology investigations. Other indicators of immunotoxicity were not present, such as the frequency of leukocyte subsets, levels of immunoglobulin and functional activity of complement, granulocytes, monocytes and natural killer cells. Further molecular and cellular investigations revealed that the therapeutic inhibited the differentiation of B lymphocytes in Cynomolgus macaques, rats and mice. The levels of more labile (i.e. short half-life) products of B cell differentiation, specifically immunoglobulin isotype class switching transcripts (switch transcripts) and post-germinal center B lymphocytes, did correlate with atrophy of follicular germinal centers, impaired TDAR and the incidence of bacterial infection, in both secondary lymphoid organs and peripheral blood. Decreases in the level of switch transcripts and post-germinal center B lymphocytes also preceded the onset of clinical signs of infection. We therefore conclude that blood switch transcripts and post-germinal center B lymphocytes are noninvasive, surrogate biomarkers of adaptive immune function and hence, could guide more effective clinical dosing.

**PL 723 ASSESSING CIRCULATING ENDOTHELIAL CELL PROGENITORS AS A SOURCE FOR POTENTIAL BIOMARKERS OF DRUG-INDUCED CARDIOVASCULAR TOXICITY IN THE RAT.**


There are currently no widely accepted noninvasive detection methods for drug-induced vascular damage. Circulating endothelial progenitor cell (CEPC) enumeration has recently gained attention as a potential biomarker of vascular injury and endothelial damage/dysfunction. The rat is commonly used in preclinical drug development toxicity testing and, as in humans, lacks a consensus noninvasive methodology for immunophenotypic identification of CEPCs. We have recognized and characterized CEPCs in rat blood using cell culture, fluorescence microscopy, flow cytometry, and gene expression analysis. A modified colony forming unit (CFU)-assay confirmed the existence of immature CEPCs in peripheral blood. Extended in vitro culture resulted in a morphology and immunophenotype consistent with mature endothelial cells as noted by positive staining for CD31, von Willebrand factor, and rat endothelial cell antigen, and negative staining for smooth muscle cell α-actin. CEPC populations (comprised of dl-ACLDL or CD36 positive cells negative for CD11b) sorted from lysed whole blood revealed heterogeneity in surface protein expression of CD31 and vWF by flow cytometry. Cell sorting of CEPC populations for subsequent gene expression analysis compared relative expression levels of nine endothelial–related and four progenitor–cell–related genes to determine endothelial and progenitor progeny. Sorted populations were consistently positive for PECAM1, EDN1, FLK1, VWF, ITGAD, CCR1, JPO, and MMP2. Taken together, our data suggests that CEPCs, as identified in rat blood by these methods, express cell-surface and gene expression markers consistent with endothelial cells and endothelial progenitor cell populations. These methods enable peripheral blood sampling of phenotypically and genotypically consistent populations of cells for use as a source for potential endothelial biomarkers of cardiovascular injury in the rat after drug administration.

**PL 724 URINARY LBPA TRACKS WITH ONSET AND REVERSAL OF DRUG-INDUCED PHOSPHOLIPOSIS.**

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Phospholipids (PLD) is characterized by the intracellular accumulation of phospholipids. The current study assesses the value of lyso-bis-phosphatidic acid (LBPA) as a biomarker to track the onset and reversal of PLD. LBPA changes were evaluated and compared to histopathology from exposure to amiodarone, a known phospholipidogenic agent. Sprague Dawley rats were dosed with vehicle (control), 15 mg/kg/day amiodarone (low-dose) or 150 mg/kg/day amiodarone (high-dose) for 14 days. Additionally, a subset of control and high-dose rats were maintained for a 12 day recovery period. Liver, lung and peripheral leukocytes were collected for histopathology evaluations. Blood was collected weekly for serum and hematologic analysis. Urine was collected daily for measurement of LBPA and creatinine (Cr). Control and low-dose animals showed no significant changes in urinary LBPA:Cr ratios, as compared to pre-treatment values. Microscopic analysis of the lung, liver and leukocytes showed no evidence of PLD in the control animals, but one animal in the low-dose group exhibited minimal PLD in the lung macrophages. In contrast, high-dose group urinary LBPA:Cr levels increased steadily from drug day (DD) 2 to DD7, representing an approximately 10-fold change above corresponding pretest levels. Foamy alveolar macrophages were observed microscopically in the lung in 5/5 high-dose animals on DD14. A substantial increase in % leukocyte vacuolation was also observed in the high-dose group at the end of the drug-phase. After discontinuation of amiodarone dosing on day 14 in the high-dose group animals there was a precipitous decrease in urinary LBPA:Cr, returning to pre-test levels within 10 days. Decreased LBPA:Cr levels in recovery paralleled the absence or marked depletion of foamy alveolar macrophages in the lungs and decreased in % leukocyte vacuolation. Collectively, these data suggest that LBPA could serve as a correlative urinary marker of drug-induced PLD.

**PL 725 CARDIAC BIOMARKER EXPRESSION IN THE URINE OF COCAINE USERS.**

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Cocaine is a powerful sympathomimetic capable of increasing heart rate, blood pressure, contractility, respiration, myocardial oxygen demand and body temperature. Its use has associated with acute myocardial ischemia and infarction, sudden death, vasocostruction and thrombodysmosis. This is accompanied with the release of cardiac biomarkers into blood, urine and other body fluids when the heart or its attendant structures are damaged. Detection of these biomarkers is used to diagnose, evaluate, and monitor patients with suspected acute coronary syndrome, acute myocardial infarction, acute cardiac ischemia or other symptoms of cardiac dysfunction. In recent years, attempts have been made to improve risk stratification using a combination of biomarkers that reflect myocardial cell damage, left ventricular dysfunction, renal failure, and inflammation. The biomarker approach is gaining acceptance in clinical settings. This study describes the effect of cocaine use on cardiac biomarker expression in the urine of cocaine users compared to non-users. Specimens were assayed for myeloperoxidase, vascular endothelial growth factor, heat shock proteins, microalbumin, aldosterone, myoglobin, creatinine, pro αtinal natriuretic peptide and C-reactive protein using enzyme-linked immunosorbant assay. Many of these markers have been assayed exclusively in serum specimens; urine specimens are non-invasive and are traditionally used to test for illicit substances. Increased expression of these markers is associated, in varying degrees of specificity, with cardiac damage, oxidative stress and inflammation. Establishing a link between biomarker expression in urine and cocaine-induced cardiac damage is the first step in developing a diagnostic tool that may permit rapid diagnosis and selection of an appropriate clinical intervention. Our research suggests that biomarkers in the urine may be an important non-invasive way to assess cardiovascular damage in users of illicit substances. This work has been supported in part by the Agency for Community Treatment and Services of Tampa.
formed to compare the levels of autonanobody expression between cases and controls. Eleven protein spots were found to be significantly higher in sera of cases than controls, with six of them being detectable only in cancer patients (Wilcoxon rank-sum test, P<0.05). Further identification of these protein spots with MS showed that levels of autoantibodies against three isoforms of α-enolase were significantly increased in sera of ESCC patients. The autonanobody against phosphorylated α-enolase was the most discriminatory marker that was detectable only in cases (6/14, 43%). α-enolase is a ubiquitous cytoplasmic glycolytic enzyme that has been shown to be overexpressed in various tumor tissues from different organ sites, including esophagus. Our initial proteomics-based analysis suggested that α-enolase is potentially immunogenic and the autonanobody against the phosphorylated α-enolase present in the sera may be a potential biomarker for early detection, diagnosis, and treatment of ESCC.

**PL 727 STEM CELL SELECTION FACILITATES ARSENIC-INDUCED MALIGNANT TRANSFORMATION VIA INNATE RESISTANCE, HYPER-ADAPTABILITY AND OVER-PRODUCTION.**

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Arsenic is a carcinogen that targets the urogenital system (UGS). Although mechanisms are undefined, arsenic drives aberrant accumulation of stem cell-like cancer stem cells (CSCs) in vivo and in vitro, indicating this is a key target population. Disruption of SC population dynamics is thought to be a critical factor in acquisition of malignant phenotype. Here, we test the hypothesis that UGS CSCs have a survival selection advantage during arsenic exposure favoring accumulation and facilitating malignant conversion. Innate and acquired resistance to acute and chronic arsenite-induced cytotoxicity and apoptosis were assessed in the human UGS SC line, WPE-stem, and compared to its maternal, parental cell line, RWPE-1. Real time RT-PCR and Western blot analysis defined selected gene expression. Arsenic-, cadmium-, and N-methyl-N-nitrosourea (MNU)-induced isogenic malignant transformants of RWPE-1 cells were also compared for acquisition of cancer SC (CSC) qualities. WPE-stem showed remarkable innate resistance to arsenic-induced cytotoxicity (LC50 = 26.9 μM) and apoptosis compared to parental RWPE-1 cells (14.6 μM) involving general apoptotic (Bel-2, Bax, caspases) and stress-related (Nrf2, HO-1, P66SHC) genes, as well as arsenic-specific adaptation genes (ABC11, GST-κ, MT). After chronic, pre-transformation (6 wk) arsenic exposure at levels (15 μM) eventually inducing malignant phenotype, WPE-stem showed a hyper-adaptability with a 282% increase in LC50 (102.8 μM) while RWPE-1 increased only 95% (28.4 μM). Quantitative assessment of CSCs in isogenic RWPE-1 transformants clearly showed a stunning overproduction of CSCs that was unique to acquired malignant phenotype after arsenic exposure but not after cadmium or MNU. These data strongly indicate that a SC survival selection during arsenic-induced malignant transformation manifests itself as a specific CSC over-abundance after acquisition of malignant phenotype.

**PL 728 CELLULAR UPTAKE OF PLATINUM NANOPARTICLES IN HUMAN COLON CARCINOMA CELLS AND THEIR IMPACT ON CELLULAR REDOX SYSTEMS AND DNA INTEGRITY.**

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Supercritical fluid reactive deposition (SFRD) was used for the deposition of highly dispersed platinum nanoparticles with controllable metal content and particle size distribution on β-cyclodextrin. The average particle size and size distribution were steered by the precursor reduction conditions, resulting in particle precipitates < 20 nm, <100 nm and > 100 nm as characterized by TEM and SEM. These particle precipitates of different size distributions were used to address the question as whether metallic platinum particles are able to invade cells of the gastrointestinal tract as exemplified for the human colon carcinoma cell line HT29 and thus affect the cellular redox status and DNA integrity. Combined focused ion beam (FIB) and SEM demonstrated that platinum nanoparticles were taken up into HT29 cells in their particulate form. The chemical composition of the particles within the cells was confirmed by energy-dispersive X-ray spectroscopy (EDX). The potential influence of platinum nanoparticles on cellular redoxsystems was determined in the DCF assay, on the translocation of Nrf2 and by monitoring of the intracellular glutathione (GSH) levels. The impact on DNA integrity was investigated by single cell electrophoresis (comet assay) including the formation of sites sensitive to formamidopyrimidine-DNA glycosylase (FPG). Platinum nanoparticles were found to decrease the cellular GSH level and to impair DNA integrity with a maximal effect at 1 ng/cm2. These effects were correlated with the particle size in an inverse manner and were enhanced with increasing incubation time but appeared not to be based on the formation of reactive oxygen species.

**PL 729 CADMIUM-INDUCED ACQUISITION OF CANCER STEM CELL-LIKE CHARACTERISTICS DURING CARCINOGENIC TRANSFORMATION OF HUMAN PANCREATIC DUCTAL CELLS.**

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Emerging evidence suggests that tumor initiation and growth may be due to a small subset of pluripotent tumor cells with high proliferative potential. Term Oncancer stem cells (CSCs), they are thought to arise from transformation of either normal stem cells (NSC) or their immediate progeny through dysregulation of normally tightly regulated, but complex process of NSC self-renewal. In light of the recent isolation of pancreatic CSCs, this study examined whether cadmium targets stem cells during pancreatic cell transformation. Normal human pancreatic ductal epithelia (PDE) cells were exposed to a non-cytotoxic level of CdCl₂ (10 μM, > 12 wk) and compared to passage-matched control cells. Floating ‘pancreaspheres’ of viable cells, shown in other cell types contain an abundance of either NSCs or CSCs, were isolated from control and chronic cadmium exposed (CCE) cells. Cadmium treatment increased floating sphere formation 5-fold and invasion 2.8-fold. Significant increases in matrix metalloproteinase 9 (MMP-9), a biomarker for tumor cells, were seen in pancreaspheres from CCE cells as early as 3 weeks of cadmium exposure, while levels in adherent cells remained constant over the course of 12 weeks. Our prior work showed that increased MMP-9 secretion occurs in treated adherent cells at 29 weeks. By real-time PCR, both treated and control floating cells showed significantly higher expression than their adherent counterparts for various genetic markers for pancreatic NSCs/CSCs and/or pancreatic cancer progression/aggressiveness such as S100P, Oct4, PSCA, and CXCR4. Time course analysis revealed a cadmium-induced time-dependent increase in these markers, possibly as a prelude to malignant transformation. These data suggest that cadmium may act to promote and maintain a CSC-like phenotype in pancreatic cancer cells during transformation. This study also reinforces the general importance of CSCs in the initiation and progression of the carcinogenic process, even with inorganic carcinogens.

**PL 730 TUMORS AND Proliferative LESIONS IN MICE INDUCED BY TRANSPLACENTAL INORGANIC ARSENIC COMBINED WITH DIMETHYLARSENIC ACID IN ADULTHOOD.**

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Arsenic is a multi-site human carcinogen and may have transplacental activity. In mice, inorganic arsenic (iAs) is a transplacental carcinogen and fetal exposure also initiates lesions promotable by other agents later in life. Dimethylarsenic acid (DMA), a methylated product of iAs, can act as a tumor promoter. Thus, we studied the promoting effects of DMA given in adulthood on carcinogenesis initiated by fetal iAs exposure. Pregnant CD1 mice (20/group) received drinking water with or without 68.5 ppm iAs (as sodium arsenite) from gestation day 8 to 18. After birth and weaning, male offspring received drinking water with 0 or 200 ppm DMA for up to 2 years. Survival and body weights were not reduced by treatment. The number of mice/group analyzed histologically was: control 49; iAs alone 45; DMA alone 25; iAs + DMA 23. Renal tumors did not occur in control or DMA alone groups but fetal iAs alone mice had 2 (a renal cell carcinoma [RCC] and adenoma) and iAs + DMA induced 4 (p < 0.05 versus control; 3 RCC, 1 adenoma). Both iAs alone (13%) and iAs + DMA (55%) had increased renal total proliferative lesions (TPL; incidence of cystic atypical hyperplasia, adenoma or RCC) over control (0%) while DMA alone (8%) did not. Fetal iAs alone (38%), DMA alone (32%) and iAs + DMA (44%) induced similar levels of urinary bladder TPL (incidence of hyperplasia, papilloma or transitional cell carcinoma [TCC]) versus control (2%). Two papilloma (iAs + DMA) and 2 TCC (iAs alone) were seen with arsenicals. RCC and TCC are rare in mice and TCC occur in arsenic exposed humans. Compared to control (6%), iAs alone (20%) tripled the rate of hepatocellular carcinoma, and iAs + DMA doubled this rate again (39%), but DMA alone (8%) had no effect. DMA alone (24%), iAs alone (38%), and iAs + DMA (48%) increased adrenal adenomas versus control (0%). Overall, DMA in adulthood promoted tumors and/or proliferative lesions initiated by fetal iAs exposure in the kidney and liver, but acted independently in the bladder and adrenal.
Depleted uranium (DU) has been extensively used in military, industrial, and medical applications. Thus, exposure of soldiers and non-combatants is potentially frequent and widespread, and is now becoming a major international public health concern. Previous studies have shown the DU has both chemical and radiological toxicity, and that the primary route of exposure of DU to humans is through inhalation and ingestion. However, there is limited research information on the potential health hazards of DU exposure to human bronchial cells. Therefore the purpose of this study is to improve our current understanding of DU’s potential toxicity to human lung bronchial cells. We determined the cytotoxicity, clonogenicity and potential transforming ability of water-insoluble DU to human bronchial epithelial cells (BEP2D). We observed cytotoxicity, foci formation and loss of contact inhibition in cells exposed to DU after 24 h. The DU-transformed cells acquired anchorage-independent growth in soft agar suggesting that DU may be tumorigenic. We are currently characterizing DU-induced transformed cell lines in which some cell lines are showing an increase in plating efficiency, sensitivity to exposure to DU, and a transformed phenotype. Our study demonstrates that transformation of human bronchial cells can be induced by depleted uranium. This work was supported by ARO grants # W911NF-04-1-0240 and W911NF-08-0333 (J.P.W.).

Depleted uranium is commonly used in military applications and is also used in civilian industry and thus exposure of soldiers and others is frequent and widespread. There is limited research information on the potential health hazards of depleted uranium exposure. Previous studies have shown that depleted uranium has both chemical and radiological toxicity. In our study we used the human bronchial epithelial cell line (BEP2D) to study the potential cytotoxic and carcinogenic hazards of depleted uranium. After a 24 h exposure we observed a concentration-dependent increase in cytotoxicity with 0.25, 2.5 and 25 μg/cm² depleted uranium inducing 79, 74 and 36% relative survival, respectively. We also found that depleted uranium was genotoxic in a concentration-dependent manner with these doses inducing with an average of 1.3, 5.0 and 9.7 DNA double strand breaks per cell (measured as H2AX foci). Our previous study demonstrated that transformation of human bronchial cells can be induced by depleted uranium. We hypothesized that depleted uranium may also have epigenetic effects, which may contribute to its carcinogenic activity. We found that the level of dimethylated histone H3 lysine 9 (H3K9), a critical mark for DNA methylation and gene silencing, was increased in human lung cells after depleted uranium exposure. This work was supported by ARO grants # W911NF-04-1-0240 and W911NF-08-0333 (J.P.W.).

It has been established that hexavalent chromium (Cr(VI)) can cause lung cancer in humans. The most potent carcinogenic form of Cr(VI) is the particulates which deposit and persist in the respiratory tract after inhalation. The particle-derived Cr(VI) ion enters the cell and reduces to reactive intermediates producing numerous types of DNA damage. Cell culture studies show that Cr(VI) intermediates form ternary DNA adducts that can be processed by nucleotide excision repair (NER) machinery and produce a DNA double strand break. However the mechanisms underlying these effects are poorly understood. We investigated the relationship of Cr(VI)-induced DNA damage and key genes involved in the repair of DNA adducts. Chronic exposure to zinc chromate caused persistent DNA double strand breaks in human lung cells. Cells exposed to 400 ng/cm² zinc chromate for 24, 48, 72, 96 and 120 hours induced 15, 20, 10, 11 and 13 DNA double strand breaks per cell, respectively. We next focused on the XPF gene, which is involved in NER and forms a complex with ERCC1, which exhibits endonuclease activity and is responsible for the 5’ incision. During DNA replication, the 5’ incision of Cr-DNA adducts results in DNA double strand breaks. We found that 24 hour exposure to zinc chromate did not change the protein expression of XPF. However, chronic ex-
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 a galdenamycin (GA) that disrupt or modify the association of the hsp90 chaperone protein with the AHR. Previous studies suggest that the TCDD-induced degradation mechanism requires the AHR to associate with ARNT and DNA and contain a functional transactivation domain (TAD). Thus, mutation was used to create an AHR with a truncated AHR (AHR-R39A) so that DNA binding would be reduced. A similar approach was used to mutate L69 and L71 (AHR-L69/71A) to reduce dimerization with ARNT. Retrotroal expression vectors were used to generate stable cell lines expressing the various AHRs as well as wild type protein (AHR-WT) in the Hepa-1 cell background. Western analysis confirmed that all stable lines expressed levels of AHR protein and ARNT that were similar. However, cell lines expressing AHR-R39A or AHR-L69/71A showed greatly reduced levels of TCDD-inducible CYP1A1 protein, confirming that the AHR-mediated signaling pathway had been disrupted. When the level of AHR was evaluated following short or long term exposure to TCDD, the AHR-R39A and AHR-L69/71A proteins showed greatly reduced levels of degradation when compared AHR-WT. Importantly, when the various lines were treated with GA, the degradation of the AHR was similar in all the stable lines. To investigate the role of the TAD domain, the AHR was truncated at amino acid 500 and expressed with a variety of TAD domains from other proteins including HIF-1, ARNT, ARNT2 and VP-16. These chimeric proteins were expressed in the Hepa-1 line and the level of TCDD and GA-mediated degradation evaluated. Collectively, the results support a hypothesis that AHR dimerization, DNA binding and a functional TAD domain are required for the ligand-dependent degradation of the AHR. Supported by NIEHS5015481

HYPOXIA POTENTIATES TCDD INDUCTION OF CYP1A1 IN DIFFERENTIATING CHONDROCYTES.

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Hypoxia has been shown to be a key regulator of chondrogenesis [1, 2]. Because both AhR and CYP1A1 pathways are transcriptionally activated by AHR, we postulate that ligand-activated AhR signaling may disrupt normal hypoxia signaling. The purpose of this study is to investigate crosstalk between the Hif and AhR pathways in differentiating chondrocytes. Primary E11.5 murine limb buds were cultured in micromass for 5 days under basal conditions. To investigate the effects of TCDD on hypoxic signaling, cells were treated with varying doses of TCDD for 18h. At this time, cells were either left in normoxia or placed in hypoxia (5% O2) for 24h. To test the effects of hypoxia on AhR signaling, cells were cultured in 20% or 5% O2 for 18h. TCDD was added to cultures for 4h. We quantified mRNA by qRT-PCR. TCDD does not interfere with hypoxic induction of VEGF. 24h hypoxia exposure increased VEGF mRNA levels. Surprisingly, hypoxia increases AhR target gene induction. TCDD-induced Cyp1a1 mRNA levels were 1.92, 1.76, and 1.96-fold higher in the hypoxia group for 1.5, 10nM doses, respectively. In this study we present the novel finding that hypoxia has the ability to increase AhR-responsive primary differentiating chondrocytes. These findings are contrary to the negative regulation by Hif-AHR interaction as reported in several studies [3-7]. These preliminary results suggest an undefined mechanism by which the AhR and Hif pathways converge in differentiating chondrocytes, but not competitively. 1. Robins, J.C. et al., Bone, 2005. 37(5): p. 513-22. 2. Wan, C., et al., Proc Natl Acad Sci U S A 2005. 102(51): p. 18601-6. 3. Zeng, N. and M.K. Walz, Cardiovasc Toxicol, 2007. 7(4): p. 282-90. 4. Khan, S., et al., Toxicol Appl Pharmocol, 2007. 225(1): p. 28-38. 5. Almen, J.W., R.S. Johnson, and S.N. Bhattacharjee. Toxicol Lett, 2005. 155(1): p. 151-9. 6. Chan, W.K., et al., J Biol Chem, 1999. 274(17): p. 12115-23. 7. Gradin, K., et al., Mol Cell Biol, 1996. 16(10): p. 5221-31.

738 TCDD, CO-PLANAR PCBs AND PAHS INDUCE COMMON AH RECEPTOR-DEPENDENT EPIDEMIC SIGNATURES IN THE CYP1A1 PROMOTER.

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Dioxin and dioxin like compounds (DLCs) are environmental contaminants released during many industrial processes including combustion. The most toxic of these compounds, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), has been identified as a possible human carcinogen and as an immune- and developmental toxicant. TCDD, DLCs and the polycyclic aromatic hydrocarbons like benzo[α]pyrene (BaP), transactivate the heterodimeric transcriptional complex formed by ligand-bound Ah receptor (AhR) and the ARNT protein. As a consequence, several cytochrome P450 genes, including CYP1A1, CYP1B1, CYP1A2 and CYP2I1 become transcriptionally induced. Previously, we have shown that recruitment of the BaP-activated AhR to the Cyp1a1 promoter of mouse Hepa1 cells requires dissociation of bound histone deacetylase-1 and modification of several histone marks. Here we find that the Cyp1a1 promoter shows a similar pattern of histone modifications accompanying gene induction, including trimethylation of histone H3 K4 and hyperacetylation of H3 K14, strongly implying that this mechanism of response to Ah receptor ligands is ubiquitous. Because DLCs have toxic effects similar to those of TCDD, albeit with different Toxic Equivalency Factors (TEF), we used chromatin immunoprecipitation to determine that TCDD, 3MC, BjF, and various DLAcs causes fingerprints of epigenetic modification similar to those elicited by BaP. In addition, dose response experiments indicate that various co-planar polychlorinated biphenyls (PCBs) caused recruitment of AhR to the Cyp1a1 promoter and increased Cyp1a1 transcription at levels proportional to their TEF, whereas PCB153, a non-co-planar PCB, showed no AhR recruitment. These results not only imply a correlation between chromatin recruitment of AhR and the resultant toxicity but also delineate an epigenetic signature for AhR-mediated gene activation. (Supported by NIH R01ES6273, NIH R01ES10807, and NIH P30 ES06906).

739 INVOLEMENT OF AMINOEVULICIN ACID SYNTHASE IN DIOXIN-INDUCED GLYCOGEN DEPLETION IN HEPG2 CELLS.

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Laboratory animals exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) develop wasting syndrome, and it has been thought that glycoegen depletion and CYP1A1, a hepatically expressed in the liver are involved in the onset of this syndrome. Heme and hemoproteins have essential roles in various cell functions, and a possible relationship between heme biosynthesis and glucose metabolism has been suggested. Treatment by glucose was found to clinically ameliorate hepatic porphyria, caused by disorders of heme biosynthetic pathway, and porphyria patients were reported by epidemiological studies to be accompanied with hyperinsulinemia. Using microarray, we found that mRNA of aminoevulicin acid synthase (ALAS), the rate limiting enzyme in the heme synthesis pathway, was dose dependently induced by TCDD in three kinds of mouse strains. We investigated how ALAS is involved in glycoegen metabolism and/or gluconeogenesis. When HepG2, a human hepatoma cell line, was exposed to 1-100 nM TCDD, glycoegen amounts in the cells were decreased dose dependently, and ALAS mRNA and protein, transfection of ALAS specific siRNA suppressed the TCDD-induced decrease in glycoegen amounts. In addition, overexpression of ALAS in HepG2 reduced glycoegen amounts indifferent from TCDD exposure. Furthermore, TCDD exposure was found to enhance the phosphorylation of GSK3-β protein, a downstream protein of insulin signal, and induce mRNAs of PEPCk and PGC1-α, both of which are involved in the regulation of gluconeogenesis. On the other hand, ALAS siRNA-transfection abolished TCDD-dependent phosphorylation of GSK3-β protein and significantly decreased the mRNA levels of PEPCk and PGC1-α that are induced by TCDD. In conclusion, the present results suggest that ALAS is involved in the regulation of glycoegen metabolism and glycoegenoses in the TCDD-exposed cells.

740 HYPOMIA MIMETIC CHEMICALS AND MICROBIAL STIMULI INTERACT TO PRODUCE A PRO-ANGIOGENIC PHENOTYPE IN HUMAN LUNG FIBROBLASTS (HFL).

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Several chemical stimuli can mimic the cellular response to hypoxia via the activation of HIF-1α transcription factor-dependent changes in gene expression. Hypoxia stimulates angiogenesis, which is regulated via the action of multiple angiogenic (CXCR3, VEGF) and angiostatic (CXCL10) factors. As several of these
Tissue regeneration is an orchestrated spatio-temporal co-ordination of multiple signaling events. Identification of signaling pathways that promote tissue regeneration will enable their modulation for therapeutic purpose. The emerging field of regenerative medicine is advancing through the evaluation of regenerative mechanisms in model organisms. Zebrafish have the ability to regenerate a number of tissues including the heart and both adult and larval caudal fin. Regeneration of these tissues despite significant structural differences, suggests conservation of the molecular pathways critical for tissue regeneration. Comparative genomic analysis across regenerating adult heart, caudal fin and larval caudal fin revealed a number of transcripts with similar expression changes. We identified retinaldehyde dehydrogenase or raldh2, a rate limiting enzyme for the formation of retinoic acid, as one of the highly induced transcripts across the three regenerative platforms. In situ localization and other functional studies using chemical probes revealed that raldh2 expression is necessary for wound epithelium and blastema formation. We report that Wnt, Fgf and Acj6 pathways, some of the key players of tissue regeneration, are also modulators of raldh2 expression during caudal fin regeneration. This is the first report indicating a role for retinoic acid signaling in early regenerative events. Our progress demonstrates that the larval zebrafish is an ideal platform to identify and define the molecular pathways underlying complex in vivo processes. Supported by NSF #0641409, NIH R01 ES10820, and NIH P30 ES00210.

PL 741 POTENTIAL NEGATIVE FEEDBACK LOOP BETWEEN NOVEL COREGULATOR KC101 AND TARGET NUCLEAR RECEPTORS.


Epidermis serves as the interface between the body and the environment and is central for protection from cutaneous toxic assaults. Perioxome proliferators (PPs) and retinoids play important roles in maintenance and regulation of epidermal barrier function, and may themselves have toxic effects. These chemicals exert their effects through distinct classes of nuclear receptors (NRs) – ligand driven transcription factors which rely on coregulator proteins to activate or repress transcription of target genes. We conducted a yeast two-hybrid screen of keratinocyte library in which we isolated a novel NR interacting protein KC101. Interaction studies revealed that KC101 associates with PP activated receptors (PPARs) and retinoic acid receptors (RARs) in the presence of their ligands, typically a feature of NR coreactifiers, but reduces receptor activity when complexed with a non-ligand dependent corepressor, which may function in combinatorial regulation of gene expression in the epidermis and other tissues. To examine the regulation of the KC101 expression, we cloned a ~6000 base pair promoter fragment and carried out in silico analysis to identify putative transcription factor binding sites which predicted several PPAR response elements (PPREs) and retinoic acid response elements (RAREs). Transcriptional activation studies revealed that KC101 promoter is positively regulated by PPARs and RARs. Electrophoretic mobility shift assays showed, PPAR and RAR binding at distinct sites in the distal portion of the KC101 promoter. Promoter-luciferase reporter studies confirmed these as response elements. Our findings reveal a potential regulatory feedback loop where KC101 expression is necessary for wound epithelium and blastema formation. We report that Wnt, Fgf and Acj6 pathways, some of the key players of tissue regeneration, are also modulators of raldh2 expression during caudal fin regeneration.

PL 742 TRANSCRIPTIONAL REGULATION OF HUMAN MULTIDRUG RESISTANCE ASSOCIATED PROTEIN 4 GENE EXPRESSION.

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Multidrug resistance associated protein 4 (MRP4, ABCC4) is an efflux transporter that plays an important role in cellular communication, signaling and drug resistance. Liver MRP4 induction in mice is associated with enhanced resistance to acetaminophen hepatotoxicity. Studies in mice have shown that Nfat22, Kupffer cell function and the non-cytotoxic receptors PPARα and CAR to hepatic MRP4 induction under certain pathological conditions and chemical treatments. Liver MRP4 induction is also seen in humans overdosed with APAP. In this study, bioinformatics and biochemical genetics approaches were used to investigate the regulation of human MRP4 gene expression at the transcriptional level. MatInspector analysis showed the presence of more than 1,000 transcription factor binding sites for over 100 transcription factors in the proximal 10 kb regulatory sequence of the MRP4 gene. Reporter gene assays in HepG2 cells showed that the basal transcription activity of the promoter-proximal region was constitutively active and that the first 100 bp are required for maximal activity. Overexpression of transcription factors Nrf1, Nfe2l2, Sp2, Stat1, Klfl10 and Tfap2a in HepG2 cells up-regulates MRP4 reporter gene expression; while Hes1, Klfl15 and Zfp161 are repressive. Overexpression of a dominant negative form of Nrf1 or site-directed mutations in putative Nrf1 binding sites suppressed MRP4 gene expression. Scanning deletion analysis of the promoter-proximal region showed the presence of a short module associated with Hes1 function. Although the interaction between the multiple transcription factors examined and the regulatory regions of MRP4 remains unclear, these observations suggest complex regulatory mechanisms for hepatic MRP4 gene expression. The interplay between the different transcription factors that regulate MRP4 gene expression in response to toxic xenobiotic treatment, oxidative stress and other stimuli remains to be elucidated. This work was supported by The National Institutes of Health Grant DK09557.

PL 743 CRITICAL ROLE OF RALDH2 EXPRESSION IN EPIMORPHIC TISSUE REGENERATION.

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Epidermal stem cells play an important role in cellular communication, signaling and drug resistance. Liver Mrp4 induction in mice is associated with enhanced resistance to acetaminophen hepatotoxicity. Studies in mice have shown that Nfat22, Kupffer cell function and the non-cytotoxic receptors PPARα and CAR to hepatic MRP4 induction under certain pathological conditions and chemical treatments. Liver MRP4 induction in mice is associated with enhanced resistance to acetaminophen hepatotoxicity. Studies in mice have shown that Nfat22, Kupffer cell function and the non-cytotoxic receptors PPARα and CAR to hepatic MRP4 induction under certain pathological conditions and chemical treatments. Liver MRP4 induction in mice is associated with enhanced resistance to acetaminophen hepatotoxicity. Studies in mice have shown that Nfat22, Kupffer cell function and the non-cytotoxic receptors PPARα and CAR to hepatic MRP4 induction under certain pathological conditions and chemical treatments. Liver MRP4 induction in mice is associated with enhanced resistance to acetaminophen hepatotoxicity. Studies in mice have shown that Nfat22, Kupffer cell function and the non-cytotoxic receptors PPARα and CAR to hepatic MRP4 induction under certain pathological conditions and chemical treatments. Liver MRP4 induction in mice is associated with enhanced resistance to acetaminophen hepatotoxicity. Studies in mice have shown that Nfat22, Kupffer cell function and the non-cytotoxic receptors PPARα and CAR to hepatic MRP4 induction under certain pathological conditions and chemical treatments. Liver MRP4 induction in mice is associated with enhanced resistance to acetaminophen hepatotoxicity. Studies in mice have shown that Nfat22, Kupffer cell function and the non-cytotoxic receptors PPARα and CAR to hepatic MRP4 induction under certain pathological conditions and chemical treatments. Liver MRP4 induction in mice is associated with enhanced resistance to acetaminophen hepatotoxicity. Studies in mice have shown that Nfat22, Kupffer cell function and the non-cytotoxic receptors PPARα and CAR to hepatic MRP4 induction under certain pathological conditions and chemical treatments. Liver MRP4 induction in mice is associated with enhanced resistance to acetaminophen hepatotoxicity. Studies in mice have shown that Nfat22, Kupffer cell function and the non-cytotoxic receptors PPARα and CAR to hepatic MRP4 induction under certain pathological conditions and chemical treatments. Liver MRP4 induction in mice is associated with enhanced resistance to acetaminophen hepatotoxicity. Studies in mice have shown that Nfat22, Kupffer cell function and the non-cytotoxic receptors PPARα and CAR to hepatic MRP4 induction under certain pathological conditions and chemical treatments. Liver MRP4 induction in mice is associated with enhanced resistance to acetaminophen hepatotoxicity. Studies in mice have shown that Nfat22, Kupffer cell function and the non-cytotoxic receptors PPARα and CAR to hepatic MRP4 induction under certain pathological conditions and chemical treatments.

Furan, a potent hepatocarcinogen and liver carcinogen in rodents, was recently shown to be present in a wide variety of heat-treated foods. Although data on human intake of furan are limited, it appears that there is a relatively narrow margin between human exposure and doses which cause liver tumors in rodents, suggesting that the presence of furan in food may present a potential risk to human health. The aim of this study was to characterize furan toxicity by assessing clinical chemistry, individual bile acids, metabolomics, histopathological and proliferative changes in rat liver after repeated oral administration of furan at doses close to human exposure. Male F344/N rats were treated with furan at doses of 0, 0.1, 0.5 and 2 mg/kg bw by gavage for up to 28 days (5x/wk). No effects on body weight and no clinical signs of toxicity were evident throughout the study. Routine clinical chemistry and metabolomics (1H-NMR and GC-MS) did not reveal signs of hepatotoxicity, other than a mild, dose-dependent increase in serum cholesterol after 28 days treatment with furan. Serum concentrations of deoxycholic acid, lithocholic acid and hyodeoxycholic acid were significantly increased in rats treated with 2 mg/kg bw, suggesting functional changes in hepatobiliary transport. Significant histopathological alterations and changes in bile duct labeling index in response to furan treatment were restricted to some parts along the edge of the left and caudate liver lobes. In these areas, a dose- and time-dependent increase in hepatocyte proliferation, accompanied by very thin foci of non-specific inflammation, was observed. In summary, results from this study suggest that even short-term treatment with low doses...
of furan may induce functional (2 mg/kg bw) and proliferative changes (0.1 mg/kg bw) in rat liver. This work was supported by FP6 of the European Union (SSPE-CT-2006-44393) and DFG (MA 3523/3-1).

**PS 745 MODULATION OF HEPATIC GENE EXPRESSION INDEPENDENT OF DNA METHYLATION IN FURAN TREATED F344/N RATS.**

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Furan is a liver carcinogen in rodents and has been found in a variety of heated food products. To better understand the mechanism of furan carcinogenicity, time- and dose-related effects of furan on the expression of genes related to apoptosis and cell cycle were assessed by qRT-PCR arrays in livers of F344/N rats treated with furan at an oral dose of 0.1, 2 and 20 mg/kg bw, 5-days per week for up to 28 days. Of 86 cell cycle-related genes, a total of 15 were statistically significantly upregulated at 0.1 mg/kg b.w. and this response was more evident than at the higher dose. In contrast, of 86 apoptosis-related genes, the greatest response was at 2 mg/kg b.w. (11 genes upregulated). There was little evidence of down regulation of any genes throughout. We found that very few gene expression changes occurred within 5 days and the changes observed at 28 days were largely reversible after a subsequent 2-weeks off-dose. The DNA methylation status of 463 genes, which were upregulated at a relatively low level. DNA methylation or gene-specific methylation changes were found at either dose level. Our results indicate that, with time after repeat dosing of furan at a relatively low dose, induction of cell cycle and apoptosis related gene expression occurred. However the modulation is reversible and epigenetic DNA methylation appears not to be a mechanism under the current experimental conditions.

**PS 746 A 13-WEEK ORAL TOXICITY STUDY OF L-SERINE IN RATS.**

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L-Serine, the nonessential amino acid, is synthesized mainly from metabolic intermediates of the glycolysis system or partially from glycine. Recently, L-Serine was reported anti-stress and anti-sleep disorder activities, and it is expected as dietary mediates of the glycolysis system or partially from glycine. Recently, L-Serine was reported as an anti-stress and anti-sleep disorder activities, and it is expected as dietary supplement for sleep-inducer. A subchronic oral toxicity study was conducted to evaluate the safety of L-serine in Sprague-Dawley rats. The test article was administered once daily by gavage in male and female rats at dose levels of 0, 500, 1500 and 3000 mg/kg body weight/day for 15 weeks. Daily clinical observations, body weight, food and water consumption were noted throughout the test article. There were no treatment-related adverse effects on clinical chemistry, hematology, urinalysis: organ weights, gross and histopathological examination. In conclusion, the administration of L-serine at levels up to 10000 mg/kg bw/day for 3 weeks was well tolerated without any signs of toxicity.

**PS 747 SAFETY AND THERAPEUTIC EFFICACY OF A NOVEL CHROMIUM(III) DINOCYCSTEINATE (CDNC).**

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Chromium(III) supplementation is widely used as an adjuvant therapy to improve glucose metabolism. It also helps in energy production and increasing lean body mass. This study was focused on determining the broad spectrum safety and efficacy of CDNC. Acute oral, acute dermal, primary dermal irritation, primary eye irritation and a dose-dependent 90-day sub-chronic toxicity studies were conducted. Both acute oral and dermal LD50s and of CDNC were found to be greater than 2000 mg/kg body weight in both male and female Sprague-Dawley rats. The primary skin irritation study in New Zealand Albino rabbits demonstrated CDNC as slightly irritating. The eye irritation study exhibited that CDNC is moderately irritating. A dose-dependent 90-day subchronic toxicity study demonstrated no significant toxicity of CDNC. In efficacy studies, Zucker diabetic fatty (ZDF) rats were supplemented with CDNC (400 mcg elemental chromium(III)/kg body weight/day) over a period of 8 weeks. CDNC significantly lowered blood glucose (24%), HbA1c (11%), CRP (20%), MCP-1 (49%), ICAM (10%) and Lipid peroxidation (20%), respectively, compared to control rats. Taken together, these results demonstrate the broad spectrum safety and efficacy of a novel Chromium(III) Dinocysteinate.

**PS 748 GENE EXPRESSION PROFILING IN PRENATALLY-EXPOSED MICE TO STUDY THE POTENTIAL OF SELENIUM TO AMELIORATE DEVELOPMENTAL METHYLMERCURY NEUROTOXICITY.**


Controversy remains regarding the safety of consuming seafood, particularly during pregnancy. While seafood is the main source of environmental contaminants such as methylmercury (MeHg), it is also rich in vital nutrients. These nutrients have important roles in health, including brain development in the foetus, but may also offset effects of seafood toxics. Selenium (Se) is a nutrient hypothesized to ameliorate MeHg toxicity. The aim of this study was to ascertain the impact of Se on MeHg-induced gene expression in a mammalian model. Whole brain microarray analysis was performed in 15d-old mice which had been exposed throughout development via the maternal diet. The three exposure groups included: MeHg (4 mg/kg bw), Se (1.3 mg/kg bw), and MeHg+Se (4 mg/kg bw +1.3 mg/kg bw). MeHg was presented as a cysteinate form, and Se as Se-methionine, the elemental species occurring naturally in seafood. Genes related to calcium binding and prolactin, key targets of MeHg neurotoxicity were significantly differentially-regulated following MeHg exposure. These genes did not appear in the combined MeHg + Se exposure, suggesting that these pathways of toxic action/response were ameliorated by the presence of Se. Instead MeHg + Se exposure resulted in significant differential expression of genes involved in cell adhesion and those possessing an immunoglobin domain. These functional clusters were also distinct from pathways regulated on exposure to Se alone. The differences were not related to changes in MeHg accumulation, as this was not significantly different between these exposure groups. Changes in gene expression were accompanied by improvements in some neurobehavioral indicators of MeHg-induced toxicity. These results suggest potentially novel mechanisms by which Se may ameliorate the negative effects of MeHg-associated with seafood consumption.

**PS 749 SIMULTANEOUS DETERMINATION OF BISPHENOL A, OCTYLPHENOL AND NONYLPHENOL PRESSURIZED LIQUID EXTRACTION AND LIQUID CHROMATOGRAPHY–TANDEM MASS SPECTROMETRY IN INFANT FORMULAS.**

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Bisphenol A (BPA) is a substance widely used for the manufacture of polycarbonate plastics and epoxy resins, which are used in plastic food containers, such as baby bottles. Nonylphenol (NP) and Octylphenol (OP) are the environmental contaminants used as intermediates to produce anionic and non-ionic surfactants. Some recent reports determined the occurrence of these endocrine disruptor compounds in foods from different markets or containers [1, 2]. The aim of this work is to develop a new rapid, sensitive and reproducible analytical strategy for determining BPA, OP and NP contents in powdered infant formulas using pressurized liquid extraction (PLE) and liquid chromatography-tandem mass spectrometry (LC–MS/MS) with a triple quadrupole (QqQ) mass analyzer. For the extraction of the sample a a new rapid, sensitive and reproducible analytical strategy for determining BPA, OP and NP in infant formulas. This work was supported by the Science and Education Spanish Ministry (AGL2007-61493). References: [1] Shao, B. et al. 2007. Food Chem. 105, 1236. [2] Marugo, N.C. et al. 2008. Food Addit. Contam. 25, 373.
Fumonisin B1 (FB1) causes NTDs when given intraperitoneally (ip) at doses of enigmatic cluster of neural tube defects (NTDs) in southern Texas during 1990-91. Corn-based foods and are toxic and carcinogenic to rodents. There is evidence suggesting that consumption of fumonisins in maize grain from genetically modified Optimum® GAT® (98140; Optimum® GAT®, registered trademark of Pioneer, A DuPont Company). and nutritional value of maize grain from 98140 or 98140+Gly/SU maize with respect to standard nutritional PMI® Certified Rodent LabDiet® 5002 were formulated by substitution of genetically-modified (GM) herbicide-tolerant maize DP-0981440-6 (98140; Optimum® GAT®, registered trademark of Pioneer, A DuPont Company). Metabolic inactivation of the herbicidal active ingredient glyphosate is conferred by genetic integration of a gene fragment from a pathogen. Therefore, V79 cells were incubated with fumonisins (5-MOP; 8-MOP; angelicin, and limettin), and irradiated with various doses of UVA-light (0-200 mJ/cm2). The slopes of these linear relationships allowed the calculation of photomutagenicity equivalency factors (PMEEF) setting the PMEEF of 5-MOP at 1.0. The PMEEFs were calculated as 0.3 for 8-MOP. 0.02 for angelicin, and 0.02 for limettin, clearly demonstrating the photomutagenic potency of this compound.

Six commercially available Zearealenone (ZEN) binders from Mexico were analyzed by equilibrium isothermal analysis to compare their adsorption capacity for ZEN at pH 2 and 6.5. Nitrogen BET surface area was determined to investigate a potential correlation with sorption capacity. The binders included Zeotek, Duoteq (Nutek), Mexsil (Karulis), Mycosorb (Alltech), Toxinoir (Norel) and Mycoad (Avimex). Binders were compared to Activated Carbon (AC), Cetylpyridinium-Low pH Montmorillonite (CP-LPHM) (w/o controls) and NovaSil (Engelhard) (- controls). Experiments to evaluate solubility and stability of Ochratoxin A (OTA) were performed as a preliminary step for the isothermal sorption analysis of ZEN/OTA binders. Isotherms for ZEN were run in triplicate for 11 different concentrations of ZEN at 0.05 mg of each binder, which was used as a control. Computer-generated isotherm data were analyzed and the semi-empirical parameters of Qmax and K were calculated. C-shaped curve characteristics were observed for most of the binders at pH 2 and 6.5 with varied t values. AC and CP-LPHM showed different patterns of sorption (L-shaped), suggestive of more effective binding properties. With OTA, 4 and 8 ppm solutions in water:acetonitrile (124.9:0.1) were stable for 1 week (with maximum absorbance observed at 333 nm) when protected from light and stored at a 2-8 °C. In conclusion, 1) none of the binders showed an effective pattern of sorption for ZEN, 2) BET data could not be correlated with Qmax values; however, it provided important insight regarding the composition of matter of the binders, and 3) OTA water:acetonitrile solution may be a suitable method for analysis of these materials is ongoing. [Supported by NIEHS P42 ES09417, PROMEP 103/5/01673]
Aflatoxins are a group of mycotoxins that have long been recognized as hazardous contaminants of food. Aflatoxin B$_1$ (AFB$_1$) is the most significant hepatotoxic and genotoxic agent. Acute dietary exposure to high levels of AFB$_1$ causes aflatoxicosis and chronic exposure to low levels of AFB$_1$ is one of the major risk factors for human hepatocellular carcinoma (HCC) in several regions of Africa and Southeast Asia. In this study, the levels of AFB$_1$-lysine adduct were measured in serum samples collected from participants in a predominantly Hispanic population in a maize-consuming region of Texas known to have an increased incidence of HCC. This cross-sectional study was conducted within adult volunteers from three zip code regions in San Antonio, Texas. In addition to a sociodemographic survey, serum samples were analysed for AFB$_1$, lysine adduct by a recently developed HPLC method. Out of the total 151 serum samples analyzed, 17.2% (26/151) had detectable levels of AFB$_1$, lysine adduct with the average level at 4.57 ± 3.28 ranging from 1.69-16.57 pg/mg albumin. Results from this study were compared to our previous findings in a southern Guangxi population known to be at high risk for AFB$_1$ exposure. Of the total 136 samples collected from participants in Fushi County, 97.1% (132/136) had detectable levels of AFB$_1$, lysine adduct ranging from 0.28 to 157.83 pg/mg albumin. These findings suggest that the study population in San Antonio had low dietary aflatoxin exposure. (Supported by NIEHS ES 09106 and NCI CA90997)

### 576 INITIAL EVALUATION OF PROTEIN EXPRESSION PATTERNS IN HUMAN LIVER CELLS TREATED WITH AFLATOXIN B1.

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2Environmental Toxicology, Texas Tech University, Lubbock, TX and 3Food Safety and Nutrition, Jiangnan University, Wuxi, China.

Aflatoxin B1 (AFB1) is the most significant hepatotoxic and genotoxic mycotoxin. Acute dietary exposure to high levels of AFB1 causes severe liver damage (aflatoxicosis) and chronic exposure to low levels of AFB1 can induce hepatocellular carcinoma (HCC) in animals and humans. Many toxicological researches have focused on evaluation of AFB1 toxic effects on single DNA or protein target; however, the overall toxicogenomic and toxicoproteomic profile of AFB1 toxicity is largely unknown. In this study, 2-dimensional gel electrophoresis based proteomic analysis was undertaken to assess the dose response and time course of the protein expression in human HCC cells (HepG2) following treatment with AFB1 at 1 μM and 5 μM for 24, 48, and 72. The differentially expressed proteins were classified according to the image analysis with ImageQuant software. No significant inhibition on overall protein expression was found in cells treated with AFB1 concentration at 1μM or 5μM up to 72 h, as evidenced by similar numbers of total protein spots detected. However, HepG2 cells treated with AFB1 had different protein expression profiling as compared to the nontreated control cells. A total of 30 distinctive protein spots (2-fold or higher or lower than controls in density) was found in cells treated with 1μM or 5μM AFB1 for 24 h. More distinctive protein expression changes were found in cells treated with AFB1 for 72 h: 60 protein spots were detected in 1μM AFB1-treated cells and 74 protein spots, in 5μM AFB1-treated cells. Both up- and down-regulations were found in protein expression profiles. Majority changes were found in proteins with their isoelectric focusing at pH 5-7. Future work will use mass spectrometry to identify these proteins. (Supported by DOD RRECOM W911SR-07-C-0069)

### 759 ACCUMULATION OF 1-DEOXYSPHINGANINE IN MAMMALIAN CELLS AND TISSUES FOLLOWING FUMONISIN INHIBITION OF CERAMIDE SYNTHASE: A NOVEL CATEGORY OF BIOACTIVE SPHINGOID BASES AND 1-DEOXIDHYDROCERAMIDES.

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Fumonisins B1 (FB1) is a fungal toxin found in maize worldwide. It is hepatotoxic and nephrotoxic in many species, a liver and kidney carcinogen in rodents, and suspected to be involved in human disease. Its mechanism of action is disruption of sphingolipid metabolism consequent to inhibition of ceramide synthases (CerS). In cultured cells, inhibition of CerS by FB1 increases sphinganine (Sa), Sa 1-phosphate (deoxySa) and a previously unidentified metabolite, believed to be a novel sphingoid base. In this study, the metabolite was analysed by mass spectrometry (MS) and assigned a m/z = 286.3123 in positive ionization mode, consistent with a 1- or 3-deoxysphinganine (deoxySa), C$_{18}$H$_{38}$NO$_2$. Based on the calculated formula, it was hypothesized that the putative deoxySa was either produced from Sa via a dehydroxylase or was produced from the utilization of alanine by serine palmitoyl transferase (SPT), the first and rate-limiting enzyme in sphingolipid biosynthesis. Comparison with a synthetic standard using liquid chromatography tandem MS (LC-MS/MS) identified the metabolite as 1-deoxysa by LC mobility and production of a distinctive m/z 44 fragment ion (CH$_3$CH(NH$_2$)-) upon collision-induced dissociation. By comparison, Sa produced a distinctive m/z 60 fragment ion (CH$_3$CH(NH$_2$)-). Inhibition of SPT with myriocin blocked the formation of both Sa and 1-deoxysa. Labeling studies using L-alanine-U-$^{13}$C$_2$ and L-serine-U-$^{13}$C$_2$ showed the preferential incorporation of alanine into deoxySa, suggesting that 1-deoxysa arises from condensation of alanine with palmitoyl-CoA via SPT.

### 757 BENZOCAINE-INDUCED METHEMOGLOBINEMIA IN MAN.

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Benzocaine (BZC) has been proposed as an alternative to tricaine methanesulfonate because it is hoped that an extended withdrawal period would not be required. Benzocaine has been known to induce methemoglobinemia in a number of mammals including humans. This poses a potential concern for humans consuming fish containing residues of benzocaine. We have assessed the potential in rats for methemoglobin formation resulting from treating rats with single oral doses of benzocaine. Initially, male and female Sprague-Dawley rats (3/sex/time point) were given a single gavage administration of 64 mg benzocaine hydrochloride per kg body weight (bw) and then euthanized at 12-min intervals up to 120 min after treatment. Plasma levels of benzocaine and Sa determined by UPLC-ES-MS/MS, were relatively low and constant (-0.1 μM) at all time points, whereas the mean methemoglobin value peaked at 20% at 24 min and then declined. Based upon these data, additional rats (5/sex/treatment group) were orally gavaged with 0-1024 mg benzocaine hydrochloride per kg bw and euthanized 24 min after treatment. Plasma levels of benzocaine increased in a linear manner from 0.01 μM at 2 mg/kg bw to 2.9 μM at 1024 mg/kg bw. Methemoglobin levels did not differ from control values (0.8%) at doses up to 32 mg/kg bw in females and 64 mg/kg bw in males, whereupon the value increased to 80% at 1024 mg/kg bw. These data indicate that oral doses of benzocaine hydrochloride <32 mg/kg bw do not cause an increase in methemoglobin in this acute exposure animal model.
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to FB, and iii) determine if endogenous 1-deoxySa is N-acylated by CerS. In LLCPK1 cells, Vero cells, and other cell lines, 1-deoxySa accumulated to high levels after
treatment with FB. 1-DeoxySa was also detected in liver and kidney of P53N5 mice
and increased in mice fed FB diets. 1-DeoxySa displayed a similar toxicity as Sa in
dividing LLC-PK1 cells. FB alone and FB plus 10 μM sphinganine co-treatment
caused a slight elevation in 1-deoxySa compared to the control, whereas cells
treated with FB plus 10 μM 1-deoxySa showed a 25-fold elevation in 1-deoxySa
compared to the FB-only treated cells and a 42-fold elevation compared to the cultures treated with only 10 μM 1-deoxySa. Conversely, in LLC-PK1 cells treated
with 10 μM 1-deoxySa, the level of total N-acyl-1-deoxySa (i.e., 1-deoxydihydroceramides) went from 32.2 to 4,634 pmol/mg protein. Co-treatment with FB reduced the increase to 396 pmol/mg protein, indicating that the endogenous 1deoxySa is acylated by CerS. 1-Deoxydihydroceramides were also easily detected in
mouse liver. These findings implicate 1-deoxy sphingoid bases in diseases caused by
FB, and they may play important roles in cell regulation.

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IMPROVED REAL-TIME PCR QUANTITATION OF
RICIN AND OTHER RIBOSOME-INACTIVING
PROTEINS.

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We previously reported an assay for ricin and other ribosome inactivating proteins
that uses quantititative real-time PCR (qPCR). The assay measures the single base
change in the rRNA sequence after Moloney murine leukemia virus reverse transcriptase (MMLV-RT) preferentially inserts an adenosine in the cDNA opposite the
specific abasic RNA site created by the toxin. We now report an improvement of
this assay that decreases the number of steps, the amount of supplies, and the time
needed for the entire process. The assay described previously involved three separate
steps: (1) RNA incubation with ricin; (2) reverse transcription of the treated RNA;
and (3) qPCR determination of the amount of altered sequence. We have now
shown that the assay can be simplified by using “one-step” quantitative reversetranscriptase PCR (qRT-PCR): toxin-treated RNA is added to the qRT-PCR reaction mix, incubated in a real-time PCR instrument for a few minutes at 50° C for
the RT step (using a reverse transcriptase based on MMLV-RT, like the original
assay), and then amplified; both the RT reaction and the qPCR amplification are
handled by a single program in the instrument. As in the original assay, reactions
are done with two primer sets: one to measure the sequence change and the other,
for a sequence close to but not including the site of ricin action, to measure the
total amount of rRNA. Enzyme activity is determined by comparing these two reactions performed on the same sample. The primer used by the RT step must not
extend into the site of ricin reaction so that the polymerase can insert an adenine
opposite the abasic site. The other primer, therefore, must be the one that matches
the altered base. This assay has been shown to detect a variety of ribosome inactivating proteins: type II toxins ricin, abrin, and shiga toxin 1; and type I toxins
pokeweed toxin and ricin A chain. It detects the enzymatic activity of the ribosome
inactivating proteins, is rapid and sufficiently sensitive to detect bioterrorism-contaminated materials and equipment, and complements immunochemical and cellbased assays.

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SAFETY ASSESSMENT OF SCLAREOLIDE AS A FOOD
INGREDIENT.

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and 2Marketing, Burdock Group, Orlando, FL.
Sclareolide (CAS # 564-20-5) is a synthetic sesquiterpene. In the food industry,
sclareolide is used as a flavoring agent to enhance the organoleptic properties of
foodstuffs, as partial fat replacer in dairy products, and as a modulator to reduce or
eliminate the aftertaste of artificial sweeteners used in food and beverages.
Sclereolide has been approved for use as a flavor ingredient by the Flavor and
Extract Manufacturers’ Association (FEMA). Sclareolide is structurally similar to
sclareol, and it is synthesized by degradation of a manoyl derivative and by ozonization of 12α-hydroxy-13-epinanoyl oxide. No toxicological studies have been conducted with sclareolide, however, some studies have been performed with sclareol.
Because of the structural similarity, the results obtained from toxicological studies
performed with sclareol are likely to approximate the toxicity of sclareolide. Sclareol
was practically non-toxic in acute studies. The oral LD50 in rats and the dermal
LD50 in rabbits exceeding 5 g/kg. At a concentration as high as 10% in petrolatum, sclareol did not produce irritation when applied to the skin of human subjects. Sclareolide was tested and found to have antibacterial activity and demonstrated cytotoxicity against breast (MCF-7), colon (CKCO-1), lung (H-1299) and
skin (HT-144) human cancer cell lines. Sclareolide has a long history of use and is
presumed safe. FEMA Expert Panel established the safe use of sclareolide at 0.05
mg/day.

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DISPOSITION OF [2, 3-14C]ACRYLAMIDE ORALLY
DOSED TO JUVENILE AND ADULT FEMALE RATS.

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Nakazawa1 and Y. Ohno1. 1NIHS, japan, Tokyo, Japan and 2Institute of Whole Body
Metabolism, Chiba, Japan. Sponsor: A. Nishikawa.
Acrylamide (ACR) was reported for testicular toxicity, neurotoxicity and carcinogenicity. International Agency for Research on Cancer (IARC 1994) labeled ACR
as a probable human carcinogen. Recently, much scientific as well as public attention has been focused on chemicals such as ACR formation during food processing,
and the consumption of ACR from food is estimated to be higher in children than
in adults. Another apprehension is the sensibility of children to ACR toxicity. There
were some reports on the disposition of ACR in male rats in relation to its testicular toxicity, but few reports in female ones. Until now there has been no report on
the metabolism of 14C-ACR in juvenile female rats. For risk assessment purposes,
we have studied on the disposition of [2,3-14C]ACR (14C-ACR) in juvenile and
adult female rats. Female SD rats of 4 and 14 wk were administered by a single oral
dose of 14C-ACR 2.5mg/kg, 50-140μCi/kg. 14C-ACR was rapidly absorbed and
excreted because 65-80% of radioactivity was excreted into urine within 72 h and
fecal excretion was smaller (9–10%). 14C-ACR was rapidly distributed over the rat
and most of organs showed the maximum concentrations at 30 or 60 min, except
for blood. The radioactivity eliminated rapidly from most of organs, but very slowly
from the blood. There was not specific tissue retention in nerve cells or mammary
glands but in blood cells after 72 h. 14C-ACR seemed more rapidly absorbed and
excreted in juvenile rats than in adult rats.

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RELATIVE QUANTIFICATION OF SEED PROTEIN
ALLERGENS FROM NONTRANSGENIC SOYBEAN
VARIETIES BY LIQUID CHROMATOGRAPHY-TANDEM
MASS SPECTROMETRY.

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Use of proteomic techniques to identify and evaluate endogenous levels of proteins
is increasing. Data are lacking on the natural variability of levels of different proteins (allergens) across varieties and different environmental conditions. These data
are critical for interpreting differences in protein levels between crop varieties. A
current approach to evaluate proteins is to separate them by two-dimensional gel
electrophoresis (2-DE) and analyze protein spots by either MALDI-TOF or tandem mass spectrometry (MS/MS). The 2-DE approach is laborious and has reproducibility issues that render it a more qualitative than quantitative approach. The
objective was to develop and evaluate a higher-throughput and potentially more
quantitative approach to analyze complex protein samples that does not employ
gels or chemical labeling. The approach involves C18 analytical liquid chromatography (LC) separation of peptides obtained by in-solution protein trypsin digestion, coupled to online nanospray ionization MS/MS; a technique commonly referred to as LC-MS/MS. Using contemporary, fast-scanning MS the frequency of
MS/MS scans (representing individual peptides as protein surrogates) is a theoretical reflection of the relative protein abundance in the original sample, termed spectral counting. After validating the linear dynamic range and reproducibility of this
approach using protein standards, soybean proteins were analyzed to compare the
relative abundance of allergenic proteins across soy varieties from multiple U.S. locations. Most of the major soy allergens: glycinins (genInfo identifier 121276,
121277, 121278, 121279, 75221455), beta-conglycinin alpha chain (121281),
Kunitz-type trypsin inhibitor (125020), Gly m Bd 28K (12697782), and 34 kDa
maturing seed protein (84371705) were identified from 1 μg of injected protein
suggesting this could be a potentially viable, high-throughput approach for monitoring variation in protein allergen levels in plants.

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SAFETY OF GERANIUM OIL AS A FOOD INGREDIENT.

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Geranium oil is the steam-derived essential oil obtained from the geranium plant,
Pelargonium graveolens, and is used by both the fragrance and food industries. It is
a popular constituent of soaps and detergents because, unlike other fragrance oils, it
is not adversely affected by the alkaline nature of soaps. Primary constituents of
geranium oil have been anecdotally used for their anti-inflammatory and antioxidant activity, making them good candidates for dietary supplement use. As a flavoring agent it is GRAS for food use by FDA, Flavor and Extract Manufacturers’
Association (FEMA) and by the Council of Europe with no set use limits defined.
The National Academy of Science and FEMA consumption calculations estimate
the per capita consumption level to be 0.234 mg/day (0.00387 mg/kg/day) and


A recent study reported finding N-Acetylaspartate (NAA) in various foodstuffs including fruits, vegetables, meats, grains, milk, coffee, tea and cocoa. In order to estimate human dietary exposure to NAA, concentrations of NAA in the foodstuffs were entered into two models: GEMS/Food Regional Diets (GEMS/Food) and DEEM™ – FCID (Exponent Inc., Washington, DC). The GEMS/Food model is based on a high level per capita estimate of food commodity utilization within a cluster of countries with similar diets. The DEEM model is based on actual U.S. consumption data. The DEEM model has the advantage of supporting subpopulation analyses. The GEMS/Food model does not allow for subpopulation analyses but can be used for comparison across countries clustered by consumption patterns.

Both models have numerous food categories, but the GEMS/Food model has fewer categories and cannot utilize food processing or other adjustment factors. A comparison of results from the two models indicated that the GEMS/Food model overestimates exposure. The estimated exposure from GEMS/Food for country cluster M (which contains the U.S.) was approximately 3X higher for NAA than the DEEM U.S. population mean analysis at 0.0264 and 0.0094 mg/kg of BW/d, respectively. This comparison highlights the differences between two models which should be considered when determining a model to use in regulatory arenas for risk assessment.
PH 3.0 and also increased rapidly as the pH increases from pH 7.5 to pH 9.0.

inactivation at 85°C in the current study to probe the effect of pH on ricin thermal inactivation kinetics.

rate of thermal inactivation for ricin holotoxin varied in acidic fruit juices and in-

The potency, resistance to heat-inactivation, and ease of isolation has led to the designation of ricin as a Category B Select Agent for bioterrorism by the HHS.

uptake. The potency, resistance to heat-inactivation, and ease of isolation has led to the designation of ricin as a Category B Select Agent for bioterrorism by the HHS.

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EFFECTS OF PREPUBERTAL EXPOSURE TO ACRYLAMIDE ON NITROOSOUREA-INDUCED MULTIORGAN CARCINOGENESIS IN RATS.

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Acrylamide (AA) has been reported to be formed in fried and baked foods with various concentrations, and human exposure levels to AA from cooked foods in childhood are estimated to be higher than those in adults. It has been documented that AA shows carcinogenic properties in several organs such as thyroid, mammary gland and tunica vaginalis in rats as well as genotoxicities in various in vitro and in vivo systems including cultured thyroid follicular cells. In the present study, to evaluate effects of the genotoxic carcinogenesis actions of AA exposed during childhood, we conducted a medium-term carcinogenicity study with prepubertal administration of AA followed by treatments of a multi-organ carcinogen and a promoting agent for thyroid carcinogenesis in rats. A total of 36 postpartum F344 rats were given drinking water containing AA at 0, 20, 40 or 80 ppm for 3 weeks in lactation period, and their weaned offspring received the same AA mixed water for 3 weeks. Offspring were then injected with N-methyl-N-nitrosourea (40 mg/kg body weight, i.p.) once at week 7. Half of the animals of 0 and 40 ppm groups were sacrificed at week 15 and the other half at week 20. The offspring of rats treated with 80 ppm of AA showed significantly increased thyroid and mammary gland tumors, whereas offspring of rats treated with 20 ppm of AA showed slightly increased thyroid gland, mammary gland, and thyroid gland tumors.

Lasalocid (cervical region), thyroids, thymus, lungs, liver, spleen, kidneys, stomach, intestines, mesenteric lymph nodes, urinary bladder, mammary gland and macroscopic abnormalities were excised and examined histopathologically. There is no treatment-related change in the incidences of preneoplastic and neoplastic lesions in target organs of AA, including brain, spinal cord, oral cavity, pituitary, thyroid, adrenals, uterus, mammary gland, clitoral gland and tunica vaginalis. The results indicate that prepubertal exposure to AA in rats does not influence the carcinogenesis in any organs.
Chronic exposure to Fumonisin B1 (FB1), a toxin produced by Fusarium fungi, has been linked to various health problems. FB1 is found in foods co-contaminated with aflatoxins in countries like China and South Africa. Previous studies have suggested FB1 may act to enhance aflatoxin (AF) carcinogenicity, but these effects have not been consistently found in all studies.

In the present study, urinary FB1 levels were assessed in a population highly exposed to aflatoxins, with the goal of identifying a urinary biomarker for FB1 exposure. Urine samples from individuals living in an area with high aflatoxicosis risk were collected. The FB1 levels in these samples were quantified using a high-performance liquid chromatography (HPLC) method. Impoverishingly, FB1 peaks were detected in 69.4% of the samples evaluated. The mean and median quantities of FB1 were 6.53 ng/ml and 6.69 ng/ml urine, respectively. These results indicate that FB1 is a potential urinary biomarker for exposure to this toxin in high-risk populations.

In another study, the toxicity and safety of N-acetylglutamate (NAG), a natural substance formed in the body, was evaluated. NAG was administered to rats at different doses over a 90-day period, and no adverse effects were observed. These findings support the further study of NAG as a potential therapeutic agent.

Viscous fiber has several demonstrable effects on the body, including delaying gastric emptying time, slowing insulin release, and altering the absorption of dietary fat and cholesterol. PGX, a soluble polysaccharide, was found to have beneficial effects on glycemic control and lipid profile in individuals with type 2 diabetes. These results suggest that PGX may be a promising dietary component for managing diabetes.

In conclusion, the study of FB1 and NAG provides insights into the development of biomarkers for aflatoxin exposure and potential therapeutic targets for diabetes management.
Conclusions: There was no involvement of MSG ingestion either in development. In the study, rats were daily fed with the AA containing foods and a diet high in MSG. Rats were fed with foods that contained MSG for the whole experimental period did not affect either pulmonary eosinophil infiltration, production of Th2 cytokines such as IL-4 and IL-5, circulatory AA contents up to 2800 μg/ml with and without metabolic activation. In the in vivo experiments, AA was non-genotoxic in a bone marrow micronucleus test in mice at doses up 1000 μg/ml in an unscheduled DNA synthesis test in rats at 2000 μg/ml kg bw daily. Taken together, these studies provide evidence that Reb A is not teratogenic or genotoxic at the doses tested.

**781** EFFECTS OF ORAL MONOSODIUM GLUTAMATE (MSG) IN ASTHMATIC MICE.


Rationale: Multicenter, double-blind, placebo controlled clinical studies testing the effects of large doses of monosodium glutamate (MSG) intake in asthmatics concluded neither consistent nor sever reactions after MSG ingestion. However, no detailed analysis have not been conducted yet using experimental animals. Thus, present study aimed to investigate the effects of MSG ingestion on development of allergic reactions using well-established murine model for asthma. Methods: Bronchial inflammation was induced in BALB/c mice by means of intraperitoneal sensitization and challenge. Bronchoalveolar lavage fluid, blood and lung specimens were harvested 24 h after the last challenge. Some of the animals were used for the measurement of airway obstruction to assess airway hyperresponsiveness to methacholine. Results: Dietary administration of MSG (0.5, 5%, w/w) for the whole experimental period did not affect either pulmonary eosinophil infiltration, production of Th2 cytokines such as IL-4 and IL-5, circulating IgE concentrations or airway hyperresponsiveness. No effects of MSG ingestion on pulmonary inflammation including secondary changes in the asthmatic mice were confirmed by histological examination. Oral gavage of MSG solution (0.5, 5%, w/w) 30 min before the assessment did not exert any acute effects either on lung inflammation or on airway hyperresponsiveness in the asthmatic mice. Discussion: There was no involvement of MSG ingestion either in development of allergic reactions or in acute responses in asthmatic model mice. These results were consistent with previous clinical studies indicating safety of MSG intake in asthmatics.

**782** IS THERE AN EFFECT OF FOOD MATRICES ON THE BIOAVAILABILITY OF ACRYLAMIDE?

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Acrylamide (AA) is classified by IARC as probably carcinogenic to humans (group 2B). The biological activity of AA is considered to be mainly determined by its mutagenic activation in genotoxic glutamic acid (GA). However, bioavailability of AA in vivo human metabolism might also be of relevance. Up to now, only few data are available to assess whether the bioavailability of AA is influenced by the food matrix. In this study we investigated to which extent French fries and bread crust, with AA contents up to 2800 μg/kg, affect the bioavailability of AA as compared to AA uptake via drinking water. Rats were daily fed with the AA containing foods and a separate group received AA in drinking water (AA-DW group) over time periods between 1-9 days. Urine and feces were collected over 24h after the last feeding, then the animals were sacrificed and blood and liver samples isolated. Levels of AA and GA were measured with no significant deviation from the levels in different groups. In conclusion, AA-val-Hb adducts were determined in the bread crust group. Under all conditions GA-val-Hb-adduct levels did not exceed those of the untreated control. The comet assay did not show significant induction of DNA damage in blood or liver cells. In summary, the bioavailability of AA after oral ingestion in French fries is comparable to that after intake in water. Hb adduct formation indicates that AA is systemically distributed whereas GA, as soon as it was metabolically generated in the liver became efficiently coupled to glutathione to be excreted as mercapturic acid in the urine. Bioavailability of AA from bread crust was slightly reduced. Thus, in rat a very effective first pass effect through the liver appears to prevent systemic bioavailability of GA. Supported by AIF, FEI and BIL.

**783** BERGAMOTTIN INHIBITS PMA-MEDIATED MATRIX METALLOPROTEINASE-9 ACTIVATION THROUGH DOWN-REGULATING OF NUCLEAR FACTOR-KB.

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Matrix metalloproteinase-9 (MMP-9) plays an important role in the invasion and metastasis of cancer cells. In this study, we examined the inhibitory effect of bergamottin (BGM) from Citrus paradisi on phorbol myristate acetate (PMA)-induced MMP-9 expression in HT-1080 human fibrosarcoma cells. BGM significantly suppressed PMA-induced MMP-9 expression in HT-1080 cells. Furthermore, as evidenced by MMP-9 promoter, BGM specifically inhibited MMP-9 gene expression by blocking PMA-stimulated activation of NF-kB. In addition, BGM suppressed PMA-induced phosphorylation of JNK and p38 mitogen-activated protein kinase (MAPK), upstream factors involved in NF-kB, whereas the phosphorylation of extracellular signal-regulated kinase (ERK) was not affected by BGM, suggesting that the primary target of BGM for suppression of the NF-kB induction is present in upstream of JNK or p38 MAPK signaling pathway. These results suggest that the suppression of MMP-9 expression, at least in part, contributes to the antitumor activity of BGM.

**784** PRUNELLA VULGARIS INHIBITS TUMOR CELL METASTASIS AND GROWTH BY MODULATING EXPRESSIONS OF MATRIX METALLOPROTEINASE-9.

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Metastasis of cancer cells to distant sites is one of the major deciding factors in cancer outcome. In fact, prognosis of cancer is mainly determined by the invasiveness of the tumor and its ability to metastasize. In this study, we assayed the preventive and therapeutic effects of Prunella vulgaris on the experimental lung metastasis induced by B16F10 melanoma cells and solid tumor induced by B16F1 melanoma cells in C57BL/6 mice. The antitumor effects of Prunella vulgaris were investigated by evaluating matrix metalloproteinase-9 (MMP-9) production in HT-1080 cells. The level of secreted MMP-9 was determined by gelatin zymography and protein expression. In B16F10 melanoma lung metastasis experiment, Prunella vulgaris showed a great inhibition effect on the number of pulmonary metastases in a dose-dependent manner. In HT-1080 fibrosarcoma antitumor experiment, Prunella vulgaris inhibited tumor growth in a dose-dependent manner. In HT-1080 fibrosarcoma antitumor experiment, Prunella vulgaris inhibited MMP-9 expression in HT-1080 cells. Furthermore, as evidenced by MMP-9 promoter, BGM specifically inhibited MMP-9 gene expression by blocking PMA-stimulated activation of NF-kB. In addition, BGM suppressed PMA-induced phosphorylation of JNK and p38 mitogen-activated protein kinase (MAPK), upstream factors involved in NF-kB, whereas the phosphorylation of extracellular signal-regulated kinase (ERK) was not affected by BGM, suggesting that the primary target of BGM for suppression of the NF-kB induction is present in upstream of JNK or p38 MAPK signaling pathway. These results suggest that the suppression of MMP-9 expression, at least in part, contributes to the antitumor activity of BGM.

**785** EUPIATILIN EXHIBITS A NOVEL ANTI-TUMOR ACTIVITY THROUGH THE INDUCTION OF CELL CYCLE ARREST AND DIFFERENTIATION OF GASTRIC CARCINOMA AGS CELLS.

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In many cases, the process of cancer cell differentiation is associated with the programmed cell death. In the present study, interestingly, we found that eupatilin, one of the pharmacologically active ingredients of Artemisia asiatica that has been re-
ported to induce apoptosis in human gastric cancer AGS cells, also triggers differenti-
ation of these cells. Treatment of AGS cells with eupatilin induced cell cycle ar-
est at the G1 phase with the concomitant induction of p21cip1, a cell cycle inhibi-
tor. This led us to test whether eupatilin may trigger AGS cells to differentiate into 
the matured phenotypes of epithelial cells and this phenomenon may be coupled 
to the apoptosis. Eupatilin induced changes of AGS cells to a more flattened 
morphology with increased cell size and granularity. It also markedly induced trefoil 
factor 1 (TFF1), a gene responsible for the gastrointestinal cell differentiation. 
Eupatilin dramatically induced redistribution of tight junction proteins such as oc-
culin and ZO-1, and F-actin at the junctional region between cells. It also induced 
phosphorylation of extracellular signal-regulated kinase 2 and p38 kinase. Blockade 
of ERK signaling by PD98059 or the dominant-negative ERK2 significantly re-
duced eupatilin-induced TFF1 and p21 expression as well as ZO-1 redistribution. 
These findings indicate that ERK cascades may mediate eupatilin-induced AGS cell 
differentiation. Collectively, our results suggest that eupatilin acts as a novel anti-
tumor agent by inducing differentiation of gastrointestinal cancer cells rather than 
its direct role in inducing apoptotic cell death.

This work was supported by the Brain Korea 21 Project in 2008.

786 SUPPRESSION OF P-GLYCOPROTEIN expression by Puerarin in breast cancer cell MCF-7/ADR.

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P-glycoprotein accounts for the most intrinsic and acquired cancer multidrug re-
stance (MDR). To inhibit the expression of P-glycoprotein is one of the effective 
ways to reverse cancer drug resistance. Puerarin, an isoflavonoid found in Pueraria Radix, has been reported to possess antiangiogenic, antiangiogenic and anti-
odiant activities. In the present study, we identified that puerarin down-regulated 
the expression of P-glycoprotein at mRNA and protein levels in MCF7adrmycin 
(MCF7/ADR), a human breast MDR cancer cell line. Puerarin suppressed the ac-
tivity of MDR1 gene promoter. Furthermore, puerarin treatment significantly in-
creases in intracellular accumulation of fluorescent P-gp substrate rhodamine 123, 
indicating that puerarin treatment leads to reversal of the MDR phenotype result-
ing from an increased accumulation of anticancer drugs by inhibiting drug efflux 
function of P-gp. These results suggest that the puerarin can be a new adjuvant 
agent for human breast cancer chemotherapy.

787 ALLOPOSE GALLOHIBIT IN MULTIDRUG RESISTANCE THROUGH DOWN-REGULATION OF P-
Glycoprotein expression.

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The major reason for the failure of cancer therapy is the development of multidrug 
resistance (MDR). The MDR of cancer cells can be mediated by the overexpression 
of P-glycoprotein (P-gp). P-gp is known to function as energy-dependent efflux 
pumps of chemotherapeutic agents by decreasing their intracellular drug accumu-
lation. We studied the effects of 1, 2, 6-tri-O-galloyl-beta-D-allopyranose (GT23), a 
gallotannin isolated and purified from the Euphorbia species, on MDR function in 
P-gp overexpressing AML-2/DX250 cells. The doxorubicin-resistant AML-
2/DX250 was selected from the AML-2/WT by chronic exposure to doxorubicin 
on an intermittent dosage schedule at sufficient intervals to permit the expression 
of resistance phenotypes. We found GT23 having inhibitory effect of MDR in P-
gp overexpressing AML-2/DX250 cells. Although GT23 was showed low cytotox-
city, GT23 decreased cell viability as chemosensitizing effects in presence of doxo-
rubin. Flow cytometry data showed that GT23 increased drug accumulation in 
a concentration-dependent manner. In addition, GT23 decreased the levels of 
rRNA and protein of P-gp through mitogen-activated protein kinase (MAPK) in 
AML-2/DX250 cells. Taken together, these results suggest that GT23 has a chemosensitizing effects by down-regulation of P-gp expression and advancing the intracellular accumulation of drugs.

788 EXPOSURE TO A PROANTHOCYANIDIN MIXTURE SIGNIFICANTLY REDUCES DIMETHYL-NITROSAMINE (DMN) – INDUCED NEPHROCARCINOGENICITY IN VIVO.

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CDC has ranked kidney cancer as the 7th leading cause of cancer mortality in 
2004, and surprisingly, NCI estimates new cases and deaths from kidney cancer 
could be 54, 390 and 13,010 respectively in 2008 alone. Prime factors that precip-
itate this scenario include air and water pollution, food-borne toxic exposures, mal-
nutrition, and lack of exercise. Constant encounters to toxic end products for clear-
anse make the kidneys extremely vulnerable to toxic/carcinogenic changes. 
Although kidney’s complex anatomy is designed to handle such consequences, 
a significant leap in tissue antioxidants can potentially protect people’s kidneys to 
a wrong path. In this context, antioxidants appear to be instrumental in cellular de-
fense mechanisms, and numerous publications attest to the fact that diets rich in 
antioxidant phytochemicals could potentially prevent cancer. This study explored 
whether or not to expose to a powerful antioxidant proanthocyanidin mixture 
(PM) would reduce nephrocarcinogenic potential of DMN in male B6C3F1 mice. 
Animals were divided into four groups (Grl: Control; GrII: PM alone; GrIII: 
DMN alone; GrIV: PM+DMN). GrI and GrII were on control diet, whereas GrII and 
GrIV were on PM-diet. In order to initiate kidney cancer, GrIII and GrIV have 
received DMN (5 mg/kg: 0.8 weeks; 10 mg/kg: 4 weeks, ip) between 0-3 months. 
Animals were sacrificed every 3 months for serum chemistry and evaluation of car-
cinogenic changes in the kidneys. Tissue biochemistry and histopathology revealed 
DMN-induced orderly neoplastic changes and presence of nuclei of diverse mor-
phology (apoptotic, necrotic and apoptotic). Co-exposure to PM along with DMN 
reduced animal mortality and carcinogenic changes in the kidneys. Minor changes in 
BUN/creatinine levels and DNA fragmentation (less than 2-A increase at 3, 6, 
9 & 12 months) were observed. These results suggest that long term exposure to 
a proanthocyanidin mixture alone does not induce any change, whereas in combina-
tion with DMN, it may delay, reduce or counteract DMN-induced nephrocarci-
no genesis in vivo.

789 EFFECTIVENESS OF SOYA ISOFlavones AND VITAMIN D IN BREAST CANCER PREVENTION.

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Abstract: Drug toxicity is a major problem of chemotherapy in breast as well as other 
cancers. Recently, it has been reported that dietary Soya isoflavones Daidzein (Da) and 
Genistein (Ge) possess similar effects like other toxic chemotherapeutic 
drugs used in prostate cancer cells. They possess structures similar to estrogen, 
and may mimic or antagonist estrogen effects in different tissues. Da and Ge may 
also be involved in gene regulation by modulating epigenetic events such as DNA 
methylation, directly or through an estrogen receptor dependent process. We hy-
pothesized these isoflavones would be able to act as demethylating drug to retard 
breast cancer like other chemotherapeutic toxic drug 5’deoxy-azacytidine (AZA).
Methods: We treated three breast cancer cell lines (MCF7, 21PFT and T47D) with 
daidzein and genistein alone and in combination with cellular differentiating vita-
moin D3 (VTD) for 96 hours. Effects were then compared to those seen with the 
known methylation inhibitor AZA. Results: Da and Ge inhibited the growth of 
all three breast cancer cell lines in a dose dependent manner. The combina-
tion of VTD with isoflavones showed an additive effect. We have found that 
isoﬂavone treatment caused reversal of methylation of several breast cancer marker 
genes including H1-h1, RAR beta and Rass-F1, associated with an induction of 
their expression levels and combination therapy of VTD showed additive activation 
of expression of these genes. Furthermore, Da or Ge treatment resulted in a signif-
icant decrease of estrogen receptor alpha (ERA) level and its downstream signaling 
as ERB levels. Indeed, breast cancer cell lines that have high ERB levels are asso-
ciated with a reduced breast cancer risk and that ERB is an 
important modulator of proliferation and invasion of breast cancer cells. 
Conclusion: These isoflavones alone and in combination with VTD can retard the 
growth of the above three breast cancer cell lines by acting as a demethylating 
non-toxic demethylating chemotherapeutic food drug at the same level of AZA.

790 LACK OF EFFECTS OF SULFORAPHANE (SFN) ON BASELINE CY34A ACTIVITY IN HEALTHY HUMAN VOLUNTEERS.


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SFN is a chemoprotective compound that occurs naturally at relatively high concentra-
tions in broccoli, and is an established inducer of phase II enzymes (e.g. sul-
foconjugates, glutathione S-transferase, and quinone reductase), and is peroxisome proliferator-activated through the NRF2/Keap1 pathway. We reported previously that SFN acts as an antioxidant and phase II enzyme inducer in human HepG2/Confluent 10 cells. We then studied the effects of SFN on the expression of the genes involved in the NRF2/Keap1 signaling pathway. We found that SFN increased the expression of the genes involved in the NRF2/Keap1 pathway. We reported previously that SFN acts as an antioxidant and phase II enzyme inducer in human HepG2/Confluent 10 cells.
LAMs appear to be promising chemopreventive agents. Based on the anti-estrogenic and antiandrogenic proper-
ty of LAMs, it was found that they were more or equally potent as the antiandrogens flutamide and cyproterone. When co-exposed to 20 nM testosterone, significant anti-androgenicity was already present around 300 nM for some LAMs (K, L and M). However, it was observed that the toxic and genotoxic potential of dusts from different hard and soft woods vary. Studies indicated that dusts from specific woods and wood products may be of particular concern to woodworkers because they always displayed the most toxic and genotoxic potential. In our studies, dusts from and wood products were collected at 15-30 minute intervals over 6 hrs, and MDZ and 6-OH-MDZ were measured by LCMS. The Areas Under the Plasma Concentration vs. time curve (AUC) of MDZ and 6-OH-MDZ were measured using WinNonLin. MDZ clearance values were within the range of values seen in other studies. CYP3A4 levels were variable between individuals, but no significant difference was noted in MDZ clearance after a week of daily consumption of soup extract. Total urinary isothiocyanate levels showed excretion of the majority of the dose given. Thus, the anti-androgenic activity of SFN and RIF interaction on MDZ metabolism. If SFN is to be developed as a chemopreventive agent then information on its interactions with other drugs is necessary.

### PS 791 TOXIC AND GENOTOXIC STUDIES OF WOOD DUSTS.

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The National Toxicology Program and the International Agency for Research on Cancer have generally classified dust wood as a known human carcinogen. This classification is based on epidemiologic studies that have clearly linked an increased risk of nasal adenocarcinoma with chronic occupational exposures to wood dust. However, few reports have addressed which wood dusts are of the greatest concern, and the potential mechanisms of wood dust action are relatively unexplored. To address some of these informational gaps we used a panel of in vitro assays to investigate the toxic and genotoxic potential of dusts from different hard and soft woods and wood products. Our studies indicated that dusts from specific woods and wood products contained a soluble compound(s) that potently activated aryl hydrocarbon receptor signaling, generated changes in mammalian cell ploidy, and induced increased transgenic gene expression in mammalian cells. In addition, our studies to identify the mutation spectra and the cell transformation capability of wood dusts are ongoing. We conclude that some wood dusts are particularly genotoxic and thus may pose potentially greater health risks than others. Teak wood dust may be of particular concern to woodworkers because it always displayed the most activity in these in vitro assays.

### PS 792 IN VITRO ANTI-ESTROGENIC AND ANTI-ANDROGENIC PROPERTIES OF LAMELLARINS.

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Lamellarins (LAMs) are natural compounds of marine origin with properties that are promising with respect to cancer chemoprevention. In the present study six LAM congeners (K, L, M, N, Y and Y-DB) were synthesized. These LAMs contain hydroxyl and methoxy groups on different positions which are known to modulate the toxic and genotoxicity of larkspur alkaloids. Anecdotal observations and pilot studies in cattle indicate that there is animal-to-animal variation in response to larkspur toxicity. The objective of this study was to determine if there is variation in susceptibility of different strains of mice to larkspur poisoning and to identify factors responsible for the variation that could then be used as a model for studies in cattle. The acute toxicity of methyllycaconitine (MLA) in ten different inbred strains of mice was compared. The rank order of susceptibility was A/J > B10 > FVB > BALB/c > C57Bl/6 > NZW > C3H > DBA > 129. The toxicokinetic profiles of MLA in the susceptible A/J and resistant 129 strains were compared in order to determine if their differences in susceptibility are simply due to differences in their ability to eliminate MLA. The differences in toxicokinetic parameters observed did not explain the differences in susceptibility. The protein expression of various nAChR subunits was also compared between the resistant 129 and susceptible A/J strains. The 129 strain of mice had twice the amount of alpha 7 nAChR subunit expression as the A/J strain, which was in direct proportion to the approximate two fold difference in LD50. There was also a significant difference in the expression of the alpha 3 and alpha 5 nAChR subunits between the 129 and A/J strains, with the 129 strain having a higher expression in each case. These data suggest that the increased susceptibility of the A/J mice could be due to a lower expression of nAChR subunits. The identification of factors that regulate the differences in susceptibility to larkspur poisoning between breeds of cattle would provide livestock producers with knowledge to use in their breeding, culling, and grazing management programs to reduce or prevent larkspur poisoning on rangelands.

### PS 793 BORIC ACID AS A NOVEL STORAGE CALCIUM RELEASE ANTAGONIST AND ENDoplASMIC RETICULUM MODULATOR IN DU-145 PROSTATE CANCER CELLS.

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Men living in countries with low and high risk for prostate cancer both exhibit microscopic prostate cancer in their 40s. However, as a consequence of local environments they exhibit more than a 100 fold difference in cancer in later life. Boric acid intake from water and food content reflect local soils, geology, and dietary and lifestyle practices. We have reported that prostate cancer is reduced by 54% in U.S. men with the highest boric acid diets and 37% in men in regions with the highest boron ground-water concentrations in Texas. In vitro studies showed that boric acid reduced NAD+ stimulated Ca2+ release in DU-145 prostate cancer cells within seconds. Here we show that boric acid induces endoplasmic reticulum stress. In addition, physiological concentrations of boric acid inhibit the release of ryanodine receptor (RyR) and inositol 1,4,5-trisphosphate receptor (IP3R) sensitive Ca2+ stores. We show that physiological concentrations of boric acid inhibit Ca2+ release in response to RyR agonists, caffeine and 4-chloro-m-cresol, as well as in response to the IP3 generating compound, m-3mFBS. In addition, prolonged treatment also lowers overall storage Ca2+ levels. Boric acid’s antagonistic actions were less effective in LNCaP prostate cancer cells and PWR1E non-tumor cells. Given the central role of Ca2+ storage and signaling in cell functions, our observations show that boric acid has promise as a cancer preventative agent and treatment as well as possible implications beyond cancer.

### PS 794 THE SUSCEPTIBILITY OF VARIOUS STRAINS OF MICE TO THE TOXICITY OF METHYLLYCACONITINE.


Although the mechanism of action for larkspur (Delphinium alkaloids) has been described, there is little information on the variation of the physiological response of individual animals to larkspur alkaloids. Anecdotal observations and pilot studies in cattle indicate that there is animal-to-animal variation in response to larkspur toxicity. The objective of this study was to determine if there is variation in susceptibility of different strains of mice to larkspur toxicity and to identify factors responsible for the variation that could then be used as a model for studies in cattle. The acute toxicity of methyllycaconitine (MLA) in ten different inbred strains of mice was compared. The rank order of susceptibility was A/J > B10 > FVB > BALB/c > C57Bl/6 > NZW > C3H > DBA > 129. The toxicokinetic profiles of MLA in the susceptible A/J and resistant 129 strains were compared in order to determine if their differences in susceptibility are simply due to differences in their ability to eliminate MLA. The differences in toxicokinetic parameters observed did not explain the differences in susceptibility. The protein expression of various nAChR subunits was also compared between the resistant 129 and susceptible A/J strains. The 129 strain of mice had twice the amount of alpha 7 nAChR subunit expression as the A/J strain, which was in direct proportion to the approximate two fold difference in LD50. There was also a significant difference in the expression of the alpha 3 and alpha 5 nAChR subunits between the 129 and A/J strains, with the 129 strain having a higher expression in each case. These data suggest that the increased susceptibility of the A/J mice could be due to a lower expression of nAChR subunits. The identification of factors that regulate the differences in susceptibility to larkspur poisoning between breeds of cattle would provide livestock producers with knowledge to use in their breeding, culling, and grazing management programs to reduce or prevent larkspur poisoning on rangelands.

### PS 795 INDUCTION OF HEME OXYGENASE-1 EXPRESSION BY SAPONINS DERIVED FROM ROOTS OF PLATYCODON GRANDIFLORUM IN HEPAC1C7 CELLS.

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Platycondon grandiflorum A. DC. (Campanulaceae) is used as a traditional oriental medicine and also as a food in Korea. Previous studies reported that the Platycodon grandiflorum, Changgil, saponin (C) has antioxidant activity. NF-κB is a transcription factor involved in the cellular protection against oxidative stress through antioxidant response element (ARE) or electrophile-response element (EpRE)-directed induction of several phase II detoxifying and antioxidant enzymes. Heme oxygenase-1 (HO-1) is known as a stress-inducible protein and functions as an anti-oxidant protein which is a key enzyme in the metabolic pathway of heme detoxification.

free diet for 1 week, then given a cheese soup with or without broccoli sprout ex-
tract containing 100 mmoles of SFN for 7 days. A cross-over design was used al-
lowing each subject to serve as their own control. The soup was well tolerated with no adverse affects reported. On days 2 and day 9 of each period blood samples were collected at 15-30 minute intervals over 6 hrs, and MDZ and 6-OH-MDZ were measured by LCMS. The Areas Under the Plasma Concentration vs. time curve (AUC) of MDZ and 6-OH-MDZ were measured using WinNonLin. MDZ clearance values were within the range of values seen in other studies. CYP3A4 levels were variable between individuals, but no significant difference was noted in MDZ clearance after a week of daily consumption of soup extract. Total urinary isothiocyanate levels showed excretion of the majority of the dose given. Thus, at the dose and time frame in this study, SFN did not cause a clinically significant change in CYP3A4 activity. A more complete study is needed in many examining the effects of SFN and RIF interaction on MDZ metabolism. If SFN is to be developed as a chemopreventive agent then information on its interactions with other drugs is necessary.
Protective Effect of the Aralia Continentalis on Carbon Tetrachloride-Induced Hepatotoxicity in Mice.

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The root of Aralias Continentalis Kitagawa has been used in traditional Korean medicine to relieve pain and to treat inflammation. The purpose of this study was to investigate the protective effects of the extract of Aralia Continentalis roots (AC) against hepatotoxicity induced by carbon tetrachloride (CCL4) and the mechanism of its hepatoprotective effect. In mice, pretreatment with AC prior to the administration of CCL4 significantly prevented the increased serum enzymatic activity of ALT and AST as well as the formation of hepatic malondialdehyde. Histopathological evaluation of the liver also revealed that AC reduced the incidence of liver lesions induced by CCL4. In addition, pretreatment with AC significantly prevented both the depletion of reduced glutathione (GSH) content and the decrease in glutathione S-transferase (GST) activity in the liver of CCL4-intoxicated mice. Hepatic GSH levels and GST activity were increased by treatment with AC alone. Heme oxygenase-1 (HO-1) is known to be induced by oxidative stress and to confer protection against oxidative tissue injuries. AC markedly up-regulated hepatic HO-1 expression in CCL4-treated mice, which might provide anti-oxidative activity in the liver. These results indicate that AC plays a critical protective role in CCL4-induced acute liver injury by promoting anti-oxidative protein expression.

Gallotannins Decrease Nitric Oxide Production Through Inhibition of NF-κB and Downstream iNOS Expression in Macrophages.

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Tannins are plant secondary metabolites and the term widely applied to large polyphenolic compounds. A variety of tannins have been isolated and have shown significant antioxidant properties. Gallotannins, a class of tannins, are a group of polyphenolic compounds of plant origin. In the present study, we examined the inhibitory effects of seven gallotannins on the LPS (lipopolysaccharide)-induced NO production and underlying mechanisms of action. Among the seven gallotannins, 1,2,3,4,6-penta-o-galloyl-beta-d-glucose (GT15) and 1,2,6-tri-o-galloyl-beta-d-allopyranose (GT23) significantly reduced LPS-induced NO production in mouse macrophage cells (J774.A1). GT15 and GT23 dose-dependently decreased gene expression and production of nitric oxide synthase (iNOS). The transcriptional activator protein, NF-κB plays a key role in immune and inflammatory responses to infection. Activated macrophages express iNOS and produce excessive amounts of NO. GT15 and GT23 also inhibited LPS-induced activation of NF-κB as indicated by inhibition of degradation of IkBα, nuclear translocation of NF-κB, and NF-κB-dependent gene reporter assay. Our results suggested that gallotannins may have an inhibitory effect on the LPS-induced inflammatory reaction. This work was supported by the Brain Korea 21 Project in 2008.

Methodology to Assess the Inhibition of α-Amylase by Plant Extracts with Anti-Diabetic Potential.

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Diabetes is a severe chronic disease caused by insufficient production of or resistance to insulin, which results in abnormal metabolism of carbohydrates, fats, and proteins. The inhibition of α-amylase activity has been suggested as a strategy for diabetes and obesity management by reducing sugar levels in blood. In this study we examined the inhibition of α-amylase by the plant flavonoid Quercetin, the tetrasaccharide Acarbose (Glucozym8®), and methanolic and aqueous extracts of Tapeinochilus ananassae (Costaceae). This plant has ethnopharmacological use in Puerto Rico and is known as “insulina”. In this study, we determined the percent of reaction of α-amylase following the production of maltose from starch at A = 540 nm after reaction with DNS color reagent, the percent of inhibition of test compounds and extracts and the IC50 for α-amylase inhibition. Results showed that methanolic and aqueous extracts of Tapeinochilus ananassae promote α-amylase activity whereas Quercetin and Acarbose are inhibitors of the enzyme at the concentrations tested (18-290 μg/mL) with IC50 207.8 μg/mL and 808.2 μg/mL, respectively. The activity observed for T. ananassae extracts has been reported with extracts of Lyrurus luteus and Pharbitis nil during the study of their plant growth stimulating activity. Acarbose is known as a non-competitive inhibitor of α-amylase activity (IC50 36 μg/mL, 19.4 μg/mL). The in vitro activity of Quercetin as inhibitor of α-amylase activity has not been reported previously. Further, in terms of using T. ananassae as α-amylase inhibitor this finding should be of importance.
TOXICOLOGICAL CHANGES ASSOCIATED WITH ADMINISTRATION OF EXTRACT OF LAGENARIA BREVIFLORA IN WISTAR RATS.


Lageneria breviflora Roberts is employed as antiviral herbal remedy in West Africa and its therapeutic effect has been confirmed by Scientists. Its toxicological effects as observed from serum and reproductive parameters of male Wistar rats administered with the plant extract was studied. 25 male rats were equally and randomly divided into a control and four test groups. Physiological saline was administered orally into rats in the control group, while rats in the test groups were administered with extract of 1000mg, 2000mg, 4000mg and 8000mg/kg bwt respectively once daily for 14 days. There was a significant (P<0.05) dose dependent increase in the serum glucose level. Higher doses of the extract increased the serum levels of triglycerides and LDL-Cholesterol, and lowered HDL-Cholesterol level in the serum. The mean serum sodium, phosphate ions, creatinine and BUN levels increased, while bicarbonate, potassium, and calcium ions decreased in the test groups. Serum enzymes AST and ALT levels in the rats were non-significantly different from that of the control group. There was significant reduction of sperm count and motility accompanied by secondary sperm cell abnormalities. Histopathological findings revealed no hepatocellular changes but marked renal and seminiferous tubules degeneration in the test rats. The extract caused hyperglycaemia and dyslipidaemia in the test animals which suggest that its continued use can predispose to diabetes mellitus and atherosclerosis. There was also evidence of electrolyte imbalance; the decreased serum bicarbonate levels may precipitate metabolic acidosis. Significant elevation of serum creatinine and BUN indicate renal injury caused by the extract. The renal injury is believed to be responsible for alterations of the serum biochemistry in the test rats. It is therefore, recommended that ethno-medicinal use of the plant be discouraged.

APPLICATION OF THE THRESHOLD OF TOXICOLOGICAL CONCERN APPROACH FOR THE SAFETY EVALUATION OF CALENDULA FLOWER (CALENDULA OFFICINALIS) PETALS AND EXTRACTS USED IN COSMETIC AND PERSONAL CARE PRODUCTS.

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Calendula flower (Calendula officinalis) (CF) has been used in herbal medicine because of its anti-inflammatory activity. CF and Calendula officinalis Extracts (CCE) are used as a skin conditioning agents in cosmetics. Although data on topical safety are available, their potential systemic effects following dermal application have not been fully characterized. The Threshold of Toxicological Concern (TTC) is a pragmatic, risk assessment based approach that has gained regulatory acceptance. Recently it has been adapted to address cosmetic ingredients safety. The purpose of this paper is to determine if the safe use of CF and CCE can be established based upon this concept. For each chemical constituent the maximum known concentration in the plant/extract, the molecular weight, and the estimated skin penetration potential were used to estimate a maximal daily systemic exposure which was then compared to its TTC value. Since the composition of plant extracts are variable, back calculation was used to determine the maximum allowable concentration of a given chemical in an extract of CF. The exposure values derived using the TTC approach are greatly conservative and are at least one to three orders of magnitude below the acceptable allowable daily exposures that rely on the classical ‘margin of safety’ approach published NOELs for some of the components of the plant. It is also a rapid and convenient method that allows evaluations of extracts where certain major components have been enriched through adjusted extractions methods or concentrated via HPLC or other methods. Using CF and CCE as examples, we have been able to clearly demonstrate the practical utility of the TTC approach to support the safe cosmetic use of a complex organic mixture such as a plant part or extract.

IN VITRO EVALUATION OF TECOMA STANS (BIGNONIACEAE) LEAF EXTRACTS USING HUMAN LIVER CELLS.


Tecomast (Bignoniaceae) a natural hered is used in complementary and alternative medicine for reducing blood glucose concentration. Natural remedies are not entirely safe and their side effects should be clearly determined. Furthermore, the anti-diabetic mechanism of Tecomast Sparks has not been elucidated. The aim of this study was to investigate the cytototoxicity of aqueous extracts of this herb in human hepatoblastoma (HepG2) and normal liver cells (THLE-2) in vitro. Cytotoxicity and IC50 values were measured by formazan assay (4, 5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. Hepatic cell glucose uptake was also measured to evaluate the potential hypoglycaemic activity in response to treatment with T. Stans using a glucose assay kit. Lysophilized T. Stans leaf extract (0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4 mg/ml) was dissolved into cell culture medium with and without Fetal Bovine Serum (FBS). The endpoints were determined following incubation for 2, 24, 48 and 72 h. Cytotoxicity and glucose uptake were dose and time dependent (P<0.001) following exposure to T. Stans without FBS. Significant inhibitory effects were observed at all concentrations of T. Stans above 0.5 mg/ml from 24 to 72 h for HepG2. In contrast, T. Stans inhibited THLE-2 above 1.5mg/ml from 24 to 72 h. The addition of FBS to the culture media increased the toxic concentration of T. Stans from 0.5mg/ml to 1mg/ml for HepG2 and from 1,5mg/ml to 2mg/ml for THLE-2 after 24 h exposure. The results indicate that while T. Stans might provide positive therapeutic benefits in glucose uptake as a complementary herbal extract, further studies are needed to determine the indication that nutrition, concentration, and duration of exposure can potentially influence the toxicity of T. Stans.

HEPATOPROTECTIVE EFFECTS OF THE ANTHOCYANIN FROM PURPLE-FLESHED SWEET POTATO AGAINST ACETAMINOPHEN-INDUCED TOXICITY IN MICE.

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The protective effects of the anthocyanin fraction (AF) obtained from Purple-Fleshed sweet potato on acetaminophen (APAP)-induced hepatotoxicities, and to determine the mechanism involved in mice. Pretreatment with AF prior to the administration of APAP significantly prevented the increased serum enzymatic activities of alanine and aspartate aminotransferase and hepatic lipid peroxidation in a dose-dependent manner. In addition, pretreatment with AF protected the APAP-induced depletion of hepatic glutathione (GSH) level. Hepatic GSH levels and glutathione-S-transferase (GST) activities were increased by treatment with AF alone. APAP-induced hepatotoxicity was also prevented by pretreatment with AF, as indicated by liver histopathology. The effect of AF on cytochrome P450 (CYP) 2E1, an major isozyme involved in APAP bioactivation, was investigated. A Treatment of the mice with AF resulted in a significant decrease in the CYP2E1-dependent aniline hydroxylation and CYP2E1 protein levels in dose dependent manner. AF exhibited antioxidant effects on Fe3+-ascorbate induced lipid peroxidation in mice liver homogenates, and in supernose radical scavenging activity. Our results suggest that the protective effects of AF against the APAP-induced hepatotoxicity possibly involve mechanisms related to its ability to block CYP2E1-mediated APAP bioactivation and induction of hepatic GSH levels and free radical scavenging effects.

THE EFFECTS OF RAYLESS GOLDENROD ON SPANISH GOATS.

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Rayless Goldenrod (Haplopappus Herophyllus) has been known to be toxic to livestock in the southwestern United States since the 1800’s. The putative toxic compound in Rayless Goldenrod has been named tremetol (a mixture of ketones and alcohols). Animals poisoned by eating Rayless Goldenrod become depressed, lethargic, stiff gaited, stand in a humped up position, and decline to move, which is indicative of muscle damage. Some livestock develop muscle tremors, especially after exercise. The objectives of this study were to determine the dose and the duration required to develop clinical signs of toxicity in Spanish goats. Four groups of four goats were dosed twice daily with dried, ground Rayless Goldenrod by oral gavage to give a daily dose of 0.5, 1, 2, and 3 percent of their body weight. Goats were exercised daily on a treadmill and EKG’s were recorded prior to, during, and following exercise. Serum enzyme concentrations of ALT, AST and CK were monitored daily. Goats dosed with 0.5 and 1% of their body weight showed no clinical signs and serum enzyme activities remained normal throughout the study. After 6 days of dosing with Rayless Goldenrod at 2 and 3% of their body weight many goats became weak, reluctant to exercise, and when forced they would stand with post-like straight legs and flexed backs and their tails flexed vertically. These animals had increases in ALT, AST and CK activities of 3, 10 and 15-fold, respectively. These severely affected animals also had nearly a two-fold increase in resting heart rate, as well as a prolonged recovery time to normal heart rate following exercise.
After 7 days of dosing the animals were euthanized, necropsied and tissues were collected for further studies. Histologic, ultrastructural, toxicokinetic, and additional biochemical studies are underway. In conclusion, we were able to reproduce Rayless Goldenrod toxicity in a goat model. This model will be used to definitively identify the toxin, define the pathogenesis of poisoning, describe the effect of poisoning and develop models to formulate prognosis for previously poisoned animals.

**806** SUPPRESSIVE EFFECT OF THE WATER EXTRACTS OF HOUTTUYNIA CORDATA ON ANAPLASTIC REACTION AND IMMUNOGLOBULIN E-MEDIATED ALLERGIC RESPONSE IN MAST CELLS.

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Mast cells are key effector cells in the early phase allergic inflammation and in diverse immunological and pathological processes. Houttuynia cordata water extract (HCWE) has been used as a traditional medicine in Korea and is known to have an antioxidant, anti-cancer and anti-allergic activities. The precise effect of HCWE, however, remains unknown. In this study, we investigated the effects of HCWE on anti-allergic effect on RBL-2H3 rat mast cell line on IgE-mediated passive cutaneous anaphylaxis (PCA) in mice. HCWE reduced the anti-dinitrophenyl (DNP) IgE-induced beta-hexosaminidase and histamine release in RBL-2H3 cells. Also, the oral administration of HCWE inhibited IgE-induced ear swelling was significantly reduced systemic passive cutaneous anaphylaxis reaction in mice. HCWE inhibited activating phosphorylation of Syk kinase, indicating that HCWE inhibits the activity of Src-family kinases in mast cells. In addition, HCWE inhibited NF-κB activation and MAP kinases, which are critical for the production of various pro-inflammatory cytokines in mast cells. Taken together, these findings suggested that HCWE has an anti-allergic activity and this might be useful for the clinical application to treat allergic diseases such as atopic dermatitis.

**807** IDENTIFICATION OF NOVEL MYCOTOXIN AND ITS CYTOTOXIC EFFECT ON HUMAN LYMPHOCYTE CELLS IN COMPARISON TO SOME OTHER MYCOTOXINS.


A hydrophobic novel metabolite was recently isolated from *P. polonicum* cultures. Its molecular mass and empirical formula were established as 390.2770 and C24H38O4, respectively, by means of chromatographic and mass spectrometric methods applied on liquid chromatography–tandem mass spectrophotometer (LC-MS/MS). The potential cytotoxicity of this novel compound was further tested in vitro, using the methylthiazol diphenyltetrazolium bromide (MTT) method. HCWE reduced the anti-dinitrophenyl (DNP) IgE-induced beta-hexosaminidase and histamine release in RBL-2H3 cells. Also, the oral administration of HCWE inhibited IgE-induced ear swelling was significantly reduced systemic passive cutaneous anaphylaxis reaction in mice. HCWE inhibited activating phosphorylation of Syk kinase, indicating that HCWE inhibits the activity of Src-family kinases in mast cells. In addition, HCWE inhibited NF-κB activation and MAP kinases, which are critical for the production of various pro-inflammatory cytokines in mast cells. Taken together, these findings suggested that HCWE has an anti-allergic activity and this might be useful for the clinical application to treat allergic diseases such as atopic dermatitis.

**808** HPLC FRACTIONATION OF AN EXTRACT OF COLA ACUMINATA.

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Cola Acuminata or Bizzy nut is a bitter, caffeine-containing nut found in the rainforests of Africa. It is used as a stimulant, an appetite suppressant, an aphrodisiac, to restore vitality and to ease hunger pains. In Jamaica, Bizzy nuts are grated and made into a tea by the ETTU people. The Bizzy nut has garnered a reputation as a rem

**809** INHIBITORY EFFECTS OF THE SAPONINS DERIVED FROM ROOTS OF PLATYCODON GRANDIFLORUM ON MAST CELL ACTIVATION.

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Mast cells are key effector cells in the early phase allergic inflammation and in diverse immunological and pathological processes. In this study, we investigated the anti-allergic effect of the saponins isolated from the roots of Platycodon grandiflorum (Changkil saponins, CKS) in RBL-2H3 rat mast cells and mice. CKS reduced the anti-dinitrophenyl (DNP) IgE antibody-induced beta-hexosaminidase and histamine release in RBL-2H3 cells. Also, CKS significantly reduced ear swelling on IgE-mediated passive cutaneous anaphylaxis (PCA) in mice. CKS inhibited phospholipase C (PLC) and antigen-induced Syk kinase in mast cells, indicating that CKS inhibits the activity of src-family kinases. CKS also suppressed antigen-induced MAP kinases, which are critical for the production of various pro-inflammatory cytokines in mast cells. The present results suggest that the anti-allergic activity of CKS is mediated through inhibiting degranulation by src-family kinase in mast cells and it may be useful for the treatment of mast cell-related immediate and delayed allergic diseases.

**810** INHIBITION OF THE SODIUM-IODIDE SYMPORTER BY PERCHLORATE: AN EVALUATION OF LIFESTAGE SENSITIVITY USING PBPK MODELING.


Perchlorate (ClO4-) competitively inhibits uptake of iodide by the sodium-iodide symporter (NIS) in laboratory animals and humans. NIS is found in many tissues, but is primarily responsible for sequestering iodide into the thyroid, enabling biosynthesis of thyroid hormones. The National Academy of Sciences (NAS, 2005) recommended the use of thyroid radioactive iodide uptake (RAIU) inhibition as the critical effect for determination of a reference dose (RfD) for ClO4-. An uncertainty factor of 10 was applied to the point of departure (0.007 mg/kg-day) to protect the most sensitive population, which was identified as the fetuses of pregnant women who might be hypothyroid or iodide deficient, to obtain an RfD of 0.0007 mg/kg-day. U.S. EPA's Integrated Risk Information System (IRIS) Program adopted the recommendations of the NAS in 2005. The present work evaluated physiologically based-pharmacokinetic models (Clewlow et al., 2007; Merrill et al., 2005) to assess lifestage relative sensitivity for use in human health risk assessment by evaluating model predicted percent RAlU inhibition by ClO4-. A six-step framework was developed for model evaluation (Chiu et al., 2007; Clark et al., 2004) was followed, and the structures were determined to be suitable for potential use in risk assessment. A limited sensitivity analysis of model parameters was conducted and minor modifications were made to model codes. Parameters describing urinary excretion of ClO4- and iodide were found to be particularly important in prediction of RAlU inhibition; therefore, a range of biologically plausible values was reviewed. The literature was evaluated. This draft analysis indicates that the near-term fetus (gestation week 40) is approximately 5-fold more sensitive at 7000 ng ClO4/kg body weight to RAlU inhibition than the average adult. (The views expressed are those of the authors and do not necessarily reflect the views or policies of U.S. EPA).
CHLOROFORM INHALATION HEALTH ASSESSMENT USING BENCHMARK DOSE MODELING: A COMPARISON OF ALTERNATIVE POINTS OF DEPARTURE.

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The current Integrated Risk Information System (IRIS) database entry for chloroform contains a reference value for chloroform via oral exposure only. Because chloroform is relatively volatile, humans can be exposed via inhalation to chloroform vapor. Chloroform is currently undergoing assessment within IRIS, which includes development of an inhalation reference value. Benchmark dose (BMD) modeling was used to quantitatively assess the impact of alternative choices on the point of departure (POD). These alternatives included the choice of endpoints selected for human extrapolation, which data sets to use as the basis for BMD estimation, and selection of the benchmark response (BMR) based on considerations of biological significance and severity of the endpoint. Data sets from a Japan Bioscience Research Center (JBRC) chronic study, three subchronic studies, and a developmental study were considered. Endpoints associated with chloroform inhalation exposure within these studies include lesions in the nasal cavity, kidney and liver, and malformation/growth retardation in the fetus. Various dose-response models available in EPA BMD software (BMDS) were fit to these study/endpoint combinations to establish PODs. BMD modeling generated a BMDL10 of 2.2 ppm for the nasal cavity endpoint (Kasai et al., 2002), a BMDL10 of 20.1 ppm for liver lesions (Yamamoto et al., 2002), and a BMDL5 of 0.97 ppm for developmental effects (Buerde and Hofmann, 1991). A BMR of 5%, rather than 10%, was used for the developmental endpoint because, as noted in EPA’s benchmark dose technical guidance (U.S. EPA, 2000), “[p]roductive and developmental studies having nested study designs often have greater sensitivity, and for such studies a BMR of 5% has typically been used”. [Disclaimer: The findings and conclusions in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.]

ANIMALS FROM INHALATION EXPOSURE TO ACRYLONITRILE.

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Acrylonitrile (AN) is widely used in the production of acrylic fibers, acrylonitrile-styrene resins, nitrile rubbers and as an intermediate in the production of other chemicals. The potential health hazard from inhalation exposure to AN has been evaluated in both epidemiological studies of occupationally exposed workers and animal studies. Neurobehavioral effects were identified as critical noncancer effects in occupational exposure studies. A LOAEL of 0.24 mg/m3 was determined, based on statistically significant performance deficits in a World Health Organization (WHO)-recommende Neurobehavorial Core Test Battery for mood, attention and speed, auditory memory, visual perception and memory, and motor steadiness in exposed workers. The LOAEL, adjusted to exposure for 24 hours/day, 7 days/week, was 0.086 mg/m3. Critical noncancer effects were observed in a 2-year inhalation bioassay of AN in Sprague-Dawley rats. Statistically significantly increased incidences of inflammatory and degenerative nasal lesions (hyperplasia of mucus-secreting cells in males and flattening of respiratory epithelium in females) occurred in rats exposed to the lowest level of 20 ppm AN in this chronic bioassay (6 hours/day, 5 days/week). Other nonneoplastic lesions with elevated incidences at the 80 ppm exposure level were gliosis and perivascular cuffing in the brain of males and females, focal nephrosis and thyroid cysts in males, and hepatic necrosis in females (which was also elevated at 20 ppm). Benchmark dose modeling was conducted on human equivalent concentrations converted from animal exposure data. The 95% lower bounds on the benchmark concentrations for 10% extra risk (BMCRL10) were determined to be 0.082 mg/m3 and 0.059 mg/m3 for nasal effects in male and female rats, respectively, and were identified as possible points of departure (POD). The PODs obtained from animal and human studies were similar. (The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.)

ORAL MINIMAL RISK LEVEL (MRL) FOR HEXAVALENT CHROMIUM.

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The general population is exposed to chromium by inhaling ambient air, ingesting food, and drinking water containing chromium. Chromium in food and water consists of mixtures of valence states that include chromium(III) and Cr(VI). Effects in humans associated with acute intoxication from ingestion of high doses of Cr(Vi) include caustic burns and hemorrhage of the gastrointestinal tract, metabolic acidosis, hepatic effects and renal failure. However, studies of outcomes of long-term exposures to humans are limited to ecological epidemiology studies that do not provide dose-response relationships suitable for derivation of oral MRLs. Studies in animals exposed to oral Cr(VI) for intermediate (e.g., subchronic) durations have reported hematological, gastrointestinal, hepatic, lymphatic and reproductive effects, with hematological effects (microcytic, hypochromic anemia) occurring at the lowest exposure levels. Microcytic, hypochromic anemia, characterized by decreased mean cell volume (MCV), mean cell hemoglobin (MCH), hematocrit (Hct), and hemoglobin (Hgb), has been reported in rats and mice exposed to serve as a biomarker of exposure but its toxicological significance is uncertain. The available database for the oral route of exposure is limited to a rat subchronic study by Mountain et al. (1953). This study reported a biologically significant reduction in RBC count (21%) at an average dose of 10.1 mg/kg/day in male Wistar rats. A NOAEL of 6.5 mg/kg-day could be established. Hemoglobin was decreased in orally dosed rats compared to controls at 6.5 mg/kg-day (5%) and 10.1 mg/kg-day (10%). These related findings may correlate with erythrocytopenia and anemia. The authors did not examine comprehensive hematological parameters. Furthermore, Zaporowska et al (1993) reported similar hematological effects in rats dosed orally to ammonium metavandenate (NH4VO3). Given the uncertainty and low confidence associated with Mountain et al. (1953) study, we further evaluated oral exposure studies of related vanadium compounds (Na2VO4, Na3VO4). Elfant and Keen (1987) reported significant decreases in percent litter survival at 7.0 mg/kg-day Na2VO4 in mice. These studies seem inappropriate surrogates for selection of a critical study for vanadium pentoxide but provide support for the proposal of 6.5 mg/kg-day (Mountain et. al. 1953) as the point of departure and decreased RBC count as the critical effect for the derivation of the RID. Studies using related vanadium compounds confirm that adverse effects may occur above an oral dose of 6.5 mg/kg-day. [Disclaimer: The views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.]

NEW CHRONIC MINIMAL RISK LEVEL FOR INHALED INORGANIC MANGANESE.

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Manganese (Mn) is an abundant element, an essential human nutrient, and a widely used component of many consumer products, including steel, fireworks, alkaline batteries, certain paints and the gasoline additive, methylocyclopentadienyl manganese tricarbonyl (MMT). In the workplace, high levels of chronic inhalation exposure to inorganic Mn have been associated with a Parkinsonian-type syndrome known as manganism which may or may not be reversible with cessation of exposure. Lower Mn exposure levels have been associated with more subtle health effects, including declines in cognitive, motor skill or male reproductive performance and cardiac difficulties. The 2000 ATSDR Toxicological Profile for Mn derived a chronic minimal risk level (MRL) for inhaled inorganic Mn of 0.4 ug/m3 using data from a study of 92 male workers in a dry alkaline battery plant exposed to manganese dioxide dust (Roels et al. 1992). An updated toxicological profile for Mn was released for public comment in October 2008. A newly revised MRL is derived from the Roels et al. (1992) study, using EPA Benchmark Dose methods to achieve a best fit to the incidence data for abnormal eye-hand coordination scores. The best-fitting model generated a BMCL10 of 0.1426 mg Mn/m3, which was selected as the new point of departure for deriving an MRL. Following adjustment to a continuous exposure basis, the point of departure was divided by a combined uncertainty factor of 100 (10 for human variability and 10 for limitations/uncertainties of the database). The new MRL of 0.003 mg/m3 is supported by data from a substantial number of other epidemiological studies of workers exposed to air concentrations ranging from about 0.1 to 2 mg Mm/M3. Significant associations with impaired performance on neurobehavioral tests were found in seven out of 10 of these epidemiological studies.
Cr(VI) compounds in drinking water or food for exposure durations ranging from 22 days to 6 months. ATSDR derived an intermediate-duration MRL of 0.005 mg/kg/day for Cr(VI) based on microcytic, hypochromic anemia. Dose-related, statistically significant decreases in MCV, MCH, Hct, and Hgb in male rats exposed to sodium dichromate dehydrate in drinking water for 22 days in a 2-year NTP study were identified as the critical effects. Benchmark dose modeling was conducted for MCV, MCH, Hct, and Hgb to derive the lower confidence limit (95%) on the benchmark dose associated with a change of 2 standard deviations from the control (BMDL2sd). Adequate BMDL was obtained for MCV, MCH, and Hgb, but not for Hct. The intermediate oral MRL was derived based on the average BMDL2sd values for MCV, MCH, and Hgb, with application of a composite uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

816 CHRONIC Минимальные РИСКОВЫЕ УРОВНИ (МРЛ) ДЛЯ ЦАДИУМА.

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For the general, non-smoking, population, dietary cadmium is the most likely route of exposure. The estimated daily intake of cadmium in nonsmoking adults living in the U.S. is 0.30-0.35 μg/kg. The most sensitive targets of cadmium toxicity in environmentally exposed populations and cadmium workers appear to be the kidney and bone following oral exposure and kidney following inhalation exposure. Adverse effect levels for renal effects appear to be similar to those observed for skeletal and respiratory effects and renal effects were used as the basis for ATSDR’s chronic oral and inhalation MRLs. The earliest indication of cadmium-induced kidney damage in humans is an increased excretion of low molecular weight proteins (e.g., β2-microglobulin, human complex forming glycoprotein [pH/C], and retinol binding protein) or intracellular enzymes (e.g., NAG). MRLs were based on a point of departure estimated from analysis of dose-response relationships from epidemiological studies of environmental exposures. Dose-response functions from each study were implemented to arrive at estimates of the minimum exposure corresponding to probabilities of 10% excess risk of low molecular weight proteinuria (urinary cadmium dose, UCCLD). The 95% lower confidence limit of this value (UCCLD) 0.35 μg/kg/day, was transformed into estimates of chronic cadmium intake that would result in the UCCLD at age 55 using a modification of the Nordberg-Kjellstrom (KN) model. The estimated dietary cadmium intake of 0.35 μg/kg/day was divided by an uncertainty factor of 3 for human variability resulting in a chronic-duration oral MRL of 0.1 μg/kg/day. For the inhalation MRL, an airborne cadmium concentration (combined with average cadmium dietary intake) which would result in a urinary cadmium level of 0.5 μg/g creatinine (UCCLD) was predicted using the ICRP Human Respiratory Tract and KN models. This air concentration of 0.1 μg Cd/m3 was divided by an uncertainty factor of 3 for human variability and modifying factor of 3 resulting in a chronic inhalation MRL of 0.01 μg/m3.

817 REFERENCE EXPOSURE LEVEL FOR 1-BROMOPROPANE. PROPOSED AS A SUBSTITUTE FOR PERCHLOROETHYLENE IN DRY CLEANING.

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Concern about the contribution of perchloroethylene to smog has led regulators in California to ban the use of perchloroethylene in dry cleaning by 2023. Candidate replacements, some already in use, include liquid carbon dioxide, mineral spirits, decamethylpentacyclosiloxane (D5), specific glycol ethers, and 1-bromopropane. An advantage of 1-bromopropane is that it can be used in existing perchloroethylene machines with little modification. It is not listed as a federal Hazardous Air Pollutant or as a California Toxic Air Contaminant. However, it is listed under California to ban the use of perchloroethylene in dry cleaning as a substitute for 1-bromopropane.

1. In a study conducted by the Environmental Protection Agency, Washington, DC.

818 ANALYSIS OF EMISSIONS SOURCE CONTRIBUTIONS OF ARSENIC, MANGANESE, AND MERCURY AND THEIR IMPLICATIONS FOR THE PROPOSED OEHHA NONCANCER REFERENCE EXPOSURE LEVELS.

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The Office of Environmental Health Hazard Assessment (OEHHA) is in the process of finalizing the 2007 draft guidance Air Toxics Hot Spots Program Technical Support Document for the Derivation of Noncancer Reference Exposure Levels (RELs). In this guidance, OEHHA proposed revisions to the methodology for deriving public health protective, eight-hour, chronic inhalation RELs and increased the stringency of health protective levels for six chemical compounds, three of which are metals. The RELs are health-based values used to evaluate noncancer risk within the risk assessment process. For hazard identification of air releases, the California Air Resource Board (CARB) maintains an inventory of toxic and criteria data for large point sources, areawide sources, and over a 100 mobile sources. The annual mass contribution by source categories were differentiated to identify high emission sources of arsenic, manganese, and mercury, thereby, facilitating the attribution of risk from relevant source exposures. To locate high emission regions of arsenic, manganese, and mercury, the proposed RELs were compared to statewide and regional air quality data. For regions with ambient concentration that exceeded the RELs, the 2006 California Toxics Inventory was analyzed to rank source categories by emissions release. State-wide background concentrations were above the RELs for manganese, and exceedances were detected in counties throughout northern and southern California. For arsenic, background concentrations exceeded the RELs in Imperial and San Bernardino counties of Southern California and in North and South Coast air basins, respectively. For all three metals, areawide sources were identified as generating higher rates of emissions release and more probable in contributing a greater extent to health risk than those of large point and mobile sources. Efforts to mitigate human health risk from the emissions of these metals will require coordination among diverse industries associated with areawide sources that include petroleum refinement and agricultural processes.
data available from human exposures (medical and forensic experience as well as subacute or chronic poisonings in inorganic arsenic in drinking water or food) and from very limited experiments on rats, respectively. PAL estimates for inorganic arsenic will be presented.

### 821 PROVISIONAL ADVISORY LEVELS (PALS) FOR PHOSPHINE (PH3)

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PALS were developed by the US EPA to represent general public emergency exposure limits for oral and inhalation exposures for hazardous materials corresponding to three severity levels and durations of 24 hrs, 30 and 90 d, and 2 yrs. PAL 1, 2, and 3 severity levels represent the threshold for mild effects, serious/irreversible/escape-impairing effects, and lethal effects, respectively. The PAL protocol has been applied to estimate oral and inhalation exposure limits for PH3. PH3 is a pesticide released as vapor from aluminum phosphide tablets used to fumigate bulk grain or it is incorporated into rodent bait as zinc phosphide and released in the gastrointestinal tract. PH3 is highly toxic by the oral and inhalation routes in humans and in animals. It is absorbed very rapidly from the respiratory and gastrointestinal tracts and inhibits mitochondrial respiration via non-competitive inhibition of cytochrome c oxidase. Although PH3 affects many organ systems in the body, its primary target organs are the gastrointestinal tract, cardiovascular system, and the central nervous system. Data were available for dermal and inhalation PAL 1, 2, and 3 values for 24 h, 30 d, 90 d, and 2 years; these will be presented.

### 822 PROVISIONAL ADVISORY LEVEL (PAL) DEVELOPMENT FOR METHYL ISOCYANATE (MIC)

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PALS were developed for hazardous materials by the US EPA to represent general public emergency exposure limits for oral and inhalation exposures corresponding to three different severity levels (1, 2, and 3) for 24-hr, 30-d, 90-d, and 2-yr durations. PAL 1 represents the threshold for mild effects; PAL 2 represents the threshold for serious, irreversible or escape-impairing effects; PAL 3 represents the threshold for lethal effects. PALS have not been promulgated nor have they been formally issued as regulatory guidance. They are intended to be used at the discretion of risk managers in emergency situations when site specific risk assessments are not available. Application of PAL protocols has been performed for MIC to estimate oral and inhalation exposure limits, as experimental data permit. Mevinphos is a restricted use organophosphate pesticide that inhibits plasma, erythrocyte, and brain cholinesterase activity in several species. Lethal reports of mevinphos poisoning in humans show that exposure results in tremors, convulsions, and pulmonary edema. Short-term non-lethal doses cause inhibition in plasma and erythrocyte cholinesterase activity, increased Achilles tendon reflex force, and decreased slow muscle fiber nerve conduction velocity. No clinical signs of intoxication were observed at non-lethal experimental doses. In rats, mevinphos exposure causes generalized tremors, miosis, salivation, lacrimation, impaired mobility, convulsions, respiratory failure. At higher concentrations, the symptoms are more pronounced and severe. Oral PAL estimates were recommended at each severity level and duration. Inhalation PAL 2 and 3 estimates were recommended for the 24-hr duration. Insufficient data were available to derive inhalation PAL values for 30-d, 90-d, and 2-yr durations. PAL estimates were approved by the Expert Consultation Panel for PALS in July 2008 and will be presented.

### 823 PROVISIONAL ADVISORY LEVELS (PALS) DEVELOPMENT FOR MEVINPHOS

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PALS were developed for hazardous materials by the U.S. EPA to represent general public emergency exposure limits for oral and inhalation exposures corresponding to three different severity levels (1, 2, and 3) for 24-hr, 30-d, 90-d, and 2-yr durations. PAL 1 represents the threshold for mild effects; PAL 2 represents the threshold for serious, irreversible or escape-impairing effects; PAL 3 represents the threshold for lethal effects. PALS have not been promulgated nor have they been formally issued as regulatory guidance. They are intended to be used at the discretion of risk managers in emergency situations when site specific risk assessments are not available. Application of PAL protocols has been performed for mevinphos to estimate oral and inhalation exposure limits, as experimental data permit. Mevinphos is a restricted use organophosphate pesticide that inhibits plasma, erythrocyte, and brain cholinesterase activity in several species. Lethal reports of mevinphos poisoning in humans show that exposure results in tremors, convulsions, and pulmonary edema. Short-term non-lethal doses cause inhibition in plasma and erythrocyte cholinesterase activity, increased Achilles tendon reflex force, and decreased slow muscle fiber nerve conduction velocity. No clinical signs of intoxication were observed at non-lethal experimental doses. In rats, mevinphos exposure causes generalized tremors, miosis, salivation, lacrimation, impaired mobility, convulsions, respiratory failure. At higher concentrations, the symptoms are more pronounced and severe. Oral PAL estimates were recommended at each severity level and duration. Inhalation PAL 2 and 3 estimates were recommended for the 24-hr duration. Insufficient data were available to derive inhalation PAL values for 30-d, 90-d, and 2-yr durations. PAL estimates were approved by the Expert Consultation Panel for PALS in July 2008 and will be presented.

### 824 HEALTH RISK ASSESSMENT OF 2-ETHYLBANOL IN DRINKING WATER

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Acceptable levels of 2-ethylhexanol in drinking water were determined based on Annex A of NSF/ANSI 61 (2007a). 2-Ethylhexanol is in the extract water of plastic pipes that convey drinking water. While limited toxicity data for 2-ethylhexanol in humans were identified, numerous studies have evaluated the toxicity of oral exposure to 2-ethylhexanol in laboratory animals. Repeated gavage exposure with 2-ethylhexanol was associated with reductions in mean body weight and altered organ weights in rats and mice in the absence of specific target organ toxicity. Rats were more sensitive than mice, since the body weight reduction occurred at lower dose levels in rats. Developmental toxicity after 2-ethylhexanol included skeletal malformation and retardation with reduced fetal body weight accompanied by signs of maternal toxicity in dams. Lifetime exposure of female mice to 2-ethylhexanol by gavage was associated with an increase in the incidence of hepatocellular carcinomas that exceeded the concurrent control incidence, but were within the historical control range for gavage studies reported by the NTP. There was no significant difference in hepatocellular adenomas or carcinomas in treated male mice or male or female F344 rats. 2-Ethylhexanol is a peroxisome proliferator, and this mode of action may be related to or influence the development of hepatocellular carcinomas. While some rodents are highly susceptible to peroxisome proliferation, humans and other primates are resistant. The weight of genotoxic evidence suggests that 2-ethylhexanol is not mutagenic or clastogenic. It was concluded that the data are inadequate for an assessment of human carcinogenic potential under U.S. EPA (2005) guidelines. The RfD of 0.1 mg/kg-day 2-ethylhexanol was based on the NOAEL of 36 mg/kg-day for reduced mean body weight and altered organ weights in rats and mice with a total uncertainty factor of 300x to account for inter- and intraspecies extrapolation and the lack of a two-generation reproduction study. The RfD was used to determine the Total Allowable Concentration in drinking water of 0.8 mg/L.

### 825 THE CHALLENGE OF SETTING REFERENCE DOSES (RFDs) FOR POLYOLS THAT ARE CONSTITUENTS OF FOODS, BEVERAGES, DRUGS, AND MUNITIONS

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Pentaerythritol (PE) was detected in a private well in Massachusetts at a concentration of 800 mg/L. In order to assess the potential human health hazard associated with the domestic use of the well water, we sought toxicological information on PE to support derivation of an RfD. PE is a sugar alcohol or polyol that is manufactured for use in explosives, pharmaceuticals and various other products. Since the toxicological database on PE was limited, toxicological data on a structurally related compound, erythritol, was also assessed to derive an RfD for PE. Erythritol belongs to a class of polyols whose toxicity has been extensively studied in both animals and humans because they are commonly used as food additives and sugar substitutes. Both compounds are well absorbed and excreted in the urine unchanged, and both seem to have similar adverse effects on the gastrointestinal (GI) tract and the kidney. The GI effects include diarrhea and increased caloric vessel, while the effects in the kidney include diuresis and increased water consumption, serum and urine electrolyte levels and cellular toxicity. It was concluded that the toxicity of PE is similar to that of erythritol and that the RfD for PE may be calculated from the RfD for erythritol.
imbalance, and changes in kidney weight and structure, including renal calcification. The estimated chronic RfDs based on animal studies are 15 mg/kg/d for PE and 17 mg/kg/d for erthylrot. The chronic RfD for erthylrot based on a human study is 22 mg/kg/d. The RfD for PE can be used to derive an allowable drinking water concentration. Typically, drinking water values are calculated using a standardized approach based on a 70 kg person consuming 2 liters of water per day and a default source apportionment factor of 20%. The challenge regarding the application of a source apportionment factor, however, is that the reported 50th and 90th percentile total daily consumption of polyols from dietary sources, as these compounds are widely used in foods and beverages, ranges from 14 to 86 and 57 to 228 mg/kg/d respectively. These values are close to or much higher than the RfD estimated for PE.

826 PFOA IN NEW JERSEY DRINKING WATER: OCCURRENCE, EXPOSURE SIGNIFICANCE, AND HEALTH-BASED GUIDANCE.

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Perfluorooctanoic acid (PFOA) is ubiquitous in the blood of humans, with a mean serum concentration in the US of about 4 ug/L. Sources of PFOA exposure include food, house dust, consumer products, and drinking water. Human exposure is of concern because of PFOA’s long half-life and toxicity. Little is known about the general occurrence of PFOA in drinking water or its contribution to total human exposure. PFOA was found in 24 of 30 (80%) of NJ public water supplies tested at concentrations ranging from <0.004-0.19 ug/L. These concentrations may contribute significantly to total human exposure, based on an approximate 100:1 ratio between the concentration of PFOA in serum and in drinking water observed in OH and WV communities with both high (<3 ug/L) and low (<0.1 ug/L) drinking water levels. A lifetime drinking water guidance for PFOA was developed based on evaluation of NOAELs and LOAELs, as well as cancer data, from animal studies identified in a USEPA draft risk assessment (2005). Since the half-life of PFOA in humans is much longer than in animals, the drinking water guidance was based on comparison of blood levels in animals and humans, rather than on administered doses. The 100:1 ratio between serum and drinking water concentrations of PFOA was used to develop health-based drinking water concentrations for non-cancer and cancer endpoints. The most sensitive endpoints were decreased body weight and hematological effects in a chronic study in female rats. The guidance value based on these endpoints is 0.04 ug/L, while the drinking water concentration based on cancer at the 1 x 10^-6 risk level is 0.06 ug/L. Recent data from animal and human studies not considered in the USEPA risk assessment, including developmental effects in mice and decreased birth weight and other measures of fetal growth in humans, further support this health-based drinking water guidance. While PFOA in most NJ water supplies was below the health-based drinking water guidance of 0.04 ug/L, several NJ water supplies exceeded this concentration.

827 HEALTH RISK ASSESSMENT OF INDOOR AIR QUALITY AT A PUBLIC HOUSING PROJECT.

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A 300-unit public housing project is located on a portion of a former petroleum storage facility that had occupied more than 100 acres in Southern California. The petroleum storage facility operated for about 40 years, closing in 1965, and the housing project was constructed in the early 1970s. Concerns about potential health effects among the housing project residents triggered several rounds of environmental site investigation beginning in the 1990s. In 2007, a regulatory agency order required a detailed investigation of soil, soil vapor, and groundwater contamination and a site-specific human health risk assessment. Because indoor air quality was a primary health concern, soil vapor, indoor air, and outdoor air samples were concurrently collected for analysis by EPA Method TO-15. Soil vapor analysis revealed petroleum hydrocarbon constituents (e.g., benzene) at concentrations of health concern. The highest concentrations occurred in the deepest soil vapor samples (52 feet below ground surface, bgs) and concentrations declined in the samples from intermediate (15 feet bgs) and shallow depths (5 feet bgs). Of the 13 volatile organic chemicals (VOCs) detected in soil vapor samples, only five were also detected in indoor air, which indicated that chemicals in soil vapor were not migrating to indoor air. Comparison of indoor air and outdoor air samples revealed that an identical suite of chemicals was detected in each medium and that the concentrations of these chemicals were equivalent. Furthermore, the indoor air and outdoor air concentrations from the subject site were equivalent to the concentrations reported for ambient air in an extensive study of background VOC concentrations in the regional airshed. In conclusion, these data indicate that residual petroleum hydrocarbon constituents in soil vapor are not migrating to indoor air at levels of health concern and indoor air quality at the public housing project is consistent with the ambient background air quality of the region.

828 HUMAN HEALTH RISK ASSESSMENT OF EXPOSURE TO PESTICIDES IN PARLIER, CALIFORNIA.

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As part of the California Environmental Protection Agency (Cal/EPA) Environmental Justice Action Plan, the Department of Pesticide Regulation (DPR) has set a goal to analyze the ambient air in a variety of communities in California for traces of pesticidal compounds. DPR selected Parlier, a small community in Fresno County and collected environmental data. Air monitoring was performed throughout the year of 2006 with the purpose of being able to determine if residents were exposed to pesticidal compounds in the ambient air at levels of concern. Thirty pesticides and breakdown products, including the majority (92.5%) of compounds that the Parlier residents exposed to were well below the reference levels considered ‘safe’.

829 HUMAN HEALTH RISK ASSESSMENT OF CONSUMPTION OF FISH FROM THE LOWER PASSAIC RIVER.

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The Lower Passaic River (LPR) in New Jersey has been substantially impacted by various human activities for more than two centuries. We conducted a human health risk assessment (HHRA) of fish consumption along the LPR in New Jersey by incorporating specific information in lieu of default exposure assumptions. Fish consumption information gathered during a year-long, intercept-style creel angler survey conducted along the LPR and representative LPR fish tissue concentrations for more than 150 chemicals of potential concern (COPCs) obtained from USEPA’s analytical LPR fish data publicly available on Oceans.usace.army.mil were used as the basis for the risk assessment. This HHRA is comprised of two phases: (1) identification of COPC groups that contribute to the majority of overall excess cancer risk and hazard estimates using deterministic and probabilistic methods, and (2) probabilistic characterization of risk using distributions of chemical concentration and cooking loss for those compounds identified in Phase 1. Phase 1 relied on CT and RME point estimates of COPC concentrations and demonstrated that PCDD/Fs and PCBs (dioxin-like and non-dioxin-like) are the greatest contributors to non-cancer risk, while non-dioxin-like PCBs are the primary contributors to non-cancer hazard estimates. Phase 1 results for total excess cancer risks for child and adult receptors were within USEPA’s acceptable cancer risk range, with the exception of RME child (3.4x10^-4 and 1.4x10^-4 for deterministic and probabilistic approaches, respectively). Phase 2 focused on PCDD, PCDF and PCBs and used distributions of chemical concentrations in fish, taking into account cooking loss. The results showed that all excess cancer risk estimates were within the acceptable risk range, although non-cancer hazard estimates for PCBs slightly exceeded a Hazard Index of 1. This HHRA of LPR fish consumption represents the most comprehensive evaluation conducted to date and demonstrates that measured COPCs are not likely to pose a health risk to people who currently consume fish from the LPR.

830 HUMAN HEALTH RISK ASSESSMENT FOR ENVIRONMENTAL APPLICATIONS OF STEEL SLAG: DIFFERENCES BETWEEN MATERIAL-SPECIFIC AND DEFAULT APPROACHES.

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Steel slag is generated from steel production, consisting of the minerals that are incompatible with steel. Its use commercially for construction and agriculture results in the potential for exposure to human populations. Certain metals in steel slag
occur at concentrations that are higher than in soils, and manganese (Mn) levels in slag are of potential human health concern. Although some metals exceed screening levels for metals in soil, there are several physical/chemical properties of slag which limit environmental mobility and bioaccessibility. These factors should be quantified in any material-specific risk assessment. Metals in slag exist as metal oxides tightly bound to the slag matrix, and heavy metals are concentrated in larger slag particles, such that concentrations lessen with decreasing particle size. A comparative health risk assessment of environmental steel slag applications, focused on incidental ingestion for a residential application, was conducted to evaluate the difference in calculated potential hazards using material-specific factors relative to default ones. Important risk assessment parameters include the use of appropriate exposure point concentrations (EPCs), considerations of bioaccessibility, factoring frequencies of exposure to slag applications, and the use of the EPA modifying factor (MF) with the Mn RfD. The assessment was conducted using EPCs based on reliable analytical methods, exposure frequencies based on time-use data, and slag-specific measures of the bioaccessibility of Mn and vanadium ranging from 20% to 70% and of 20% to 30%, respectively. Exposures to metals were quantified and compared to the EPA Mn RfD without use of the MF because Mn in steel slag is not associated with drinking water exposures or exposures to neonates, and uptake of Mn is tightly regulated by homeostatic controls. The comparative assessment demonstrated that exposures to metals in slag are below the level of potential concern with a hazard index of 0.8, and that hazards are approximately 10-times lower than that estimated using default approaches.

831 AN ASSESSMENT OF THE POTENTIAL HEALTH HAZARDS ASSOCIATED WITH EXPOSURES TO CHRYSTOTILE ASBESTOS IN DRYWALL ACCESSORY PRODUCTS.

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Historically, short fiber chrysotile asbestos was added to industrial joint compounds and consumer spackling and patching compounds to serve as a reinforcing agent designed to control shrinkage and cracking as the materials dried. As is the case with numerous historic asbestos-containing products, some concerns have emerged regarding the potential health risks associated with former use of these compounds. In this study, we summarize: 1) the published and unpublished literature describing fiber type and fiber length data for historic drywall accessory products, 2) animal toxicology data involving inhalation exposures to short-fiber chrysotile, and 3) epidemiologic and other data available for workers engaged in drywall related occupations. These factors should be quantified and compared primarily to short-fiber chrysotile. The results of our analysis indicate that: 1) the vast majority of asbestos fibers in joint compound were likely to have been less than five microns in length, and were, therefore, too short to pose a risk of disease, 2) laboratory animal studies evaluating long-term exposures to short chrysotile consistently report an absence of asbestos-related diseases, even at exposures in great excess of those experienced by career drywallers, and 3) mortality studies of drywall workers do not indicate an increase in mortality from asbestos-related disease. We conclude that the weight of evidence suggests that drywallers and, consequently bystanders to drywalling activities, were not historically at risk for developing asbestos-related disease.

832 EVALUATION OF RISK FOR FETAL LIMB DEFECTS FROM OCCUPATIONAL EXPOSURE TO MANCOZEB AND ETHYLENE THIOUREA DURING PREGNANCY.


Ethylene thiourea (ETU) and Mancozeb, which metabolizes to ETU in the environment, are two pesticides used on tomato farms. ETU is associated with developmental toxicity in rats, including some forms of limb defects, while Mancozeb is associated with fetal effects, but only at maternally toxic doses. We evaluated the potential for these pesticides to cause limb defects in human fetuses from maternal exposures to a hypothetical farm worker. Using information about recommended application rates, treatment regimens, and the environmental fate of the applied pesticides, we estimated potential worst-case maternal exposures during the critical period for limb development (gestation days 28-42) and compared to doses that cause developmental toxicity in animals. To estimate maternal dose we examined the following exposure pathways: 1) inhalation of sprayed pesticides, 2) dermal exposure to sprayed pesticides, 3) incidental exposure to soil, 4) dermal contact with soil, 5) incidental ingestion of residue on plant, 6) dermal exposure to residue on plant, and 7) inhalation of fugitive dust. The United States Environmental Protection Agency (US EPA) air dispersion model, AERMOD was used to determine pesticide concentration in air (for inhalation exposure) and deposition rate (for dermal exposure). To evaluate potential risk, we used a margin of exposure (MOE) approach. Using our multi-exposure pathway analysis, we demonstrated that MOEs for Mancozeb for each pathway ranged from over 1,000,000 and that MOEs for ETU for each pathway ranged from over 5,000,000, indicating a negligible risk of fetal limb defects from occupational exposure (via maternal exposure) to these pesticides.

833 POTENTIAL FOR OCCUPATIONAL EXPOSURES OF HAIRDRESSERS TO VINYL CHLORIDE IN HAIRSPRAY (1967-1974).

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Vinyl chloride was used as a propellant in a small percentage of aerosol hairspray products in the U.S. from approximately 1967 to 1973. Since that time, questions have arisen regarding whether occupational exposures of hairdressers to vinyl chloride in hair salons would be sufficient to increase the risk for developing hepatic angiosarcoma (HAS) which has been linked definitively with vinyl chloride exposures among industrial workers with significant chronic vinyl chloride exposures. Since the hairspray products used during this time are no longer available to use for simulation purposes and existing measurement data are sparse, a modeling approach was used to estimate the potential for exposures to hairdressers during their time period. A transient two-zone model along with comparison estimates from the steady-state imperfect mixing model were used to estimate the airborne concentration of vinyl chloride for both individual hairdressers spraying hairspray and background concentrations of vinyl chloride in hair salons over time, because the emission rate of hairspray was not constant. Near field and far field concentrations of vinyl chloride were modeled over time for representative small, medium, and large salons as well as a representative home salon. Ventilation and air movement characteristics, air exchange rates, salon size, the number of hairdressers, and the number of customers were determined using published literature and variability in these parameters was also considered using Monte Carlo techniques. The modeling results were also compared against airborne exposure measurements in the literature for a number of products with the potential to produce airborne concentrations in hair salons. The results indicate that the lifetime cumulative doses via inhalation of vinyl chloride for hairdressers for the time period in which it was used in hairspray products are more than 10 to 100 fold lower than those found among industrial manufacturing workers who developed HAS.

834 INHALATION RISKS OF MERCURY IN INDOOR AIR FROM BENEFICIAL USE OF COAL COMBUSTION PRODUCTS (CCPS) IN BUILDING MATERIALS.

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Coal combustion products (CCPs), including coal fly ash (CFA) and the gas desulfurization (FGD) residues, are receiving increasing use as substitutes for traditional substances in building materials. For example, approximating 30% of U.S. wallboard is manufactured from FGD synthetic gypsum, while CFA has gained widespread usage as a partial replacement for Portland cement in concrete. Questions have been raised regarding the presence of mercury (Hg) in CCPs and the potential for Hg releases from CCP building materials into indoor air, and we conducted a screening-level assessment to estimate worst-case inhalation risks from indoor air exposures to Hg for two CCP utilization scenarios: (1) FGD synthetic gypsum wallboard used in a school classroom or home, and (2) CFA concrete blocks used in a school classroom. For CFA concrete, we relied on data from recent laboratory studies of Hg emissions during dry curing of concrete at near-ambient temperatures to calculate two Hg release rates to indoor air, one based on the maximum 28-day curing rate from these studies to represent worst-case emissions and one based on extrapolated long-term emissions to represent upper-bound, long-term emissions. For wallboard, we calculated a Hg release rate based on laboratory flux chamber measurements for synthetic gypsum wallboard samples. We estimated indoor air Hg concentrations using these Hg release rates and conservative values for other parameters (e.g., air exchange rates, material loading ratios) in a steady-state indoor air model. Even using parameters intended to overstate potential exposures, we predicted indoor air Hg concentrations that are generally consistent with or below ambient background Hg levels. Moreover, predicted indoor Hg concentrations were well below estimated inhalation toxicity criteria (hazard indices ranged from 0.0002 to 0.016). Thus, based on our findings, we conclude that CCPs in concrete and wallboard building materials are unlikely to result in Hg exposures in either classroom settings or in residential homes that pose a health concern.
The beneficial use of coal combustion products (CCPs) conserves natural resources, reduces landfill waste, and may also improve the quality and performance of construction materials. Concerns remain, however, about potential exposures to Hg in CCPs, including coal fly ash (CFA) used in concrete and structural fill, as well as the use of CCPs as cement replacement in wallboard. Moreover, with potential regulations requiring that Hg emission reductions, Hg concentrations in CCPs are likely to increase. Therefore, to examine potential exposures to Hg emissions from CCPs, we conducted a screening-level risk assessment to determine worst-case inhalation exposures to Hg for two outdoor exposure scenarios: (1) CFA used as structural fill material, and (2) CCP concrete and wallboard disposed of in a construction & demolition (C&D) landfill. For the structural fill scenario, we considered both fugitive dust emissions containing inorganic Hg and volatilization of elemental Hg from the CFA. For the landfill scenario, we considered only volatilization of elemental Hg from CCPs in concrete and wallboard. We estimated outdoor Hg air concentrations using US Environmental Protection Agency’s (US EPA’s) Wind Erosion Model and the Screen 3 Model applying conservative, worst-case assumptions. To estimate potential risks, we compared the modeled air concentrations to toxicity criteria to determine if exposures to children and adults were above a level of health concern. Our results showed that Hg exposures via inhalation from the CCP materials are near or below ambient Hg levels and several orders of magnitude lower than health-based toxicity criteria. Our findings thus indicate that upper-end exposures to Hg from the beneficial use of CCPs in structural fills, and from disposal of building materials containing CCPs, are not expected to pose an inhalation health risk.
critical adverse effect (ovarian toxicity) with a clear NOAEL of 250 ppm (47 ppm when adjusted for continuous exposure) was identified. A benchmark dose evaluation also resulted in a BMDL value of 49 ppm. A total uncertainty factor of 100 was used: 10 (intraspescies variability), 10 (subchronic to chronic), and 1 (inter-species) because of mouse/human differences in VCH disposition and similarity between VCH and 1,3-butadiene metabolism. A non-cancer reference value of 0.47 ppm (470 ppb) was derived. Based on the proposed MOA, this value is expected to be adequately protective against ovarian tumors.

Emissions related to airport operations, including particulate matter and gas-phase hazardous air pollutants (HAPs), can affect local air quality, and may pose a health concern for individuals at the airport and in surrounding communities. Airport managers are increasingly asked to evaluate potential health risks for airport employees and nearby residents. However, for many HAPs, there are insufficient data to adequately conduct such evaluations. The aim of this analysis was to prioritize airport HAPs in terms of emissions and toxicity, to identify areas for future research. Emissions were estimated for 56 HAPs emitted from aircraft and ground service equipment. We calculated risk-based concentrations (RBCs) for cancer and non-cancer endpoints for 30 HAPs, using published toxicity criteria along with standard methodology recommended by the U.S. EPA. We then quantitatively ranked these 30 HAPs based on the quotient of emissions relative to the RBC. For airport-related HAPs without published toxicity criteria, we searched for published, peer-reviewed test data, with no conclusions of safe compound or published toxicity criteria. For nine of these HAPs, we identified sufficient information, or toxicity criteria for suitable surrogates, to qualitatively assess emissions relative to potential toxicity. For the 30 HAPs with published toxicity criteria, the quantitative ranking indicates that acrolein represents the most significant health risk. Additional prioritized HAPs include formaldehyde, 1,3-butadiene, naphthalene, benzene, acetaldehyde and ethylbenzene. A significant finding was that several HAPs without published toxicity criteria may, in terms of emissions and potential toxicity, pose a health risk comparable to that of the prioritized HAPs. These HAPs include the following aldehydes: crotonaldehyde, glyoxal, methylglyoxal and propanal. Additional toxicology studies for these aldehydes would provide valuable information for conducting more rigorous, quantitative analyses. (Analysis funded by ACRP Project 02-03).

Use and disposal of household consumer products have become important topics within the field of risk assessment. Sodium hypochlorite (bleach) is used in a variety of consumer cleaning products. Although bleach has been used as a household cleaner for over 50 years, public concern about the potential environmental risks associated with the use of bleach-based cleaning products continues. The purpose of this assessment was to evaluate the potential human and ecological health risks associated with halogenated and other chemical byproducts that are generated as a result of residential bleach use. A total of 23 halogenated organic and two inorganic (chlorate, perchlorate) compounds were considered in this human and ecological health risk assessment. Using conservative models (EFAS and EUSES) and input parameters, our risk assessment revealed that down the drain disposal of bleach results in exposures well below predicted or currently available no effect health benchmarks. Conservatively predicted tapwater, surface water, soil, and sediment concentrations for the halogenated organics and inorganics were below their respective health screening criteria by a factor of at least 10 to 1000 times for the vast majority of chemicals evaluated. Underlying uncertainties were evaluated for chemicals within a factor of 10. Overall, these results suggest a large margin of safety for the secondary exposure to halogenated organics in treated tapwater, wastewater, surface water, soil, and sediment derived from household drain disposal of liquid bleach. These findings show that household usage of bleach contributes a negligible fraction of halogenated organics to surface water as compared to municipal and industrial wastewater treatment processes and would not necessitate further testing or risk reduction. As such, this assessment demonstrates that halogenated organic byproducts formed as a result of residential bleach use are highly unlikely to cause adverse health or ecological effects.

482 HAZARD EVALUATION OF 6-2 FLUOROTOLEMOR ALCOHOL (6-2 FTOH), 1, 1, 2- TETRAHYDROPERフルオロオクトANOL.

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6-2 Fluorotolomer alcohol (6-2 FTOH) was evaluated for acute, genetic, subchronic, reproductive, developmental, and aquatic toxicity. 6-2 FTOH was slightly toxic by the oral and dermal routes with an oral LD50 of 1750 mg/kg and a dermal LD50 > 5000 mg/kg in rats, and did not produce a dermal sensitization response in mice. 6-2 FTOH was not mutagenic in the bacterial reverse mutation test or in the mouse lymphoma assay and was not clastogenic in a chromosome aberration assay in human lymphocytes. 6-2 FTOH was moderately toxic to aquatic organisms. The 96-hour LC50 in fathead minnows was 4.8 mg/L, 48-hour EC50 in Daphnia magna was 7.8 mg/L, and 72-hour EC50 in Pseudokirchneriella subcapitata was 4.5mg/L. In subchronic, one-generation reproductive, and developmental toxicity studies, 6-2 FTOH was administered to rats by oral gavage. Mortality was observed in the subchronic study at 125 and 250 mg/kg/day; the deaths occurred after about three weeks of dosing and continued sporadically. Other effects observed at these doses included decreased body weights, changes in organ weights, and pathological findings involving the liver and kidneys as well as effects on the teeth. Observations at 125 and 250 mg/kg/day in the reproductive study included reduced litter size, increased pup mortality during lactation, reduced uterine weights, and reduced offspring weights. Effects in the developmental study were limited to increased incidences of skeletal body variations, low maternal body weights and food consumption at 125 and 250 mg/kg/day. There was no maternal mortality nor were there any test substance-related gross observations in the developmental study. There was no effect on fetal body weight or litter sex ratio and there were no effects on the incidences of fetal resorptions or other malformations at any dose level. These data indicate a conservative risk assessment for 6-2 FTOH based on the proposed MOA, this value is expected to be adequately protective against ovarian tumors.

PROBABILISTIC LEAD RISK ASSESSMENT FOR A COMMUNITY WITH AN OPERATING SMELTER.

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Human health exposure assessment in communities with major operating air emission sources requires consideration of unique site conditions when using generic intake models. A U.S.EPA probabilistic lead model was used to estimate the contribution of lead via multiple exposure pathways for children. Site-specific data from a community in a developing country with an operating smelter were incorporated into the model for estimating lead intake from the diet, drinking water, dust, air and soil. Due to the unique living conditions of this community and on-going dust emissions from the operating smelter, the model was modified to account for intake of lead via indoor and outdoor dust. Default lead bioavailability values were not modified. Blood lead data collected for children under the age of six were used to calibrate the model and to back-calculate soil and dust ingestion rates. In the community closest to the smelter, intake of lead via ingestion of outdoor and indoor dust was estimated to contribute approximately 69% and 14%, respectively, of total lead intake while soil ingestion had an estimated contribution of 9%. Dietary intake of lead was measured in a duplicate diet study and contributed approximately 9% of total lead intake, greater than intake reported in developed countries. The modified model was then used to predict future blood lead levels if smelter emissions were reduced. An update to the risk assessment conducted after stack and fugitive lead emissions were reduced showed good agreement with the significant observed decline in blood lead levels of young children. In the updated models, intake of lead via ingestion of outdoor dust was reduced relative to other pathways and was estimated to contribute 56%. The indoor dust and soil contributions increased to 23% and 12%, respectively. Dietary intake decreased to approximately 6% and the contributions of air and water were nominal.

PROBABILISTIC RISK ASSESSMENT OF INCREMENTAL RISK BETWEEN SITE AND BACKGROUND ARSENIC IN SOIL.


A site in the Northeastern United States with soil arsenic concentrations resulting from natural background conditions and contamination from chemical manufacturing activities was evaluated through deterministic and probabilistic risk assessments. The deterministic risk assessment using default assumptions found that even background arsenic levels ranging up to 20 mg/kg would be of concern for cancer risks, making it difficult to identify areas requiring remediation. Thus, a probabilistic risk assessment (PRA) was performed to yield more useful quantitative and qualitative evaluation.
ive risk information to compare site and background conditions and to support the remediation process. Background conditions were estimated using data from a nearby town not impacted by chemical manufacturing activities and considering differential land use weighting (e.g., proportion of historical orchard use because ar- senical pesticides are often used in orchards). Background samples were divided into land use subsets (orchard, wooded-agricultural, commercial-industrial, and residential-public) and each subset was tested for distribution goodness-of-fit. Following selection of an appropriate distribution, a larger data subset of 1,000 forecast values was generated for each land use category using Oracle’s Crystal Ball® software. Finally, a background condition data set of the same size as the site data set was generated by sampling from each of the forecast data subsets according to property type/land use weighting factors based on historical land use in the site study. A generalized threshold exposure point (CTE) and reasonable maximum exposure (RME) were identified as the 50th and 95th percentiles of the distributions, respectively. RME based on FRA was substantially lower than RME based on determinis- tic risk assessment. The incremental risks between site and background conditions were calculated for CTE and RME at the different exposure points during exposures

Due to rapid recovery of ChE inhibition following exposure to carbaryl, acute exp- osure has been the focus of risk assessments. To refine the assessment via inhal- ation, red blood cell (RBC) and brain ChE activity was determined following acute exposure to a dust aerosol of carbaryl. Immediately after exposure, animals were euthanized, and blood and brains were sampled. ChE activity was analyzed using a Hitachi 912 Serum Chemistry Analyzer. The assay method used was based on a modification of the Ellman reaction. The time to peak inhibition was determined in a time-course study with male rats exposed to 500 mg/m3. Both RBC and brain ChE was significantly and rapidly inhibited after 15 minutes of exposure (94 and 28%, respectively) with a plateau being reached following 1.5 hours of exposure. Subsequently, groups of 5 male and 5 female albino rats were exposed to concentra- tions of 240, 120, 60, 30, 10 and 0 mg/m3 for 3 hours to determine the No- Observable Effect Level (NOAEL) of Carbaryl inhalation exposure. The range of MMAD was 1.4–1.7 microns (MMAD). Mean RBC and brain ChE activity for males and females showed a dose-related decrease at exposure levels of 60, 30, 10 and 0 mg/m3, with a saturated response at levels of 240 and 120 mg/m3. At the lowest exposure level of 10 mg/m3 (0.01 mg/L), ChE activity was considered com- parable to control based on less than approximately 10% inhibition and lack of any statistical difference. This exposure level is equivalent to 1.0 and 1.4 mg/kg body weight for males and females, respectively. Prior to the conduct of this study, the risk from inhalation exposure associated with residual use of a dust formulation was extrapolated using the oral toxicity NOAEL of 1.1 mg/kg/day. However, the ex- trapolation from an oral route toxicity study to exposure from inhalation intro- duced uncertainty in the risk assessment. The results of this study demonstrated comparable toxicity between the inhalation and oral routes of exposure thus reduc- ing the route specific uncertainty.

Development of a SAFE DOSE for CHLORPYRIFOS: FOCUS ON HUMAN KINETIC VARIABILITY.

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Chlorpyrifos (CPF) is a widely used pesticide that inhibits cholinesterase (ChE). ChE inhibition by the CPF metabolite, CPF-oxon, is believed to be the most sensi- tive effect in all animal species evaluated and in humans from previous evaluations. Three human studies (i.e., Coulston et al., 1972; Kicikici et al., 1999; Nolan et al., 1984) form an adequate basis for determining the safe level, and the resulting point of departure is a NOAEL for RBC cholinesterase inhibition of 0.1 mg/kg/day in the Coulston et al. (1972) study. There is considerable variability in activity of paraaxonase 1 (PON1), the enzyme that metabolizes CPF-oxon, due to genetic polymorphisms and variability in gene expression. We considered the impact of this variability, together with other sources of variability on the dose of CPF-oxon, and thus on the kinetic component of the human variability uncertainty fac- tor. Published (Timchalk et al. 2002) PBPK simulations have found that variability in PON1 in adults due to the Q192R genetic polymorphism, coupled with vari- ability in enzyme levels, had minimal impact on the dose of CPF-oxon at doses in the range of the current or any proposed RD3. Unpublished simulations submitted to EPA (Poet and Timchalk 2008) that took into account infant levels of PON1 and the metabolic status of pregnant women found <50% impact on an effect measure (BuChE inhibition) in these potentially sensitive populations at doses in the range of the RD3. The actual impact of low PON1 levels is likely to be lower than the estimates from these studies, since the PBPK model did not account for the recent finding that human serum albumin is also able to detoxify CPF-oxon and can compensate for low levels of PON1 (Sogorb et al. 2008). Based on these considerations, the default uncertainty factor of 3 for kinetic variability is more than adequate, but the data are not sufficient to use chemical-specific data for a data-derived extrapolation factor. A health-protective RD3 can be derived from the NOAEL of 0.1 mg/kg/day and a factor of 10 for human variability.

A RISK MANAGEMENT BASED APPROACH TO THE EVALUATION OF SYNERGY.


The potential for synergy between chemicals has been a concern for toxicologists assessing mixture toxicity for many decades. This poster presents a simple and flex- ible framework for determining the impact of specific findings of synergy on the management of risks posed by mixtures. The framework was developed by asking the question “What characteristics of synergistic effects are required to cause the current system for managing the chronic human non-cancer effects of mixtures to underestimation?” To answer this question the models of mixture toxicity that do not consider synergy (additive and independence models) are examined and the doses of mixture components permitted under each model are determined. This is performed in a two step process of first looking at the prediction of safe levels for test animals and then extrapolating to the predictions of the safe levels for sensitive humans. This approach demonstrates that both additive and independence models keep doses of components of mixtures to fractions of their corresponding no effect levels in sensitive humans. The framework allows assessors to determine if a study showing synergy would change risk management decisions either directly (showing that effects would occur at doses permitted under additive or independence models) or indirectly (showing that synergy at higher doses is also likely to occur at the permitted doses). A graphical description of this framework was developed and applied to the Moser et al. (Toxicol Sci 92:235-45, 2006) finding of syn- ergy. Using the framework, the permitted doses were shown to be 36 (independence model) and 78 (additive model) fold lower than the lowest tested dose by Moser et al. and that the observed synergy declined with dose. This framework en- ables one to clearly and easily distinguish between mixtures where synergistic effects are a concern from those where current risk management systems already afford ample protection.

IN VITRO METABOLISM OF BIPHENOL A BY HUMAN AND RAT LIVER MICROSONES.


Biphenol A (BPA) has been widely used in manufacturing both polycarbonate plastics and epoxy resins. BPA is considered an estrogen disruptor, according to a series of in-vitro and in-vivo studies. The toxic effects of BPA in humans are un- known, but because of its widespread use, the potential for human exposure is high. Therefore, understanding the metabolic fate of BPA is necessary for risk as- sessments. Results from animal studies demonstrate that BPA glucuronide conjug- ate is a major BPA metabolite found in urine and feces. Thus, BPA and its conjuga- te can be good biomarkers for assessing human exposure to BPA. Since free-form BPA is a pharmacologically active species, measuring the free form of this com- pound in biological fluids (e.g., blood, serum, and milk) would be particularly im- portant for risk assessments. However, BPA may be present in numerous consumer and household products, and the potentially contaminated samples with BPA could pose a challenge for measuring free-form BPA. Therefore, looking for different BPA metabolites is scientifically interesting. We investigated the in-vitro metabolism of BPA using rat liver and human liver microsomes (BD GentestTM, Woburn, MA). After incubating BPA with the microsomal preparations, the BPA metabolites were firstly extracted by on-line solid phase extraction, and then separated and detected by high-performance liquid chromatography-tandem mass spectrometry. Based on their chromatographic behavior and mass spectral fragmentation patterns, we tentatively identified several BPA metabolites and oxidative products. One of the prod- ucts is believed to be a product of BPA with a hydroxyl functional group on the chelating ring (catechol BPA). We also compared the metabolism pathways and kinetics data of BPA with rat enzymes and human enzymes. Our findings suggest that in addi- tion to BPA glucuronide, oxidized BPA metabolites may potentially be used as bio- markers to assess exposure to BPA.
849 GUIDANCE FOR SELECTING CRITICAL STUDIES AND UTILIZING CHILD SAFETY FACTORS IN DEVELOPING CHILD SPECIFIC REFERENCE DOSES (CHRRDS) FOR CALIFORNIA SCHOOL-SITE RISK ASSESSMENT.


California Health & Safety code 901(g) promulgated in 2000 a policy to protect school children from harmful exposure to environmental contaminants. The law directs the Office of Environmental Health Hazard Assessment (OEHHA) to identify chemical contaminants commonly found at school sites which are of concern because of child-specific physiological sensitivities, and to develop child-specific health guidance values for school-site risk assessment. Developing child-specific health standards in the form of chRRDs is new and there is no national science policy to provide guidance. Therefore, OEHHA formulated the following guidance for chRD development: 1) Use the most sensitive species and developing organs as endpoints, 2) target perinatal and pubertal studies in organs during critical windows 3) also consider chemicals that interfere with specific cellular migration, differentiation, and proliferation pathways to produce functional deficits as adults, as well as producing pathological changes, 4) integrate information from EPA, FIFRA, and peer-reviewed studies to comprehend the mechanism disrupting a normal physiological process in young animals to determine the appropriate critical study for the chRD, and 5) if only adult studies are available, add child safety factors (cSF). Default cSF are expedient when numerous chemicals need child-protection values at once. Emphasizing mechanisms in children has allowed OEHHA to focus on pharmacodynamic (PD) and pharmacokinetic (PK) differences in the developing organs of children compared to adults on a case-by-case basis. Studies in young animals do not usually require cSFs because PK and PD are already incorporated. A cSF is only used if critical studies 1) employ adults, or 2) have a high LOAEL, and other studies in young animals with less robust data indicate a lower LOAEL for that chemical. This approach informs the concept of child safety factors with a scientific basis. OEHHA will present cases illustrating use of this guidance in our chRRDs.

850 HAZARD EVALUATION OF FLUOROTELOMER-BASED URETHANE POLYMERIC PRODUCTS.

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The objective of these studies was to evaluate the acute, genetic, and ecotoxicity of three fluorotelomer-based urethane polymeric products derived from short-chain raw materials (A, B, C). In addition, the subchronic, reproductive, and developmental toxicity in rats of the fluorotelomer-based urethane polymeric product (C) used as a stain and soil-resistance finish for textiles was also evaluated. All three products have low acute toxicity (oral LD50=5000 mg/kg; not classifiable for skin or eye irritation; not a skin sensitizer). At the highest achievable aerosol concentration of 1363 mg/m3, a single exposure to Product B resulted in no deaths. Products B and C were negative in the bacterial reverse mutation test and mouse lymphoma assay (Product C). Product C exhibited low concern for acute aquatic hazard in fish and green algae and moderate concern in Daphnia, whereas Product B exhibited low concern in Daphnia. In a 10-day biopersistence screen, there was very low uptake of Product C. Levels in the blood of male rats daily were generally very low and only slightly below the estimated limit of quantification and tissue concentrations in liver and fat were marginally higher. Product C was administered near oral gavage at dosages of 0, 50, 250, or 1000 mg/kg/day (90-day subchronic toxicity and one-generation reproduction), and at the same dosages on gestation days 6-20 (developmental toxicity). The NOAEL for subchronic and reproductive toxicity was 1000 mg/kg/day, based on no test substance-related effects on in-life, neurobehavioral, ophthalmological, or anatomic pathology parameters, or on any reproductive toxicity study, the NOAEL for both maternal and fetal toxicity was 1000 mg/kg/day, based on a lack of test substance-related effects on any maternal or fetal parameter. No fetal malformations were observed. Overall, these materials are considered to have low potential to produce human health effects.

852 IN VITRO EFFECTS OF A NOVEL NANOPARTICLE, ZIRCONIUM PHOSPHATE.

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Synthetic zirconium phosphate (Zr(OH)4; ZrP) particles have a layered inorganic framework with high surface area and are candidate carriers for drug delivery. ZrP particles are prepared by refluxing a zirconium salt and phosphoric acid in the presence of HF at a ratio of 1:4:2 for 6 days. Because ZrP particles are 60 nm in length in one dimension, they can be classed as nanoparticles. X-ray diffraction analysis showed formation of a crystalline α-ZrP phase. The structure of ZrP resembles that of naturally occurring zontoclase, however, its toxicity profile in a biological system had not been evaluated. We used human cell lines (A549, lung epithelium; AGS, gastric epithelium) to evaluate the toxicity of ZrP in vitro. Cells were cultured to a confluent state and were then exposed for 24 or 48 h to ZrP suspensions ranging from 0.5 to 100 μg/ml (0.13 to 26.5 μg/cm²). Control cells were untreated or treated with Aerioxide® TiO2, P25 (Degussa) or Min-U-Sil 5 (US Silica). Cytotoxicity was evaluated using MTT assay and LDH release. In A549 and AGS cells, ZrP particles caused a greater dose-dependent decrease in mitochondrial activity than did TiO2 or silica. Like nanosized TiO2, ZrP particles showed marked aggregation that progressed over time in culture at pH 7.4. Scanning electron microscopy of ZrP particles that were suspended in medium and then dried confirmed this aggregation. LDH release occurred in a dose-dependent manner in A549 and AGS cells but was variable across trials, probably because particle aggregation reduced the effective dose. Low concentrations of ZrP particles reduced apoptosis in AGS cells cultured as evidenced by reduced levels of caspase 3 and 7 but did not affect A549 cells. At high ZrP concentrations, caspase levels were comparable to those of control cells. Thus, apoptosis may not underlie the observed reduction in cell proliferation. In general, additional in vitro studies and treatment of the ZrP particles to decrease aggregation at physiologic pH is essential prior to their in vivo use.

853 UNDERSTANDING THE SIZE AND SHAPE DEPENDENCE GENO, CYTO AND PHOTOTOXIC EFFECTS OF FRESHLY PREPARED SILVER NANOMATERIAL.


Silver nanomaterials are rapidly becoming a part of our daily life in the form of cosmetics, food packaging, bandage, shocks, clothing and laundry detergents and others. As the nanotechnology field continues to develop, assessing nanoparticle toxicity is very important for advancing nanomaterials for day to day life application. Here we report geno and cytotoxicity of silver nanomaterial of different size and shape on HaCaT keratinocytes, cell line using Comet and MTT assay. We also compare the results with the toxicity of AgNO3, which is the starting material for UV light on intake of silver products in skin cell may produce argyria, we also reported the photo-toxicity of silver nanomaterial of different size and shape on HaCaT keratinocytes, cell line using Comet and MTT assay. We also compare the results with the toxicity of AgNO3, which is the starting material for
hation with 100 μM concentration and also not phototoxic even after 30 minutes of UV light exposure. On the other hand our data show silver nitrate is highly toxic even at the concentration of 10 μM. We have also shown that silver nanorods are highly toxic due to the presence of CTAB as coating material, where as PS coated nanorod is not toxic.

**854 INCREASED INTRACELLULAR CALCIUM CONCENTRATIONS IN HUMAN BRONCHIAL EPITHELIAL CELLS EXPOSED TO ULTRAFINE ZINC OXIDE PARTICLES.**

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Zinc oxide nanoparticles caused a dose- and time-dependent cytotoxicity in a human bronchial epithelial cell line, BEAS-2B. This toxicity was observed at concentrations as low as 5-6 μg/mL; there was a steep decline in cell numbers at ZnO concentrations between 6-10 μg/mL. Exposure to ZnO nanoparticles increased intracellular calcium levels in a dose- and time-dependent manner. Treatment with an antioxidant N-acetylcysteine (NAC) prevented cell loss and diminished the increase in intracellular calcium concentration, findings consistent with an oxidative stress-mediated cytotoxicity. Calcium concentrations were elevated by as much as four fold compared to control cells, and the elevation went down when the cells were subsequently treated with NAC. Naphthide, an L-type voltage-gated calcium channel blocker, attenuated the intracellular calcium elevation caused by ZnO toxicity, suggesting that the elevation of intracellular calcium concentration depended on an influx of extracellular calcium. Thus, these findings indicate that exposure of BEAS-2B cells to 20 nm ZnO particles causes a dose- and time- dependent cytotoxicity reflected in cell viability reduction, elevated oxidative stress and increased calcium content that is dependent on the influx of extracellular calcium.

**855 ZINC OXIDE NANOPARTICLES: IT’S THE CONTACT THAT KILLS.**

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Previously, we evaluated the toxicity and transcriptional responses to 6 manufactured nanoparticles in colon cell lines. These manufactured nanoparticles are used in consumer products, and therefore, there could be considerable occupational exposure. NanoZnO displayed the most toxicity and demonstrated the most pronounced transcriptional response. This transcriptional response suggested exposure to elemental Zn, and therefore, perhaps the toxicity was Zn toxicity. Therefore, we sought to determine if the nanoZnO toxicity was due to the dissolution of ZnO to elemental Zn and determine the mechanism of the cell death upon exposure to the nanoZnO. In addition, we utilized two size ranges of ZnO particulate matter to evaluate the effects of size/surface area. First, we set out to determine: 1) if cell and particulate matter contact was required for ZnO toxicity, and 2) if ZnO dissolution to free Zn was dependent on the cells. We used a set of three experimental conditions: 1) the ZnO was separated from cellular contact using a dialysis device with a 10 kD cutoff to ensure no ZnO particulate matter could interact directly with cells, 2) transwell with 0.4 micron pores that would allow greater interactions with cellular products but still separate the cells and the particulate matter, and 3) ZnO particulate matter in direct contact with the cells. We measured the Zn concentrations in the media by ICP spectrometry and cell viability by PI exclusion. We found that the ZnO toxicity was only observed when the particles were in contact with the cells irrespective of Zn levels in the media, suggesting that contact and potentially uptake is required for cellular toxicity. We have found that ZnO induces apoptosis as measured by Annexin V cytomteric analysis. We have found that ZnO disrupts mitochondrial function and induces superoxide production in the mitochondria. In addition, all of the toxic effects are dependent on particle size as the larger ZnO particulate matter always demonstrated reduced toxicity compared to the smaller ZnO nanoparticles.
the rubpy-doped particles, indicating that plasma Veg may have adhered to the particles and facilitated their uptake into the developing eggs via vitelligenin receptor mediated endocytosis. The rubpy-doped particles appeared to be concentrated in yolk granules, as determined by confocal microscopy. This novel assay can be used to screen nanomaterials for their potential to interfere with egg development and embryogenesis.

### SOT 2009 Annual Meeting

#### 859 SERUM PROTEIN COATED GOLD NANOPARTICLES IN THE PERFUSED HUMAN TERM PLACENTA


Nanoparticles (NPs) have become incorporated in our daily lives from environmental to medicinal exposures. Unfortunately, pregnant women have not been a focus of health risk assessment. NPs that enter the systemic circulation become coated with serum proteins, e.g., human serum albumin (HSA) and immunoglobulin G (IgG). During pregnancy, the placenta plays a critical role as a conduit or filter for xenobiotics. HYPOTHESIS: Gold (Au) NPs pre-coated with HSA or IgG (F105) will interact with the human placenta in a manner consistent with the catabolism of HSA or the receptor-mediated placental transfer of IgG. HSA or IgG proteins were adsorbed onto the surface of 5 mm irradiated Au NPs. Au NPs (5ug Au/ml, hydrodynamic diameter for HSA- or IgG-Au NPs $\pm$ 8.4 or 7.3 nm, respectively) were transfused at 2.6 $\times$ 10$^6$ into the maternal circulation of the decidual perfused human term placental lobule in vitro. Passages were performed for 14 hrs with an initial control period of 2 hrs followed by maternal and fetal transfusions. HSA and IgG NP exposures did not significantly alter the blood gases, no fetal oxygen transfer, flow rates and fetal perfusion pressure compared with control values. HSA-chromatoidin contractions were measured in maternal and fetal perfusates. Circulating Au NPs significantly decreased in the maternal circuit depending upon the protein coating: IgG-Au NPs decrease 9.83$\pm$0.65% (n=2), while HSA-Au NPs decreased 1.92$\pm$1.1% (n=3). We conclude that (i) perfusion of the placenta with nanogold particles did not alter normal placental function, and (ii) protein coatings of the NPs do influence their distribution regardless of surface chemical composition. A concentration-dependent effect on hemo lysin was observed with substantive hemolysis ($>10\%$) occurring at the highest nanoparticle concentrations ($>220$ µg/ml). Silver ion release in PBS particle solutions increased 20-100 times greater for the nanostructures compared to the microparticles. These data suggest that the increased surface area and release of silver ions from silver nanoparticles may contribute to hemolysis. Thus, blood-contacting medical devices that use silver nanoparticles need to be carefully monitored for their hemolytic potential.

#### 860 NANOPARTICLE SIZE AND COMPOSITION AFFECT ADSORPTION OF HUMAN PLASMA PROTEINS.

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On contact with biological fluids, nanomaterials will adsorb proteins that modify their surface and play an important role in cellular recognition and response. Developing an understanding of the processes that affect binding of specific proteins and the relationship of protein binding and response will improve our ability to predict biocompatibility of absorbed nanomaterials. To investigate these questions, label-free proteomic techniques were used to identify and quantify proteins bound to 3-5, 20, and 40 nm gold particles as well as DeGussa P-25 titania particles after incubation in human plasma. Results demonstrate dramatic differences in the human plasma proteins that bind to gold and titania nanoparticles. F2-prothrombin, complement factor 1 and 4, and kininogen 1 were highly enriched on titania nanoparticles and were nearly absent on gold particles. Albumin, serotransferrin and alpha-2 macroglobulin were much more abundant on gold particles. Comparing binding of proteins to various sizes of gold particles revealed 32 proteins for which particle size statistically affected binding. Several proteins exhibited increased adsorption to smaller particles, including fibrinogen, serpinG1 and a 104kDa protein (IP00799995). Other proteins exhibited decreased binding to smaller particles, including apolipoproteinB100, paraoxonase, complement C9, and cluseterin. These results clearly demonstrate that nanoparticle size and composition affect their protein corona and are likely to impact biological response. These studies were supported by funds from the Center for Nano-Bio Sensors at the University of Florida.

### SOT 2009 Annual Meeting

#### 861 IN VITRO HEMOLYSIS TESTING OF SILVER NANOPARTICLES IN HUMAN BLOOD.

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Materials used in blood-contacting medical devices must be evaluated for their potential to damage red blood cells since released hemoglobin may induce toxic effects to the kidneys and other organs. Due to its antimicrobial efficacy, silver is increas-

#### 862 NOVEL MOLECULAR PATHWAYS INDUCED IN FUNCTIONALIZED FULLERENE EXPOSED HUMAN EPIDERMAL KERATINOCYTES (HEK).

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Engineered nanomaterials have been extensively used for diagnostic, therapy and drug delivery based on their unique physicochemical properties. Since native C60 is hydrophobic, many hydrophilic C60 fullerene derivatives have been synthesized to facilitate clinical applications. However, the interaction of fullerenes with biological systems is not completely understood. In the present study, we investigated the bioimpact of three chemically modified fullerene derivatives, hexa carbonyl fullerene adduct (Hexa-C60), tri carbonyl adduct fullerene adduct (Tris-C60) and gamma (3-cyclodextrin caged C60 (CD-C60). Key biological responses, such as cellular viability, ROS generation, proliferation and cell cycle responses were evalu-

#### 863 IN VITRO METHODS TO PREDICT IMMUNE-MEDIATED TOXICITIES OF DEXTRAN-BASED NANOMATERIAL PRECURSORS IN RATS.

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While nanomaterials hold great promise as drug delivery platforms, biological con-

sequences resulting from the interaction of nanomaterials with the immune system have not been fully elucidated. Initial in vitro toxicity studies with hydrophobic der-

ivatives of dextrans of different molecular weights were conducted in Sprague-

Dawley rats. Intravenous injections for seven consecutive days resulted in dose-re-

lated toxicity with 5000 MW and 10000 MW dextran derivatives. Toxicities ranged from an increase in the number of circulating neutrophils and lymphocytes, to death after 3 days in the highest dose group of the 5000 MW derivative. Renal glomerular thrombosis was observed in the high dose group and disseminated intravascular coagulation was suspected. We hypothesized that complement and macrophage activation contributed to these observed effects, and our goal was to

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Silica-based mesoporous materials offer great promise in biomedical applications, such as vehicles for drug delivery, in transfecion devices, and so on. However, there is increasing concern about the putative adverse effects of nanomaterials on biological systems. The induction of oxidative stress has been postulated as a common paradigm for cytotoxicity of engineered nanomaterials. Moreover, our recent studies have shown that microsomal glutathione transferase 1 (mGST1) may protect cells from a variety of cytotoxic drugs and oxidative stress. Here, we hypothesized that mGST1 can also protect against oxidative stress caused by engineered nanomaterials. To this end, a comparison was made between commercial Luidox® colloidal silica and de novo synthesized mesoporous silica particles. Structural and textural characterization of the particles was performed using powder X-ray diffraction and transmission and scanning electron microscopy, and size distribution and surface charge was determined by dynamic light scattering and zeta potential measurements, respectively. No obvious cytotoxic effects were observed for mesoporous silica particles in the nano- and micrometer size range using the human breast cancer cell line, MCF-7 as a model system. Luidox® silica particles, on the other hand, induced dose- and time-dependent toxicity, as assessed by Trypan Blue exclusion and the MTT assay. Importantly, the toxic effect of colloidal silica particles was reversed by overexpression of mGST1. The protective role of mGST1 was further examined by detection of oxidative DNA damage. Overall, our studies contribute to the understanding of the toxicity of nanoparticles, and point to potential anti-oxidant strategies to overcome such toxicity.

866 ASSESSMENT OF QUANTUM DOT (QD) UPTAKE AND TOXICITY IN SVEC4-10 MURINE ENDOTHELIAL CELLS.

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Semiconductor quantum dots have emerged as a new class of fluorescent nanoparticles. Control over the physical and chemical properties during the production of QDs has made them a popular choice for use in optoelectronics, photovoltaics, and probes in biomedical imaging. With an increase in QD production and availability, questions regarding QD toxicity have arisen as a major concern. A growing base of literature has emerged identifying important parameters for QD persistence and toxicity in biological systems; specifically that size and unique chemical structure dictates QD toxicity. The goal of this research was to assess the in vitro toxicity of aptahilamic polymer stabilized QD (QD-TOPO-PATM) toward murine endothelial SVEC4-10 cells. QD uptake was measured at 6 and 24 h using both flow cytometry (FACS) and fluorescent microscopy showing a time and dose-dependent increase. Mutations in cell viability (MTT) were measured yielding no effect of up to 200 nM QD for 24 h of exposure. Subsequent analysis using FACS showed no signs of necrosis or apoptosis using 7-AAD and Annexin V probes. Cell glutathione (GSH) levels following exposure to QD at 2.5 and 20 nM for 24 h were measured using either monochlorobimane (MCB; conjugated to GSH by glutathione-S-transferase (GST)) by FACS, or biochemical assay. Interestingly, neither NAD(P)H (by FACS) nor GSH levels (biochemical assay) showed any differences over 24 h suggesting that GST activity may be down-regulated or compromised following QD exposure. The preliminary results of this study suggest that polymer stabilized QD are readily taken up and have no adverse effect on viability in SVEC4-10 endothelial cells but may compromise GST function. This work was supported by NIHES grants R01ES016189, T32ES07032 and P30ES07033.

867 SHAPE MATTERS: DIFFERENTIAL CYTOTOXICITY OBSERVED IN GOLD NANOSPHERES AND NANORODS.

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Gold nanoparticles are being used in sensing, imaging, and treatment of certain cancers and as novel gene and drug delivery agents due to their unique physical and chemical properties. Despite being easily accessible and found in a wide range of consumer products, the cytotoxicity of these nanomaterials has not been thoroughly examined. Many of the unique properties that must be evaluated prior to making claims of biocompatibility are size, surface area, morphology, shape, surface charge, crystal structure, etc. The majority of nanotoxicity studies have focused on size and very few have assessed the role that shape plays in determining nanoparticle toxicity. This study evaluated the cytotoxicity of gold nanoparticles with different shapes. Gold nanorods 51 nm in length with varying diameters were compared to gold nanospheres 12 nm in size and toxicity was assessed in the human keratinocyte (HaCaT) cell line. The cells were treated with varying concentrations (0-100 μg/ml) of the gold nanomaterials for 24 h and then biocompatibility was tested using cell viability and membrane integrity as endpoints. The MTS assay which measured mitochondrial function and directly relates to cell proliferation demonstrated that the 12 nm nanospheres did not disrupt cell proliferation while the nanorods regardless of size reduced proliferation. A similar trend was observed with the LDH, membrane leakage assay which measures membrane integrity. In addition, the nanorods were toxic at very low doses and did not display much of a dose-dependent effect. Four out of the six nanorods were toxic at the lowest dose of 5 μg/ml with the other two displaying toxicity as early as 10 and 25 μg/ml respectively. Based on the initial observations of this study, shape seems to play a role in contributing to nanotoxicity.

868 OXIDATIVE STRESS-RELATED TOXICITY OF SILICON DIOXIDE, CERIUM OXIDE AND SILVER NANOPARTICLES IN HUMAN CELL LINES.


In this study, we investigated the potential harmful effect of the exposure to silicon dioxide (SiO2) and cerium oxide (CeO2) and silver nanoparticles through in vitro toxicity assay using human cells, with a focus on the involvement of oxidative stress.
as the toxic mechanism. Nanoparticle-induced oxidative stress was assessed by examining formation of reactive oxygen species (ROS), apoptosis, and the induction of antioxidant enzymes, such as superoxide dismutase (SOD) and heme oxygenase-1 (HO-1). Subsequently, to understand the molecular mechanism of nanoparticle-induced oxidative stress, the involvement of oxidative stress-responsing transcription factors, such as, nuclear factor-kappaB (NF-kB) and nuclear factor-e2-related factor-2 (Nrf-2), and mitogen-activated protein (MAP) kinase signal transduction pathway was studied. Taking the overall results into account, nanoparticles exert their toxicity through oxidative stress as they cause a significant increase in cellular H2O2 concentrations. However, SO2 nanoparticles lead a moderate induction of HO-1 via the Nrf-2 extracellular signal-regulating kinase (ERK) MAP kinase pathway, whereas exposure to CeO2 nanoparticles leads to a strong induction of HO-1 via the Nrf-2 MAP kinase pathway. Further studies on the mechanism by which nanoparticles induce the Nrf2 MAP kinase pathway are warranted to better understand the nanoparticle-induced oxidative stress, as are studies with dose-response and time-course studies. The characterizations of nanoparticle in test media (i.e. aggregated or single forms, intensity of uptake into the cells) and their influence on cytotoxicity need to be investigated. Acknowledgment: This work was accomplished through the supports of the Ministry of Environment as “The Ecoscience 21 project”.

PS 869 IMMUNE SUPPRESSION IN MRSA-INFECTED HUMAN ALVEOLAR CO-CULTURES TREATED WITH ALUMINUM NANOPARTICLES.

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The goal of this study was to evaluate the biological response following exposure to aluminum nanoparticles (Al NP). Based on their uses in jet fuels and munitions, the most likely scenario for exposure is inhalation. Since nanoparticles have been shown to be capable of penetrating deep into the alveolar regions of the lung, we investigated the in vitro response to nanoparticles. Three types of Al NPs were evaluated and they were aluminum (Al), aluminum oxide (Al2O3) and aluminum oleic acid (Al-OA). Following a 24 h incubation, the most likely scenario for exposure is inhalation. Since nanoparticles have been shown to be capable of penetrating deep into the alveolar regions of the lung, we

PS 870 COMPARATIVE IN VITRO STUDIES ON THE PROINFLAMMATORY EFFECT OF CARBON BLACK PARTICLES ON MACROPHAGES AND DENDRITIC CELLS.


Previously, we have shown that nano size (14 and 40 nm in diameter) carbon black particles (CBP) induce airway inflammation in vivo. In order to better understand how CBP affect lung health, two main airway cells types likely to interact with inhaled particles, alveolar macrophages (AM) and dendritic cells (DC), have been exposed to CBP in vitro and followed for proinflammatory endpoints and/or maturation. Mouse AM (RAW 264.7 cell line) and DC (bone marrow-derived dendritic cells from BALB/c mice) were exposed to different concentrations of CBP, phosphate buffered saline (PBS; vehicle control) or lipopolysaccharide (LPS, positive control) for 4 and 24 hours. The inflammatory mediators nitric oxide (NO) and tumor necrosis factor alpha (TNFalpha) together with cytotoxicity (MTT test) were measured in AM cultures. DC maturation and activation was determined by expression of co-stimulatory molecules (CD40, CD80 and CD86), and by cytokine production (IL-10 and IL-12).

The exposure of AM to both 14 and 40 nm CBP induced a clear dose-dependent increase in TNFalpha production when compared to PBS exposure. In contrast, only 40 nm CBP induced a dose-dependent upregulation of costimulatory mole-

PS 871 CHARACTERIZATION AND BIOCOMPATIBILITY OF GREEN SYNTHESIZED SILVER NANOPARTICLES.

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There are currently ~1,000 commercially available products which contain some form of silver nanotechnology, ranging from topical creams and cosmetics, to anti-microbial socks and household cleaners. Previous studies have indicated that silver nanoparticles (Ag NPs) have a size dependent cytotoxicity where the smaller particles are the most toxic; however, it has also been shown that the Ag NPs dramatically alters the toxicity. In light of these studies and the ever increasing probability of dermal and inhalation exposure to Ag NPs, it is imperative to create nanoparticles which can be synthesized in a cost-effective, environmentally friendly manner, and pose minimal threat to workers. Therefore, the goal of this study was to synthesize biocompatible and environmentally friendly Ag NPs. Ag NPs were synthesized using wet chemistry with epinephrine (Epi) as a reducing agent, and biocompatibility was assessed using the human keratinocyte cell line (HaCaT) and rat alveolar macrophages as in vitro models for dermal and inhalation exposure, respectively. Characterization was performed using transmission electron microscopy (TEM) to yield the primary particle size and morphology, and dynamic light scattering (DLS) was used to identify agglomeration in the exposure media (EM). Biocompatibility was evaluated using Cytoviva imaging to determine internalization, the MTT assay to assess cell proliferation, and the LDH assay to evaluate membrane integrity at concentrations of 0, 50, and 100 µg/mL. The EpiAg NPs had a primary particle size of 11.5 ± 4.7nm, 25.8 ± 15.8nm, 11.9 ± 3.9nm, 17.3 ± 7.0nm, and 24.2 ± 6.5nm depending on the ratio of Ag to reducing agent (1:1, 2:1, 10:1, 10:3, and 10:1). Furthermore, all of the EpiAg NPs agglomerate to some degree once dispersed in EM. Overall, the EpiAg NPs regardless of reducing agent ratio or size appeared to not disrupt cell proliferation (over how long??) despite being internalized by the cells with minor membrane disruption.

PS 872 NANOSILICAS WITH VARIOUS SURFACE CHARGE INDUCE THE DIFFERENT PROFILE OF CYTOKINE PRODUCTION ON MACROPHAGE.

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Nanotechnology has produced a diverse array of nanomaterials such as carbon nanotubes, fullerene derivatives and quantum dots. The advent of nanomaterials has provided incredible opportunities for biomedical applications. For example, nano scaled silica (NS) is used in various fields such as engineering, electronics, foods, and drugs. On the other hand, the potential adverse effects of nanomaterials, such as NS, on human health remain to be established. We have tried to clarify the property and safety of nanomaterials in vivo and in vitro. In this study, we compared the effect of various NSs with different surface charge on innate immune responses using Raw264.7 cells, mouse macrophage cell lines. We used the NSs with a diameter of 70 nm with neutral (NS70(±)), anionic (NS70(-)) and cationic (NS70(±)) charge on the surface. At first, we examined the effect of NSs on the production of proinflammatory cytokines, TNFα, in macrophage. NS70(±) alone induced the significant level of TNFα. Next, we examined the effect of NSs on the production of proinflammatory cytokines in LPS-stimulated macrophage. LPS-induced activation resulted in elevated levels of IL-6 and TNFα compared with unstimulated Raw264.7 cells. NS70(+) and NS70(±) enhanced LPS-induced production of TNFα, whereas NS70(±) suppressed LPS-induced TNFα production. In contrast, all NS70(±) suppressed LPS-induced IL-6 production significantly. These results indicated that NSs modified the immune responses and surface charge was important factor.
CHARACTERIZATION OF SELECTED NANOPARTICLES AND THEIR RELATIONSHIP TO TOXICITY IN LUNG EPITHELIAL CELL LINE.


The aim of this study was to investigate the relationship between characterization of selected nanoparticles (NPs) (Boron, Copper, and Tungsten, W) and their toxicity in human alveolar basal epithelial cells (A549). Various sizes of these particles were suspended in water, phosphate buffered saline (PBS) and 0.9% saline at room temperature for Dynamic Light Scattering measurements. All NPs in de-ionized water showed the smallest hydrodynamic aggregate sizes while PBS produced 2-3X larger sizes and 0.9% saline the largest values of 3-9X size in water. Since the major route of exposure of these materials would be inhalation while handling NPs at work places and in manufacturing settings, lung epithelial cells were chosen as a simple model to study effects of nanoparticles on lung cells. The cells were exposed with different concentrations (0, 10, 50, 100, and 200 μg/mL) of particles (Cu <100nm; B 1μm; 60 nm and 4, 9, and 26 μm W powder). Results of MTS assay for cell viability of Cu revealed that dose-dependent MTS reductions were observed at 50 min post exposure; at 5, 10, 20, 40, 60 and 80 μg/mL. MTS reductions were 20, 35, 60, 80, 85, and 90% reduction of control, respectively. No MTS reduction was observed in B and micron sizes of W. Reactive oxygen species (ROS) generation was studied to see if these NPs induced oxidative stress. Nano and micron sizes of W showed several fold increases of ROS production, especially 60 nm W which displayed ~4 fold increase at 6 hr time point. No significant increase in ROS was observed in B and Cu. In summary, nanoparticles studied showed induction of ROS generation except Cu, which could cause oxidative stress in the cells when exposed with these particles. In summary, these data showed that the nanoparticles under study exhibited chemical-, size- and concentration-dependent and specific toxicity.

ASSESSMENT OF THE BIOLOGICAL EFFECTS OF SILVER NANOPARTICLES IN CULTURED HUMAN CELLS.


Silver nanoparticles (nano Ag), whose antimicrobial properties are well known, have been extensively used as antibacterial agents and deodorants. Despite the widespread use of nano Ag products, relatively few studies have been undertaken to determine the biological effects of nano Ag exposure. We evaluated the toxicity of nano Ag and examined influences on the expression of several genes by nano Ag. We observed apparent cytotoxicity in HeLa cells exposed to nano Ag (5-10 nm) or silver nitrate for 24 h. The IC50 values for nano Ag and silver nitrate were 92 and 17 μg Ag/ml, respectively. Furthermore, nano Ag as well as silver nitrate induced apoptosis in HeLa cells. These results indicate nano Ag possesses an obvious cytotoxicity. To obtain the clue to the mechanism of action, we examined the expression of typical stress response genes coding for metallothionein-2A (MT-2A), heme oxygenase-1 (HO-1) and 70 kDa heat shock protein (HSP70) after nano Ag exposure. Nano Ag induced the expression of MT-2A and HO-1 almost as effectively as Cu, known to be a strong inducer of these genes. HSP70 expression was slightly increased by nano Ag. These results may suggest the occurrence of metal ion, oxidative stress and/or protein denaturation by nano Ag exposure.

LIMITATIONS AND RELATIVE UTILITY OF SCREENING ASSAYS TO ASSESS ENGINEERED NANOPARTICLE TOXICITY IN A HUMAN CELL LINE.

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Carbon black (CB), single-walled carbon nanotubes (SWCNT), fullerenes (C60), nC60, and quantum dots (QD) were used in vitro to determine their cytotoxicity with classical toxicity assays. Classical dye-based viability assays such as neutral red (NR) and MTT produce invalid results when treated with some nanomaterials (NM) due to interactions and/or adsorption of the dye or dye product. In this study, human epithelial keratinocytes (HEK) were exposed in vitro to CB, SWCNT, C60, nC60, QD, and Min-U-Sil 5 that served as a particle control to assess viability with Trypan Blue (TB), Live/Dead (LD), calcine AM (CAM), NR, MT, CellTiter 96 AQueous One (96 AQ), alamar Blue (ab), CellTiter-Blue (CTB), CytoTox OneTM (CTO), and flow cytometry. Viability linearity (R² value) for each assay was determined with HEK plated at concentrations ranging from 0 to 25,000 cells per well in 96 well plates. HEK were treated with serial dilutions of each NM for 24 h and assessed with each of the viability assays above. Dye-based assays varied a great deal, depending on the interactions of the dye/dye product with the type of NM. Based on the above studies, the optimal assay was 96 AQ. Unlike small molecules, NM can interact with different assay markers to cause variable results with these classical assays and might not be suitable for assessing cytotoxicity of engineered NM. Therefore, multiple assays may be required when determining nanoparticle cytotoxicity for risk assessment. (Supported by the US Air Force Office of Scientific Research, FA 9550-08-1-0182)

ARE CELLULOSE NANOPARTICLES TOXIC TO HUMANS AND FISH?

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Nanoparticles are estimated to be a $1 trillion per year industry by 2010. One of the particular interests for industry is in extracting cellulose nanoparticles from wood to be used as reinforcing agents in bioplastics, and to replace the plastic presently used in frozen desserts. The Toxicity of cellulose nanoparticles is currently unknown therefore it is important to assess its potential harm to humans and the environment. In our study we used human skin fibroblast cells (BJ/ tert), and medaka fish fin cells (OLHN-12) to test the potential cytotoxicity of cellulose nanoparticles. We exposed the cells to a wide range of potentially usable concentrations of cellulose ranging from 0.5-1000 μg/cm² for a period of 24 hours. The fish cells were exposed to another range of 50-1000 μg/cm² for a period of 24 hours and a period of 120 hours. Currently we have observed mild cytotoxicity from cellulose nanoparticles to human skin cells at doses of 50-1000 μg/cm². For the fish cells we found moderate cytotoxicity at 1000 μg/cm² for 24 and 120 hours. Due to the lack of much significant toxicity on human skin cells and that the only toxicity on fish cells is an extremely high dose; the data suggests that cellulose may be suitable for use on consumer products. This work is supported by the Educational Outreach Award of the Maine EPSCoR FRB Research Project (J.F.W.)

MECHANISM OF QUANTUM DOT NANOPARTICLE UPTAKE IN A HUMAN CELL LINE.

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Quantum dots (QD) have been used in biological and medical applications, but the mechanism of QD uptake cell remains unknown. Soluble QD555 nanoparticles consist of a CdSe core and a ZnS shell with a carboxylic acid surface coating. These QD are ellipsoid in shape and have a hydrodynamic diameter of 18nm-22nm. Clathrin, caveolae, lipid rafts, early endosomes, late endosomes, and lysosomes were labeled to observe their co-localization with QD. The mechanism of QD555 uptake by human epithelial keratinocytes (HEK) was investigated with 27 endocytic inhibitors associated with seven classical and very specific endocytotic pathways for HEK. Clathrin and eps15 did not co-localize with QD at 1h, but co-localization occurred between QD and lipid rafts. QD555-COOH partially co-localized with EEA1 at 1h and gradually translocated into CD63/LAMP1-positive compartments indicating that early/late endosomes participated in QD uptake. Inhibitor experiments supported that QD endocytosis by HEK was not related to the clathrin-mediated pathway, macropinocytosis or HEK specific melanosome transfer pathway, but was highly dependent on the density lipoprotein receptor/scavenger receptor mediated pathway and may be regulated by lipid rafts or G-protein linked pathway. Finally, at the high concentration of 20nM QD, minor toxicity was detected at 24h by the Live/Dead assay, and actin filaments increased in the cytoplasm. Here, we report on the QD endocytic pathway mechanism for HEK. Size, surface coating and charge of the QD nanoparticles are extremely important parameters to determine QD uptake in mammalian cells and may help to develop rational targeted nanotherapeutics. (Supported by U.S. Air Force Office of Scientific Research, FA 9550-08-1-0182)

ASSESSMENT OF FULLERENE TOXICITY IN A DYNAMIC Rotating Cell Culture SYSTEM IN A HUMAN CELL LINE.

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In vitro screening methods with adherent cell cultures have been used to assess the toxicity of numerous chemicals and pharmaceuticals. However, repetitive dosing for long periods of time is difficult to conduct in regular in vitro cell culture models due to the limitations of cell growth. We have used a rotating cell culture system...
to study the effects of microgravity and biological interactions of C60 fullerenes with human epidermal keratinocyte (HEK). The cells are in constant motion (dy-namic) without direct contact of large agglomerates of nanomaterials sitting directly on the cell surface. A single dose of 0.1mg/ml, 0.2 mg/ml, or 0.4mg/ml of C60 was studied in proliferating HEK for 10 days, assayed for viability, and cellular morphology and C60 uptake evaluated by transmission electron microscopy (TEM). Additional studies were conducted to assess the effects of repetitive daily dosing using this rotating system with the medium dose of 0.2mg/ml C60 for 10 days. Samples were taken every 48 h for TEM and cellular viability was assessed by MTT. The single dose study showed a slight decrease in viability, with a statistically significant (53.4% ± 0.6) decrease in viability after daily dosing for 10 days. Ultrastructural evaluation depicted the C60 within cytoplasmic vacuoles of the cells with a slight increase in uptake over 10 days. Control cells had normal morphology and growth. These findings demonstrate that cells in a microgravity environment can extend the life span of the cells so repetitive daily dosing can be conducted to assess longer exposure times for the study of nanomaterials to mimic multiple exposure scenarios in an occupational setting. (Supported by the US Air Force Office of Scientific Research, FA 9500-08-1-0182)

879 OXIDATIVE STRESS AND APOPTOSIS IN HUMAN CELLS EXPOSED TO AG AND TiO2 NANOPISTARTICLES.

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The objective of this project is to study the mode of action of nanoparticles (NP) in vitro systems and investigate the toxicity as a function of particle number and surface area. The toxicity of Ag NPs/V/P coated length: 81 nm; aspects ratio = 1.2 and TiO NP (nanocrystalline; 7.5,12 and 18 nm) were investigated in A549 human lung epithelial and THP-1 monocyotic cells. Cellular toxicity was assessed by the MTT and LDH assays. Induction of reactive oxygen species (ROS) was determined by DCFH-DA and by expression of heme oxygenase (HO-1) by RT/PCR. Aporia was measured by the Annexin V and tunnel assays using flowcytometry. Ag NP induced toxicity in A549 cells in a dose-dependent manner (10-100 ug/ml) after 24 hrs incubation, whereas toxicity was only observed above 50 ug/ml after 4 hrs incubation. Ag NP increased ROS in a dose-dependent manner after 4 hrs incubation. The reduction of ROS was dependent on the ratio of particles/cell number. Ag NP also induced significantly the expression of HO-1 at doses above 10 ug/ml, whereas other genes involved in cellular defence against oxidative stress, e.g. catalase was not induced. The toxicity of all 3 TiO NPs in A549 was in a concentration (ug/ml) and surface area (200-10000 mm2/ml) dependent manner. Using the annexin V/proapondiol (PDI) assay, Ag NP was found to induce both apoptosis and necrosis in THP-1 cells in a dose/time and cell number dependent manner. Using the tunnel assay a significant effect was observed only at concentrations higher than 50 mg/ml. Summary: Ag and TiO NP induced ROS and loss of cell viability in a dose dependent manner, and cell death occurs primarily by apoptosis. In case of TiO, our results suggest that the toxicity should be expressed not alone by mass.

880 ASSESSMENT OF SILVER NANOPISTARTICLES IN HUMAN EPIDERMAL KERATINOCYTES AND IN VIIVO PIG SKIN.

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Products utilizing the antimicrobial properties of silver (Ag) nanoparticles range from surgical tool coatings, surface and personal sanitizing sprays, toothbrushes, and infant pacifiers. The purpose of this study was to evaluate the cytotoxic effects of nanosilver in human epidermal keratinocytes (HEK) in vitro. Ag samples included: uncoated, unwatered colloidal 20nm, 50nm and 80nm of 0.2mg/ml in deionized water; uncoated, washed colloidal 20nm (2.86mg/ml), 50nm (3.45mg/ml) and 80nm (2.79mg/ml) in deionized water and 25 and 35nm coated Ag. Using the tunnel assay a significant effect was observed only at concentrations higher than 50 mg/ml. Summary: Ag and TiO NP induced ROS and loss of cell viability in a dose dependent manner, and cell death occurs primarily by apoptosis. In case of TiO, our results suggest that the toxicity should be expressed not alone by mass.

881 EFFECTS OF SILVER NANOPARTICLES (AG-45) UPON CORONARY ENDOTHELIAL CELLS.

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Nanoparticles (NP) are small scale engineered materials (<100 nm) with unique beneficial, physical, chemical and mechanical properties, however, their potential to produce adverse or beneficial effects is still not fully evaluated. This investigation was designed to determine whether silver nanoparticles (Ag-45 nm) induce any selective and specific biological effects upon coronary endothelial cells (CEC), such as proliferation, cytotoxicity, and nitric oxide production (NO). CEC were cultured and exposed to increasing concentrations (0.1–100 ug/ml) of Ag-45 nm for 24 hrs. Ag-45 induced dual effect upon cellular proliferation, an inhibition at low concentrations and stimulation at high concentrations, and these actions were directly associated with NO production and inversely related with cytotoxicity. Confocal microscopic studies showed that Ag-45 nm exposure, did not change CEC morphology, and were distributed widely in cellular surface, even with high concentrations of this agglomerate nanomaterial (NM). In addition, physical characterization of these Ag-45 nm, using TEM, demonstrated that the real range of this NM, compromise a heterogeneous group of silver sizes, from 10-90 nm. These results clearly demonstrate that Ag-45 nm show selective and specific effects on this vascular bed, depending on the concentration, and that opposite effect could be result of the heterogeneity of sizes. However, the protective effects we found could be mediated by the production of NO, a free radical produced mostly by endothelium from blood vessels and, mediated angiogenesis and vascular tone.

882 DESIGN, SYNTHESIS, & BIOLOGICAL EVALUATION OF NANOPARTICLE DRUG DELIVERY SYSTEMS.

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The prevalence of nanotechnology in medicine has grown exponentially in recent years – moreover, intense exploration into the toxicology of nanoparticles (NPs) has paralleled this growth. It is known that inorganic nanoparticles – such as those made from silica – catalyze the production of damaging free radicals. This undesirable toxicity limits the medical applications of inorganic nanoparticles. However, functionalization of the reactive nanoparticle surface may mask the potential for free radical generation. Free radical scavengers – in particular, 2,6-tetramethylpyridine-N-oxyl (TEMPO) – may be covalently attached to nanoparticles via an acid-labile oxazoline moiety, which selectively hydrolyzes to release the antioxidant payload upon lysosomal incorporation. This new release strategy may increase the biocompatibility of inorganic nanoparticles via reduction of oxidative stress pathways. As an extension, the oxazoline release mechanism may be utilized to deliver a myriad of small molecule drugs, as it is amenable to drug attachment via peptide coupling and esterification. Silica nanoparticles were prepared and their surfaces were functionalized with the oxazoline-TEMPO antioxidant release system. In addition, the nanoparticles were conjugated to fluorescein, thus permitting to monitor subcellular localization via fluorescence microscopy. The nanoparticles were coated with phosphatidylserine to allow for efficient uptake. Macrophages (RAW264.7) were incubated with these nanoparticles; rapid and extensive uptake was seen within 0.5-1 hours. The nanoparticles concentrated in lysosomes and mitochondria, with a significant reduction in superoxide generation. In summary, the toxicity associated with inorganic nanoparticles may be mitigated by surface modification with small molecule antioxidants, thus increasing their biocompatibility and prevalence in medicine and drug delivery. Supported by NIAID U19AI068211 and NIOSH OH08282.
883 ASSESSMENT OF ALUMINUM NANOPARTICLE INTERACTIONS IN HUMAN EPIDERMAL KERATINOCYTES.
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Aluminum nanoparticles (Al NP) have been used in drug delivery, as a surface coating, as an ingredient for solid rocket fuel, and in military explosives and artillery. Although Al NP have been suggested for many civilian and military applications, the health and safety of these nano size particles are not known. Size characterization was determined by dynamic light scattering and transmission electron microscopy (TEM). To understand the biological activity of Al NP, human epidermal keratinocytes (HEK) were exposed to 50nm and 80nm nanoparticles ranging in concentrations from 0.0004mg/mL to 4.0mg/mL in a humidified 5% CO2 atmosphere at 37°C. Media was harvested at 24 h to assess the inflammatory mediator interleukin (IL)-8. HEK viability was determined with MTT, alamarBlue, and CellTiter 96AQueous One (96 AQ) assays. There was no significant decrease (p<0.05) in viability relative to the controls with the 50nm and the 80nm Al NP at all treatment concentrations in the three assays, which suggests that the Al NP are not cytotoxic to HEK. TEM depicted AlNP localized within the cytoplasmic vacuoles of the cells. IL-8 data normalized to the cell viability was variable; indicating possible nanoparticle interactions with the cytokine assay. (Supported by the US Air Force Office of Scientific Research, FA 9550-08-1-0182)

884 PREPARATION AND STABILITY TESTING OF ORAL FULLERENE C60 FORMULATIONS WITH PARTICLES IN THE NANTOMETER AND MICROMETER SIZE RANGES.
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To allow for studies of the effect of size on the toxicity of nanoscale materials there is a need for the control and characterization of the size of materials in the dosing formulations used in toxicity studies. The purpose of this study was to develop methods for the preparation of oral gavage formulations of C60 with two different particle size ranges in support of studies as part of the NTP Nanotechnology Safety Initiative. The C60 as received was made up of 10 – 20 μm particles agglomerated into clusters approximately 100 μm in diameter. Several methods for reducing the particle size of the material into the desired ranges were attempted. All of the methods, except grinding in oil, resulted in reagglomeration of the C60. A formulation with nanometer-sized particles was produced by grinding C60 in Cremophor® EL for 7 days followed by dilution to a final formulation of approximately 1:1.8 Cremophor:ethanol:water. The formulation was filtered through a series of filters with descending pore sizes. The formulation with micrometer-sized particles was prepared similarly, except it was only ground for approximately 15 minutes and was not filtered. Both formulations were analyzed for particle size by laser diffraction and concentration by HPLC/UV. The nanometer and micrometer-sized C60 formulations were found to have an average particle sizes of 211 nm and 16.6 μm, respectively. Both formulations were homogenous at concentrations of approximately 30 mg/mL. The formulations were reanalyzed for concentration and particle size after 42 days of storage at room temperature and no significant changes in either were observed. This study shows that homogenous, stable, oral gavage formulations of the fulleren C60 with different particle sizes can be prepared in a mixture of Cremophor, ethanol, and water. This work was supported by NTP Contract N01-ES-05456.

885 SILICA-BASED NANOPARTICLE UPTAKE AND CELLULAR RESPONSE IN PRIMARY MICROGLIA.
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Silica-based nanoparticles are currently being developed for imaging and diagnostic applications in medicine. Nanoparticles have been used to visualize brain tumors, thus they are able to cross the blood-brain barrier and enter the brain parenchyma. silica-based nanoparticles are more biocompatible than quantum dots that contain toxic metals like cadmium, however, there is a paucity of knowledge on their cellular target(s) in the brain and how they affect cellular function(s). Microglia are the immune-compotent cells of the brain. They have neuroprotective and immunocompetent functions in order to maintain neuronal health. In this study, primary microglia from rat brain were used to determine if silica-based nanoparticles are phagocytized and if they affect microglia function. Primary microglia were isolated and plated as a density of 250,000 cells per plate. After overnight incubation, they were exposed to 10, 100, or 1000 silica-based nanoparticles per plate. These silica-based nanoparticles are embedded with a fluorescent dyes, allowing tracking of cellular uptake. Fluorescent confocal microscopy of nanoparticle-exposed microglia indicated that there was intracellular accumulation at all concentrations tested. Using a MTS assay, the concentration of nanoparticles tested produced no significant effect on cell viability. However, exposure to nanoparticles resulted in a dose-dependent increase in the generation of reactive oxygen species in microglia. Microglia are known to express inflammatory cytokines and enzymes such as TNF-α and COX-2, respectively. Analysis of TNF-α and COX-2 gene expression using real-time quantitative PCR showed a reduction in TNF-α gene expression at all concentrations tested and a significant increase in COX-2 gene expression only at the highest concentration. The consequences of these changes to microglia function are currently under investigation. (Supported by the Institute for NanoBiotechnology at Johns Hopkins and NIEHS-T32 ES07141)

886 THE TRANSPORT OF TiO2 NANOPARTICLES IN HUMAN LUNG EPITHELIAL CELLS AND ALVEOLAR MACROPHAGES: HOW DOES AGGREGATION STATE INFLUENCE CELLULAR UPTAKE MECHANISMS?
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The use of nanomaterials in today’s society is increasing at a rapid pace. However, with the uses of a new technology comes the hazards and risks associated with its exposure. Here, a previously well-characterized titanium dioxide (TiO2) nanoparticle sample is further examined to determine the toxic properties associated with cellular uptake in human lung epithelial cells and alveolar macrophages. Literature shows a variety of mechanisms associated with the uptake of nanoparticles; however, we suggest that nanoparticle aggregates (termed nanogaggregates) and defined primary nanoparticles (those particles that have little to no aggregation) yield differing cellular responses. A time-course study incorporating transmission electron microscopy (TEM) was used to examine the aggregation state of the nanoparticles upon cellular translocation. In addition, a variety of cellular uptake mechanisms (i.e. transmembrane diffusion vs. endocytosis) were examined. Furthermore, the fate of the particles and time for clearance was studied. Results indicate that the TiO2 nanoparticles enter the epithelial cells or macrophages in either of two forms (aggregates or primary particles). It was found that larger aggregates were incorporated into vesicles such as endosomes, while primary particles were found in the cytosol. Ongoing studies will determine if these cytosolic nanoparticles interact with proteins or other charged subcellular components due to their unique electron configuration and surface charges. The chemical identity of TiO2 inside the cells was confirmed using energy dispersive X-ray spectroscopy (EDS). Elucidating the interaction between cellular substrates and nanoparticles is crucial to developing a mechanistic approach to the conflicting toxic responses of TiO2 and other nanoparticles.

887 NANOCHEMISTRY, IN VITRO-A NEW DIMENSION TO NANOTOXICOLOGY USING NANO-SIZED CARBON BLACK AND IRON OXIDE.
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Exposure to a variety of different classes of nanomaterials is significant to both consumers and the environment. Little effort has been put forth on the hazard assessments of co-exposure to nanomaterials, i.e. exposure to two or more nano-sized materials (as consumer product usage or occupational exposure scenarios). This study was designed to investigate the effects of the chemical reactions between nano-sized carbon black and nano-sized iron oxide in both ex vivo and in vitro conditions. We exposed human lung epithelial cells and alveolar macrophages to nano-carbon black and nano-iron oxide alone, as well as in co-exposures. We expected that the carbon black and iron oxide co-exposures would induce a greater cytotoxicity and oxidative stress than in single nanoparticle exposures. Additionally, we characterized the carbon black and iron oxide nanoparticles in an effort to develop a structure-activity relationship. To assess cytotoxicity in culture, we used enzymatic and metabolic indicators (Resazurin and MTT dye) and cell counts (Coulter counter) to evaluate cell viability. Protein, membrane, and DNA oxidation was used as indicators of oxidative stress. Results show that in cell-free conditions the physical and chemical characterization profiles of nano-carbon black and nano-iron oxide are different. A decreased cytotoxic response of cells exposed to nano-sized iron oxide alone versus co-exposure with nano-carbon black was apparent. In regard to the REDOX chemistry, the reaction between carbon black and iron oxide resulted in a reduced form of iron in solution (namely Fe2+), causing a significant increase in cellular oxidation. These results illustrate the need to not only identify the toxic
profiles of single nanoparticle exposures, but in order to effectively cope with health and environmental concerns, we must also know how these sole nanoparticles interact with other particles in biological and ecological environments.

Silicon nanowires (NW) are anisotropic crystals with semi-conductor capabilities and are currently being utilized in the production of biosensors, gas sensors, and field electric transistors. Because of their composition and large length to diameter ratio, silicon NW-induced toxicity may differ from other forms of nanoparticles. A variety of different forms of nanoparticles have been shown to produce toxic effects in cellular and animal models through the induction of oxidative stress. The potential for oxidative damage after exposure to silicon NW has not been investigated.

The goal of this study was to assess the generation of free radicals by silicon NW. Electron spin resonance (ESR) was used to monitor hydroxyl radical production in an acellular system by measuring Fenton-like reactions in the presence of hydrogen peroxide. In addition, the ability to produce radicals after cellular exposure was also determined by ESR. These measures of particle surface reactivity of silicon NW (20 nm diameter x 10 μm length) at two different concentrations (0.2 mg/ml or 0.1mg/ml), were compared to those of titanium dioxide (TiO2) NW (0.1 mg/ml or 0.05 mg/ml), amosite asbestos fibers (0.2 mg/ml or 0.1 mg/ml), and lead chromate particles (PbCrO4) as a positive control (0.5 mg/ml). For in vitro studies, two cell types were used to measure reactive oxygen species (ROS) production, primary rat macrophages harvested from the lungs of male Sprague-Dawley rats and the RAW 264.7 macrophage cell line. ESR results showed no presence of hydroxyl radical or ROS production with silicon NW, in either the acellular or cellular systems. Hydroxyl radicals were observed in PbCrO4 and amosite asbestos. However, radicals were not seen in TiO2 NW. These findings indicate that silicon NW may not induce a significant increase in oxidative stress and associated damage.

We used amorphous (monodisperse) spherical silica nanoparticles with a diameter ranging from 2, 16, 60, 104 to 340 nm. Particles were incubated with epithelial cells and macrophages (A549 + THP-1) at a concentration of 5 μg/cm² cell cultures surface or at 10 cm² particle surface area/cm² for 24 h whether or not in the presence of confluent EAHY926 cells in an insert introduced above the bioculture after 12 h. At 24 h, supernatants were recovered and TNFalpha, IL-6, IL-8, MMP-1alpha and MIP-Ibeta were measured by means of a bead assay.

Quantification using the FACS array showed significant increases for TNFalpha, IL-6, IL-8 and MIP-1alpha for the 2 nm particles in all conditions tested. The larger particles did not induce cytokine expression at 5 μg/cm², but at dosing 10 cm² particle surface area/cm² (and thus a large mass) also the larger particles induced a response. In the biocultures, TNFalpha, IL-8 and IL-6 were most prominent, while in the EAHY926 cells also MIP-Ibeta was significant increased. This work clearly shows the role of size and surface area in response to nano-silica. Work was financed by the Belgium Ministry of Scientific Policy in the frame of the ‘Science for sustainable development’ programme (S’NANO project, contract number SD/HE/02A).

Nanoparticle colloidal suspensions are used for both industrial and medicinal purposes. To resolve some of the outstanding toxicity concerns, we are utilizing a model that more accurately mimics the response of the liver in an ex vivo cell culture system, namely co-cultures of primary hepatocytes, Kupffer’s cells, and lymphocytes isolated from C57BL6 mice. The manufactured inorganic metal nanomaterials include colloidal silver (Ag), copper (Cu), and nickel (Ni). The sizes, characterized in the dry state using BET surface area analyses and in the wet phase using dynamic light scattering and electron microscopy, vary with time, temperature, and media. Further, the pH of each suspension changes over time. We also found metal ions leaching from each material via atomic emission spectroscopy. These dynamic physico-chemical properties influence the biological response in two distinct pathways: heavy metal cationic effect versus a nano-size effect. Cells were exposed to the three metal nanomaterials, independently. Post-exposure time points ranged from 4 hr to 1 wk. Colortometric cytotoxicity was measured using MTT and reazurin dyes. Inflammatory response was determined by cytokine profiles (TNFalpha, and IL-6). A stable PXR-transfected luciferase reporter cell line (JBC, Gu) was used to test the effects of the nanomaterials on the pregnane X transcription activity. Nano colloidal Ag, Cu, and Ni treatment enhanced the PXR ligand Rif-induced reporter gene activity, suggesting an alteration of the xenobiotic metabolism. The mechanism for the interaction will be further analyzed. Results from cells ex-
posed to Ag exhibit no significant change in cytotoxicity; decrease in IL-6; increase in TNF-alpha. Cells exposed to Cu and Ni exhibit increase in cytotoxicity; IL-6 and TNF-alpha. We conclude that all three nano metal colloidal systems behave differently in physico-chemical properties and immune-toxicological responses.

**893 SYNTHESIS, CHARACTERIZATION, AND CYTOTOXIC EFFECTS OF MANGANESE NANOPARTICLES.**
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Recently we have shown that nanoparticles such as Mn, Ag and Cu can be neurotoxic by generating ROS and depleting dopamine concentration in PC-12 cells. We have also found significant changes in genes associated with oxidative stress and apoptotic pathways. In the present study we synthesized Mn nanoparticles (Mn-NP) using different solvents (decaffeinated tea, isopropyl alcohol and caffeine). These NP were subsequently characterized using Dynamic Light Scattering, Laser Doppler Velocimetry and TEM. Mn-NP when reduced with different solvents produced semi-spherical, poly-dispersed particles with an average size of 97.6 nm in decaffeinated tea, 32 nm in isopropyl alcohol, and 25 nm in caffeine. Astrocytes respond to the synaptic release of various neurotransmitters, and their dysfunction has been implicated in Mn-induced neurotoxicity. Therefore, we evaluated the cytotoxicity of Mn-NP using astrocytes and neuronal cultured cells. Mn-NP also produced a time-dependent increase in the expression of nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor with a key role in regulating oxidative stress response. Morphological evaluation showed a time-dependent effect of Mn-NP on cell morphology. The most interesting effect was found in cells treated with Mn-NP in tea. Eight hrs post-treatment, astrocytes appeared deformed and increased in size by 24 hrs. However, with other media, the effects were not as pronounced. Similar effects were found in neuronal cultures, where Mn-NP in tea produced the most pronounced dysmorphic effects. These data suggest Mn-NP effectively produce neurotoxicity by affecting astrocytic and neuronal functions, most likely by generating oxidative stress and neurotoxicity.

**894 THE ENDOTHELIAL AND ADIPOCYTE CELL SPECIFIC ROLE OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR (PPAR) IN DMBA-MEDIATED BREAST TUMOURIGENESIS.**
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Peroxisome proliferator-activated receptor (PPAR) plays a role in tumourigenesis. Previous studies with PPARγ (α/δ) mice suggest PPARγ normally suppresses di-methylbenz[a]anthracene (DMBA)-induced breast, and other, tumour progression; however, the mechanisms remain unknown. Conditional adipocyte (A) and endothelial (E) cell-specific PPARγ knockout mice (PPAR-A KO and PPAR-E KO respectively) were used to evaluate whether PPARγ signaling normally acts to prevent DMBA-mediated breast tumour progression in a stromal cell-specific manner. Twelve week old PPARγ KO mice and their congenic wild-type (WT) controls were randomly assigned to one of two treatment groups. All mice were treated by gavage once/week for 6 weeks with 1 mg DMBA and maintained on a normal chow diet. At week 7, mice in each group were divided into those continuing normal chow, and those receiving a PPARγ agonist. Both PPAR-A-KO and PPAR-E-KO mice showed a significant increase in breast tumour multiplicity and larger DMBA-mediated mammary tumours compared to PPAR WT controls. In contrast, DMBA+Rosi treated PPAR WT, but not PPAR-A-KO or E-KO mice, appear to be protected from these effects. These results suggest normal PPARγ signaling in adipocytes and endothelial cells may contribute to the suppression of DMBA-mediated breast tumour growth; 2) add evidence supporting a protective role for PPARγ activation in reducing environmental chemical-mediated breast tumour progression; and 3) may facilitate development of more effective drugs against breast cancer.

**895 RAT CARCINOGENICITY STUDY WITH GW501516: A PPAR DELTA AGONIST.**
Safety Assessment, GlaxoSmithKline, King of Prussia, PA, 2Safety Assessment, GlaxoSmithKline, Ware, United Kingdom and 3Huntingdon Life Sciences, Huntingdon, United Kingdom.

GW501516, a non-genotoxic PPARγ agonist, was assessed for carcinogenic potential by daily administration (oral gavage) to Han Wistar rats for a period of 104 weeks. Males were given 0, 5, 15 or 30 mg/kg/day for the first 6 weeks of the study. For the remainder of the study males were given 0, 20 or 40 mg/kg/day. Females were given 0, 3, 10 or 20 mg/kg/day for the entire study. GW501516 produced test article-related neoplastic findings in multiple tissues at all doses. Increased mortality was seen with females given GW501516 at all doses and uterine endometrial adenocarcinoma contributed to death in a high proportion of these animals. Neoplasms considered test-article related occurred in the liver (hepatocellular adenoma at ≥ 10 mg/kg/day), urinary bladder (transitional cell carcinoma in males given 20 and 40 mg/kg/day), thyroid (feline cell adenoma at ≥ 3 mg/kg/day and carcinoma in males at ≥ 20 mg/kg/day), tongue (squamous cell papilloma in males at 5 mg/kg/day and 40 mg/kg/day), stomach (squamous cell papilloma in males at ≥ 3 mg/kg/day and a female at 20 mg/kg/day, and carcinoma in a male at 40 mg/kg/day and a female at 3 mg/kg/day), skin (inverted squamous cell papilloma in males at ≥ 5 mg/kg/day and females at 3 or 20 mg/kg/day),Harderian glands (adenoma in males at ≥ 5 mg/kg/day and adenocarcinoma in a male at 40 mg/kg/day), testis (interstitial cell adenoma at 40 mg/kg/day), ovary (Sertoli cell adenoma at ≥ 10 mg/kg/day) and uterus (polyp and endometrial adenocarcinoma at ≥ 5 mg/kg/day). Some of the tumor types observed in this study have not been reported with either PPARα or PPARγ agonists and may reflect tumor promotion mediated through PPARα agonism.

**896 MOUSE CARCINOGENICITY STUDY WITH GW501516: A PPAR DELTA AGONIST.**
Safety Assessment, GlaxoSmithKline, King of Prussia, PA, 2Safety Assessment, GlaxoSmithKline, Ware, United Kingdom and 3Huntingdon Life Sciences, Huntingdon, United Kingdom.

GW501516, a non-genotoxic PPARγ agonist, was assessed for carcinogenic potential by daily administration (oral gavage) to CD1 mice for 104 weeks at doses of 0, 10, 30, 60 or 80 mg/kg/day. Survival was decreased at doses ≥ 30 mg/kg/day. Neoplasms considered related to test article occurred in liver (hepatocellular carcinoma at ≥ 30 mg/kg/day and adenoma at ≥ 10 mg/kg/day), stomach (squamous cell carcinoma at all doses) and combined squamous cell tumours at all doses (squamous cell papilloma and carcinoma, and keratoacanthoma). There have been conflicting reports in the literature regarding the effects of PPARγ on epithelial cell proliferation. The results of this study demonstrate an increase in proliferation in certain epithelial cell populations, but do not support a role for PPARγ in colon cancer tumours. The squamous cell tumours observed in this study have not been reported with either PPARα or PPARγ agonists and may reflect tumor promotion mediated through PPARα agonism.

**897 LIGAND-ACTIVATION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-BETA/Delta INHIBITS THE PROLIFERATION OF HUMAN PANCREATIC CANCER CELLS.**
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Pancreatic cancer is the fourth leading cause of cancer-related deaths in the United States, owing to its extremely aggressive nature and resistance to chemotherapeutic regimens. In the present study, we report that activation of the nuclear receptor PPAR-β/δ decreases human pancreatic cancer cell growth. The proliferation of three human pancreatic cancer cell lines - BxPc-3 (high COX-2 expression), Panc-1 and MIA PaCa-2 (COX-2 negative) - was inhibited by the PPAR-β/δ selective agonists, Ciprofibrate and Rosiglitazone, respectively, but most effectively by the PPARγ/β/δ-selective agonist GW501516. Treatment of pancreatic cancer cells with a PPAR-β/δ-selective agonist significantly decreased mRNA levels of the cell cycle regulatory gene, cyclin D2, compared with control cells at 24 hours. Furthermore, GW501516 treatment significantly reduced the TNF-α-induced mRNA expression of pro-inflammatory mediators in MIA PaCa-2 and BxPc-3 cells, several of which are known to influence cell proliferation. PPAR-β/δ interacts with both PPARγ/β/δ and PPARα, but was not dependent on ligand. PPAR-β/δ is unique among the PPAR isofoms in its association with the transcriptional repressor, Bcl-6. We hypothesize that PPAR-β/δ activation results in Bcl-6 shuttling from the receptor complex to its known consensus sequence in the cyclin D2 promoter where it influences pancreatic cancer cell proliferation.
898 COMBINING LIGNIN ACTIVATION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-β/δ (PPARβ/δ) AND INHIBITION OF CYCLOOXYGENASE-2 (COX2) ACTIVITY EXERTS BOTH CHEMOPREVENTIVE AND CHEMOTHERAPEUTIC EFFECTS ON SKIN TUMORIGENESIS.

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PPARβ/δ-null mice exhibit exacerbated skin tumorigenesis and lignin activation of PPARβ/δ can inhibit chemically-induced skin tumorigenesis. In the present studies, the effect of combining lignin activation of PPARβ/δ with inhibition of COX2 activity on skin tumorigenesis was examined using in vitro and in vivo methods. Modest chemopreventive effects of the COX2 inhibitor nimesulide were observed in wild-type mice but not in similarly treated PPARβ/δ-null mice. In addition, better chemopreventive effects of the PPARβ/δ ligand GW0742 were observed in wild-type than PPARβ/δ-null mice. Increased efficacy of chemoprevention was also observed by combining GW0742 with nimesulide, and a significant decrease in tumour multiplicity was only observed in wild-type mice following co-administration of GW0742 and nimesulide. In wild-type mice with pre-existing papillomas, co-administration of GW0742 and nimesulide caused an increase in the efficacy of decreasing tumour multiplicity and that this effect was not seen in similarly treated PPARβ/δ-null mice. No effects on tumor size were seen in the above experiments. Treatment of neoplastic mouse keratinocyte cell lines with a combination of GW0742 and COX2 inhibitors resulted in enhanced inhibition of cell proliferation. Results from these studies demonstrate that combining ligand activation of PPARβ/δ with inhibition of COX2 activity can increase the efficacy of attenuation of skin carcinogenesis. These results also suggest that this approach may be suitable for both chemoprevention and chemotherapy.

899 OVEREXPRESSION OF THE PROSTAGLANDIN E2 RECEPTOR SUBTYPE 1 (EP1) PROMOTES SKIN TUMOR DEVELOPMENT THROUGH COX-2 INDUCTION.

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Prostaglandin E2 (PGE2) has been shown to promote skin tumor development. EP1 is one of four PGE2 receptors. EP1 mRNA levels were increased after a single or four topical treatments of 12-O-tetradecanoylphorbol 13-acetate (TPA) on skin as well as in 7,12 dimethylbenz[a]anthracene (DMBA)/TPA-induced tumors. To determine whether the EP1 receptor levels affect skin tumor development, we generated B6.C3F1 transgenic mice which overexpress EP1 in the basal layer of the epidermis. The skins of these mice are histologically indistinguishable from wild type mice. To determine the role of EP1 in skin tumor development, a DMBA/TPA skin carcinogenesis protocol was used. EP1 transgenic mice had a reduced tumor multiplicity and a reduced tumor incidence compared to wild type mice, but had a higher papilloma to carcinoma conversion rate. In DMBA only skin carcinogenesis protocol, EP1 transgenic mice developed more tumors than wild type mice. The effect of EP1 on cell proliferation was measured in vitro. After TPA treatment, this proliferation was increased in both EP1 transgenic mice and wild type mice to a similar extent. However, 5 days after DMBA treatment, there was about 2 fold more K67 positive cells in the basal layer of the epidermis of EP1 transgenic mouse skin than wild type mice. To confirm that the enhanced tumor formation in transgenic mice is in fact PGE2 dependent, mice were given the selective cyclooxygenase-2 inhibitor Celecoxib or a control diet starting 1 week before DMBA treatment. Surprisingly, there was no lesion formation at all on mice that were fed Celecoxib. Histological sections of skin from Celecoxib fed mice showed a fairly normal skin structure 2 weeks after DMBA treatment compared to pronounced pseudocarcinomatous hyperplasia in control mice. By western blotting, COX-2 and phospho-c-Jun were increased in untreated EP1 transgenic mouse skin. Therefore, our results suggest that increasing PGE2 production through c-Jun and COX-2 induction and promotes tumor development. Supported by CA100908 to JEK.

900 PPARβ/δ REGULATES AHR SIGNALING IN SKIN.

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Peroxisome proliferator-activated receptors (PPARs) are ligand activated transcription factors with cell-specific biological functions. Previous evidence has shown that PPARβ/δ can attenuate skin tumorigenesis, which could be due in part to modulation of carcinogen metabolism. This hypothesis was examined using PPARβ/δ-null mouse models, and surprisingly, PPARβ/δ-null mouse skin and primary keratinocytes were refractory to 7,12-dimethylbenz[a]anthracene (DMBA)-dependent induction of cytochrome P450 1A1 (CYP1A1) or CYP1B1 mRNA and protein. Similar results were obtained with the human HaCaT keratinocyte cell line stably expressing shRNA against PPARβ/δ. The difference in CYP1A1/B1 expression was also observed with other polycyclic aromatic hydrocarbons (PAHs), and this effect did not appear to be due to differences in the relative level of the AHR or accessory proteins, which are known regulators of CYP expression. Interestingly, Phase I and other non-AHR-dependent genes show a similar expression profile upon PAH treatment in the absence of PPARβ/δ expression. To begin to delineate the mechanism by which PPARβ/δ regulates AHR signaling, ligand binding and the nuclear translocation of AHR were examined, but no difference in these responses were observed between genotypes. Glutathione S-transferase (GST) pull-down of AHR with PPARβ/δ suggests there is not a functional interaction between the two receptors, although this must be more thoroughly examined in detail. The occupancy of the CYP1A1 promoter in response to PAH was examined by chromatin immunoprecipitation (ChIP), and in the absence of PPARβ/δ expression, reduced AHR occupancy of the CYP1A1 promoter was observed. These findings suggest a novel mechanism in mouse and human skin where PPARβ/δ modulates AHR-dependent signaling and potentially alters the carcinogenic effect induced by PAH, likely through modifying the balance between bioactivation and clearance of PAH. (Supported by CA97999, CA124533, J.M.P., ES04869 (G.H.P.), and an NSF GRFP).

901 INVOLVEMENT OF REACTIVE OXYGEN SPECIES AND MACROPHAGES IN TGGOLITAZONE (TGZ)-INDUCED HEMANGIOSARCOMAS.

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Hemangiosarcomas are malignant vascular tumors, rare in humans but relatively common in mice (especially in liver). In mice, these tumors are inducible by both genotoxic and non-genotoxic agents. The mechanism for hemangiosarcoma induction remains unclear. We have previously demonstrated that Kupfer cell (the macrophage of the liver) activation secondary to chronic hemolysis, and ROS and VEGF production are involved in 2-butoxy ethanol induced hemangiosarcomas. To clarify the role of hemangiosarcoma induction by TGZ, we investigated the VEGF gene expression status using real-time PCR. Following exposure to TGZ, the mRNA level of VEGF in endothelial cells increased 1.4-fold at 24 hr, and 2.6-fold at 48 hr. The increase of VEGF levels was 1.7- and 4.9-fold in endothelial cells co-cultured with macrophages only and macrophages pretreated with TGZ for 48 hr, respectively. Taken together, these results suggest that TGZ stimulates endothelial cell growth through induction of ROS and upregulation of VEGF gene expression. In addition, these findings support the involvement of ROS and macrophages in TGZ-induced hemangiosarcomas. Supported in part by NIH CA100908 to JEK.

902 EVALUATION OF THE EFFECTS OF THE PPARγ AGONIST TROGLITAZONE ON CYTOTOXICITY AND MITOGENESIS OF ENDOTHELIAL CELLS: DIFFERENCES BETWEEN HUMAN AND MOUSE.

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Long-term treatment with high doses of troglitazone (TG) increased the incidence of hemangiosarcomas in various tissues in mice. We observed increased endothelial cell (EC) proliferation in the liver and brown and white adipose tissues in female B6C3F1 mice treated with 400 and 800 mg/kg for 4 weeks. The purpose of this study was to determine if TG directly increases EC proliferation in human (HMEC1) and in mouse (MFP MVEC) microvascular endothelial cells. Cytotoxicity occurred in a dose dependent manner in HMEC1 and MFP MVEC at high doses of TG. The LC50 on day 3 was 17.4 μM in HMEC1 and 92.2 μM in MFP MVEC.
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Macrophage reverse cholesterol transport halts the progression of atherosclerosis and promotes its regression. Hydrolysis of cholesteryl esters in macrophages is the rate limiting step in this process. The best candidate enzyme for this hydrolytic activity is cholesteryl ester hydrolase (CEH), also known as carboxylesterase 1 (CES1). CES1 has an important role in xenobiotic metabolism. Here, we studied the inhibitory effects of several oxysterols, cholesterol, and fatty acids of physiological origins on the carboxylesterase activity of THP1 cell line and the recombinant human CES1. Our results for oxysterols using THP1 cells show: (1) 27-hydroxycholesterol is a potent inhibitor (IC50 ~8nM); (2) 24(S),25-epoxycholesterol has moderate inhibitory activity (IC50 ~5μM); (3) 22(R)-hydroxycholesterol, 24-hydroxycholesterol, 25-hydroxycholesterol, and 7-ketocholesterol all exhibit minimal inhibitory activity; (4) cholesterol has no inhibitory effect. 27-Hydroxycholesterol appears to act as a non-competitive inhibitor of pure CES1 with Ki ~7μM. Furthermore, unsaturated fatty acids are better inhibitors of CES1 than saturated fatty acids, with arachidonic acid being the most potent fatty acid tested (IC50 ~1.7μM). The degree of unsaturation seems to correlate with inhibition potency (rank order of unsaturated fatty acids: arachidonic acid > linolenic acid > linoleic acid). In general, short-chain saturated fatty acids are better inhibitors than long-chain saturated fatty acids. Our results suggest that oxysterols and fatty acids might influence cholesterol metabolism in macrophages by directly inhibiting the cholesteryl ester hydrolase activity of CES1, thus reducing the rate of cholesterol efflux from these cells. [Supported by NIH R18ES015348]

**906 EVALUATION OF ELECTROCARDIOGRAMS IN TOXICOLOGY STUDIES: HOW MUCH IS ENOUGH?**


In a typical toxicology study, approximately one minute of electrocardiographic (ECG) data is obtained at specified time points while the animals are either physically restrained or anesthetized. The emergence of noninvasive ECG monitoring devices has enabled continuous monitoring for an extended period of time; from two hours up to 24 hours continuously or ranging from 120 to 1400 one-minute intervals. These novel techniques enable the collection of more than 100 times the typical study data thereby increasing the amount of time and effort it takes to analyze the data for a study. The hypothesis from the sensitive formulation is does the sensitivity increase by detecting changes in measured intervals of continuous data collection when compared to the measured intervals of only a few ECG complexes. In this study, 32 cynomolgus monkeys (n=6/group/sex) were given a single oral dose of 0, 10, 50, 175 mg of monofloxacin/kg of body weight. ECG data were evaluated by measuring 20 consecutive ECG complexes within each one minute segment for each pre-determined time point: predose, 2, 4, 6, 18, and 24 hours postdose. A comparison was made between the data set of 20 consecutive measured ECG complexes versus the data set generated with five minute averages of measured ECG complexes. Measurements for the five minute averages were achieved with the use of semi-automated ECG template analysis software. The 20 consecutive measured ECG complexes resulted in the mean covariate differences of QTc ranging from 26 to 50 msec (or ~8 to 15% increase). However, the five minute averages of measured ECG complexes resulted in the mean covariate differences of QTc ranging from 12 to 62 msec (or ~4 to 19% increase). This indicates that by increasing the number of measured ECG complexes from 20 consecutive waveforms to five minute averages, the sensitivity to detect QTc increases.

**907 PLASMA NITRITES, ENDOTHELIAL FUNCTION AND THE EFFECTS OF DIESEL INHALATION.**

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Plasma nitrite (NO2-) has been proposed as a biochemical marker of endothelial function. The rapid oxidation of NO2- to nitrate brings into question its usefulness in clinical studies. In order to determine the viability of NO2- as a biomarker, we...
have assessed its stability in plasma over time and the effect of sample storage. By addition of exogenous NO2- to freshly prepared human plasma we have determined that NO2- consumption is second order and that the rate is low below 1µM at 4°C. As human plasma NO2- concentrations are approximately 10nM, one would predict that samples can be kept on ice for at least 3 hours. We have confirmed this using plasma from healthy subjects. Here we show that snap freezing at -80°C and thawing did not significantly reduce NO2- concentration; indicating that samples can be stored for later analysis. Brachial artery occlusion and release, a hyperemic stimulus, demonstrates plasma NO2- in healthy subjects. We have calculated the physiologic measure of endothelial function, brachial artery forearm-mediated dilation. Nitrite levels prior to hyperemic stimulus are similar in diabetic and healthy subjects. Following stimulus, diabetic groups do not significantly increase NO2-. Endothelial dysfunction may also occur in response to particular exposure, such as diesel, which may be exacerbated in the presence of disease. As diesel exhaust contains oxides of nitrogen, inhalation may lead to artificial changes in NO2-. Therefore, we determined if these oxides contribute to plasma NO2- in the absence of exogenous NO2- addition and consequent promotion of angiogenesis through up-regulation of VEGF expression.

Conclusions: B[a]P inhibited ischemia-induced angiogenesis in mice, and this effect was greatly diminished by ablation of the AHR. Our observations suggest that B[a]P up-regulated the expression of metallothionein in the ischemic hindlimb of wild-type mice were reduced by exposure to B[a]P. These various effects of B[a]P were markedly attenuated in AHR-null mice. These various effects of B[a]P were markedly attenuated in AHR-null mice. To the plasma membrane NADPH oxidase system, there are other important sources (presumably mitochondria) of reactive oxygen species (ROS) that contribute to the enhanced oxidative stress status in the H9c2 cells exposed to B[a]P. Alternatively, the so-called ‘non-NADPH oxidase’ scavenger receptor, such as sequestration (SR), and yet other unknown activities that need further investigation. 

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Inhibition of Ischemia-Induced Angiogenesis by Benzo[a]pyrene in a Manner Dependent on the Aryl Hydrocarbon Receptor.

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Objectives: Smoking is an important risk factor for ischemic heart disease and peripheral vascular disease. We have investigated the effect of benzo[a]pyrene (BP), a carcinogen of tobacco smoke and an agonist for the aryl hydrocarbon receptor (AHR), on hypoxia-induced angiogenesis. Methods and Results: Ischemia was induced by femoral artery ligation in wild-type and AHR-null mice, and the animals were subjected to analyses of 29 healthy subjects post diesel exposure on a weekly basis. We show that exposure to B[a]P up-regulated the expression of metallothionein in the ischemic hindlimb and markedly inhibited ischemia-induced angiogenesis in wild-type mice. The amounts of interleukin-6 and of vascular endothelial growth factor (VEGF) mRNA in the ischemic hindlimb of wild-type mice were reduced by exposure to B[a]P. These various effects of B[a]P were markedly attenuated in AHR-null mice. Conclusions: B[a]P inhibited ischemia-induced angiogenesis in mice, and this effect was greatly diminished by ablation of the AHR. Our observations suggest that the loss of the inhibitory effect of B[a]P on ischemia-induced angiogenesis appears in AHR-null mice may be attributable to maintenance of interleukin-6 expression and consequent promotion of angiogenesis through up-regulation of VEGF expression.

Investigation of Intracellular Calcium Change in Cultured Cardiomyocytes Using Laser Scanning Cytometry.

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Increased intracellular Ca2+ concentration is involved in several separate mechanisms of drug-induced cardiac ventricular arrhythmia. We developed and optimized an in vitro model for evaluating intracellular Ca2+ concentration ([Ca2+]i) using the mouse cardiomyocyte cell line HL-5 by laser scanning cytometry and Fluo-4 AM as a fluorescent indicator. Ionomycin (INM), a Ca2+ ionophore, was used as a control and compared with effects elicited by the positive controls, Bay-K 8644 (BK, an L-type channel activator), and thapsigargin (TG, a sarco/plasmatic reticulum Ca2+ ATPase inhibitor). Both instant and delayed effects on 

Ca2+ (i), were assessed. Instant effects on [Ca2+]i were measured at 30 s intervals ranging 2 min before and after treatments. Delayed effects were assessed at 0.5, 30, 120 and 360 min post treatment. In instant assays, INM robustly increased [Ca2+]i by 80% compared with vehicle control. BK and TG instantly increased [Ca2+]i by 40%. The effect with BK treatment was more sustained than with TG. In delayed assays, treatment with INM immediately prior to measurement at each timepoint increased [Ca2+]i to similar levels, ensuring functionality of the model. BK caused a rapid increase of [Ca2+]i, which slowly decreased over 360 min. TG caused similar [Ca2+]i increase but it diminished to the vehicle level by 30 min. TG induced cytotoxicity by 120 min and cell death at 360 min. This novel, sensitive in vitro imaging method successfully detects changes in [Ca2+]i in HL-5 cells occurring via different mechanisms, and has the potential for use in addressing cardiac toxicity issues arising in drug development.

Mainstream Cigarette Smoke (M) Affects the Cardiovascular System of Spontaneously Hypertensive Rats (SHR).


Cigarette smoke elevates the risk for cardiovascular disease. This study investigates the effects of M (450, 900, and 1350 µg total particulate matter (TPM)/day; 5 days/week; 90 days) on cardiac functional and phenotypic changes, as well as on...
cardiovascular gene expression. Langendorff analysis of heart function revealed a statistically significant decrease in baseline perfusion pressure for the SHR groups exposed to 900 and 1350 ug TPM/day in comparison to sham (p < 0.05). Exposure of SHR to 900 ug TPM/day resulted in a statistically significant reduction in post-ischemic recovery after 30 minutes of global ischemia compared to sham (p < 0.05). Ratios of both heart weight and left ventricular weight to (1) body weight, (2) brain weight, and (3) tibia weight were statistically significantly increased for the 900 and 1350 ug TPM/day groups compared to sham (p < 0.05). Affymetrix gene chip analysis indicated that MS induced changes in a panel of ventricular genes. This was confirmed by PCR analysis (e.g., increased tissue inhibitor of metalloproteinase 1 in the 1350 ug TPM/day compared to sham animals; p < 0.05). Multi-analyte profiling of 67 serum proteins revealed statistically significant changes in several markers after exposure to 900 ug TPM/day including increases in interleukin 10, macrophage chemotactic proteins 1 and 3, stem cell factor, and tissue inhibitor of metalloproteinase 3 (p < 0.05 versus sham). A statistically significant increase in the platelet activation marker 2, 3 dinor thromboxane B1 was detected in urine of SHR exposed to 1350 ug TPM/day compared to sham (p < 0.05). The data suggest that this model system may be useful in dissecting the mechanisms involved in smoke-induced cardiovascular disease and potentially for screening reduced-risk tobacco products.

913 GENDER DIFFERENCES IN THE PULMONARY REGENERATIVE RESPONSE TO NAPHTHALENE-INDUCED BRONCHIOLAR EPITHELIAL CELL INJURY.

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Substantial epidemiological evidence suggests that gender affects the incidence and severity of several pulmonary diseases, such as chronic obstructive pulmonary disease and lung cancer, which are more common in non-smoking females than in non-smoking males. Previous studies have shown gender differences in the susceptibility to naphthalene-induced lung injury in mice where the damage to non-ciliated secretory bronchiolar epithelial (Clara) cells was found to occur earlier and to be more extensive in females than in males. However, very little is known about whether there are any gender differences in the subsequent lung repair response.

The aim of this study was to investigate whether gender plays an important role in the pulmonary regenerative response to naphthalene-induced Clara cell ablation. Adult male and female mice were injected ip with either a low (50 mg/kg), medium (100 mg/kg), or high (200 mg/kg) dose of naphthalene, and lung tissue regeneration was examined by immunohistochemical staining for the cell proliferation marker Ki-67 and the mitosis marker phosphohistone-3. Histopathological analysis showed that Clara cell injury was more prominent in the lungs of female mice as compared to male mice after injection with any of the three different naphthalene doses. Cell proliferation and mitosis in both the terminal bronchiolar epithelium and peribronchiolar interstitium of female mice was found to be significantly greater than that of male mice after treatment with the low and medium doses. However, after treatment with the high dose, lung regeneration was delayed in female mice, while male mice mounted an adequate and timely regenerative response. Taken together, these findings show that there are clear gender differences in naphthalene-induced lung injury and repair.

914 PULMONARY EMBOLI AND DOSE-RELATED IMPAIRMENT OF CARDIOPULMONARY FUNCTION BY THE INTRAVENOUS INJECTION OF PRECIPITATED CALCIUM SALTS IN ANESTHETIZED RATS.


If chemical incompatibility exists, crystals may precipitate in intravenous admixture solutions. Respiratory distress and pulmonary emboli secondary to accidental infusion of precipitates of calcium salt have been reported in patients receiving parenteral nutrition. However, the relationship between the amount of precipitate and its effects has not been studied. In this study, we examined the effects of intravenous injection of calcium salt reagents on cardiopulmonary function in rats. Anesthetized rats (n = 5) were injected intravenously with calcium hydrogen phosphate suspension (CaHPO₄, 10, 30, or 60 mg/kg), calcium carbonate suspension (CaCO₃, 10, 30, or 60 mg/kg), or saline solution. Respiratory rate, heart rate, arterial pressures, and arterial blood gases were measured for 90 min after injection. Histopathological changes in the lungs were evaluated at 90 min. The injection of CaHPO₄ resulted in dose-related decreases in arterial pressures and oxygen partial pressures in arterial blood (PaO₂) at 30 and 60 mg/kg and death in three of five animals at 60 mg/kg, although the effects were insignificant at 10 mg/kg. Dose-dependently, the CaHPO₄ also caused increases in lung weight, and histopathologically, crystals obstructed the pulmonary microvasculature at all the dose levels. No significant changes were observed in other parameters. The effects of CaCO₃ were similar but weaker than those of CaHPO₄, and no animals died. It was concluded that intravenous injection of precipitated calcium salts caused pulmonary emboli and dose-related impairment of cardiopulmonary function in anesthetized rats.

915 ARSENATE AUGMENTS CHROMIUM-EVOKED PULMONARY INJURIES AND SIGNALING IN MICE.


Chromated copper arsenate (CCA) has been used worldwide as a wood preservative that can cause adverse impacts on human health. Although chromium (Cr) and arsenic (As) are known to cause respiratory diseases in humans, combined effects of these metals have not been elucidated in vivo. Herein, male C57BL/6j mice were intratracheally instilled arsenate (As(V)), Cr(VI) and/or their mixture and sacrificed 2, 7, or 14 days after instillation to determine pulmonary toxic injuries. We found that the cotreatment of Cr(VI) with As(V) caused pulmonary inflammation and subsequent fibrosis, as assessed by lung histopathology, mRNA expression (type I collagen and ccr2), and leukocyte count and cytokines/chemokines expression (IL-6, MCP-1, MIP-1 beta, RANTES, and IL-10) in bronchoalveolar lavage (BAL). Exposure to Cr(VI) rather than As(V) had a potential to induce pulmonary toxicity, although each metal resulted in no overt impact. Concurrent As(V) exposure worsened Cr(VI)-induced acute pulmonary injuries with increases in IL-6 level and LDH activity in BAL, and neutrophil sequestration and capsaicin activities in the lung. We further provide evidence that oxidative stress and MAPK signaling were involved in response to combined treatments: enhanced ROS generation and increased both reduced and oxidized glutathione contents, along with increases in mRNA levels of catalytic subunit of glutamylcysteine ligase, glutathione peroxidase 2, and heme oxygenase-1; and phosphorylation of ERK and JNK. These findings suggest that acute pulmonary responses were enhanced by co-administering Cr(VI) with As(V), followed by the development of fibrosis, and these effects might be associated with oxidative stress and MAPK signaling.

916 UNDERSTANDING THE GENETIC BASIS OF OZONE SENSITIVITY IN THE NEONATAL MOUSE LUNG USING AN INTEGRATIVE GENETIC AND GENOMIC APPROACH.

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Ozone (O₃) is a powerful oxidant and respiratory irritant that leads to airway inflammation and pulmonary dysfunction. Although a greater susceptibility to inhaled O₃ in children is not established, rodent studies have shown neonates to be more sensitive than adults. Further, mouse studies in our lab showed clear and quantitative age and interstrain differences in response to O₃ inhalation, and classic and in silico linkage analyses revealed significant quantitative trait loci (QTLs) associated with O₃-induced lung inflammation and injury in neonates (15 to 16d old). Using micro-array technology in the current study, we integrated gene expression data with our QTLs to name candidate genes that may play a role in age-related, O₃ sensitivity. Adult and neonatal mice from resistant (AjI) and sensitive (Balb/cJ) strains were exposed to 5h 0.8ppm O₃ or air. One hour later, lungs were removed and snap-frozen, RNA was extracted, and cDNA was prepared and hybridized to Affy Mouse 430 2.0 gene chips (n=9 mice/group; 3 samples pooled/chip (3 chips/group); 36 total chips). Expression fold-changes (O₃/Air) were calculated and analyzed using MeVv4.1 software. A 2-way ANOVA revealed significant age×strain interactions for 3200 of 40,000 gene probes. Post-hoc testing coupled with linear expression matrix (LEM) visualization of our QTL regions narrowed this list to <5 genes, some of which (Foxp1 and 2 (chr 6), Adm and Smp30 (chr 7)) were identified in our in silico haplotype mapping study. Validation of these candidate genes through RT-RT PCR is currently underway along with further expression analysis using DAVIDv6 to identify biological pathways and functions associated with these genes. Transgenic and KO mouse models are also being planned. Overlapping genetic and genomic studies to examine age-related O₃ sensitivity in mice has given us greater insight into the gene-specific etiology of this complex trait and may provide us with a deeper understanding of comparable pulmonary disease in children.
917 DIESEL EXHAUST PARTICLE EXPOSURE INDUCES ANGIOPENESIS.

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Background: Epidemiologic studies have shown a strong link between air pollution exposure and cancer morbidity and mortality. Although multiple mechanisms have been proposed, there are no studies mechanistically evaluating diesel exhaust, primarily generated from automobile sources, on angiogenesis that cancers are dependent upon for their growth. We hypothesized that exposure to diesel exhaust particles (DEP) induces angiogenesis both in vivo and in vitro. Methods: C57BL/6 mice that underwent unilateral hindlimb ischemia surgery were exposed either with DEP or filtered air 6 h/5 d/week in a whole body exposure chamber for 2 months. In vitro studies of capillary sprouting were done by exposing aortic rings and confluent human umbilical vein endothelial cells (HUVECs) to various concentrations of DEP (5, 15, 30 μg/ml). Gene expression of major angiogenesis regulators vascular endothelial growth factor (VEGF) and Heme oxygenase (HO)-1 was performed using quantitative RT-PCR. Results: DEP exposure induced 6-fold increase in vascular volume in the ischemic hindlimbs and 4-fold increase in the non-ischemic hindlimbs compared to the filtered air by micro-CT imaging. DEP exposure also enhanced CD31, α-smooth muscle actin, and NG2 proteoglycan expression in the ischemic hindlimb muscle demonstrated by immunohistochemical staining. Capillary sprouting assay showed marked increase in capillary tube numbers (60%), tube length (70%), and sprouts (50%) when compared to DEP vs. non-dep. with aortic rings were incubated with DEP vs. in vitro compared to the controls. DEP exposure also increased VEGF and HO-1 mRNA expression in in vitro endothelial cell culture co-cultured with DEPs vs. RT-PCR. Conclusion: DEP exposure induces angiogenesis through upregulation of VEGF and HO-1.

918 DOWNREGULATION OF POLYAMINE BIOSYNTHESIS BY DIETARY RESTRICTION ATTENUATES BLEOMYCIN-INDUCED PULMONARY FIBROSIS.

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We have observed previously that bleomycin (BLM) administration results in up-regulation of lung polyamine (PA) and hydroxyproline (HP) biosynthesis suggesting that BLM-induced pulmonary fibrosis may be associated with PA metabolism. Thus, we postulated that PA downregulation may reduce BLM pulmonary toxicity. However, studies using the metabolic inhibitor difluoromethylornithine to deplete PA were unsuccessful and enhanced BLM toxicity. In an earlier report (Elayyaraj et al, 1988), we observed that food restriction (FR) downregulate PA metabolism and cell replication and reduce oxygen toxicity in the lung. In this study we examined whether FR would attenuate pulmonary BLM toxicity. Sprague-Dawley rats were fed (FR) or food restricted (RF) to 20% of the average FR animals’ dietary intake for 7 (FR-7) or 21 days (FR-21) followed by intratracheal instillation of BLM (1.5 unit/rat) or saline. After treatment, all rats were FF for an additional 14 days recovery then euthanized. We found that with BLM treatment, body weight decreased 40% and 20%, p<0.05 in FF and FR-7, respectively, but remained unaltered in FR-21 rats. PA biosynthesis evaluated by downregulation of PA biosynthesis.

919 ACONITINE CHALLENGE TEST REVEALS A SINGLE EXPOSURE TO AIR POLLUTION CAUSES INCREASED CARDIAC ARRHYTHMIA RISK IN HYPERTENSIVE RATS.


Epidemiological studies demonstrate a significant association between arrhythmias and air pollution exposure. Sensitivity to aconitine-induced arrhythmia has been used repeatedly to examine the factors that increase the risk of such cardiac electrical dysfunction. In this study, we are the first to use a technique known to elicit arrhythmia to examine whether inhalation exposure to particular air pollutants or gaseous irritants increases the risk of arrhythmia being triggered in a rat model of hypertension. Spontaneously hypertensive rats implanted with radio telemetry were exposed for 3 hours to 3mg/m3 (high), 1mg/m3 (med) or 0.45mg/m3 (low) metal-rich synthetic residual oil fly ash (s-ROFA), 3ppm acrolein, or filtered air. Arrhythmogenesis was assessed 24 hours later in urethane-anesthetized animals by continuous intravenous infusion of aconitine while heart rate, ECG and ventilatory parameters were monitored. Sensitivity to aconitine was measured as the threshold dose of aconitine required to produce ventricular premature beats (VPB), ventricular tachycardia (VT), and ventricular fibrillation (VF). Rats exposed to filtered air developed VPBs, VT, and VF successively during aconitine infusion. Rate exposed to low, med and high s-ROFA, and acrolein developed VPBs, VT, and VF at significantly lower doses of aconitine than air-exposed animals. The cumulative dose of aconitine required to elicit these responses was lowest in animals exposed to acrolein followed by high s-ROFA. Paradoxically, arrhythmia was elicited at lower doses of aconitine in low s-ROFA-exposed animals than med s-ROFA. These findings suggest that a single exposure to a toxic air pollutant, whether particulate or gaseous, can increase the sensitivity of the cardiac electrical conduction system and trigger arrhythmia; which poses a particular health risk to individuals with cardiovascular disease. (This abstract does not reflect EPA policy.)

920 DETERMINATION OF THE SENSORY IRRITANT POTENTIAL OF UCAR™ N-PROPYL AND UCAR™ N-BUTYL PROPIONATE IN SWISS WEBSTER MICE.


UCAR™ n-propyl and UCAR™ n-butylic propionate are solvents commonly used in automotive refinishes, OEM coatings, appliance coatings, cleaning fluids, and printing inks, as well as polymerization solvents for high solids acrylic resins. Since these products are not expected to be used by consumers and right engineering controls during manufacturing limit exposure, the human irritation response has not been well characterized. To better understand potential levels for irritation an RD0 assay, species mechanical causing a 50% reduction in baseline respiratory rate (ASTM Method E 981-04), was conducted to determine the sensory irritant potential in mice. The RD0 value can be used to evaluate occupational exposure limits (OEL) and empirical evidence indicates that the threshold limit value (TLV) for many irritant chemicals falls within 0.01 – 0.1 RD0. Preliminary studies indicated that the RD0 in Swiss Webster mice was 535 ppm for n-propyl propionate. Lethality was observed at the highest exposure levels; however mice exhibited no clinical signs, including no signs of sedation or other central nervous systems (CNS) effects, at any exposure concentration. Sensory irritants stimulate nasal trigeminal C-fibers and decrease breathing rate. At high doses capsaicin induces receptor-mediated degeneration of sensory neurons, thus blocking irritant-induced respiratory depression. Groups of mice, with and without intravenous capsaicin pretreatment, were exposed to 0, 535 or 1000 ppm n-propyl propionate according to ASTM Method E 981-04. Exposures to chlorine and formaldehyde were included as controls to evaluate previously determined values. The response to chlorine and formaldehyde indicated that the capsaicin pretreatment was effective in attenuating the chlorine- and formaldehyde-induced respiratory depression in Swiss Webster mice. Capsaicin pretreatment also attenuated the n-propyl propionate response indicating that the irritant properties of this chemical act through the trigeminal sensory irritant pathway.

921 PREVENTION OF BLEOMYCIN-INDUCED LUNG FIBROSIS AND DYSFUNCTION BY PROSTAGLANDIN E2.

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Experimental and clinical data support the involvement of cyclooxygenase-derived eicosanoids in the pathogenesis of lung fibrosis. The present study demonstrated the effects of increasing the circulating concentration of the potentially anti-fibrotic prostaglandin E2 (PGE2) in an experimental model of lung fibrosis and dysfunction. Male C57BL/6 mice were implanted subcutaneously with osmotic minipumps designed to deliver PGE2 (1.44 μg/hour) or vehicle (1.5% ethanol in saline) continuously for 28 days. Seven days after pump implantation, mice were given a single dose of bleomycin (1 mg/kg body weight) or saline by oropharyngeal aspiration. Lung mechanics and indicators of lung inflammation and fibrosis were assessed 21 days following dosing with bleomycin or saline. Pilot studies revealed that serum PGE2 was increased two- to three-fold for up to 28 days following implantation of minipumps containing PGE2, confirming the utility of this method of prolonged administration. Compared to mice dosed with saline, mice dosed with PGE2 showed a significant decrease in lung collagen and blunted lung collagen accumulation and forced expiratory volume in one second (FEV1) compared to saline dosed mice. PGE2 treatment also improved survival and decreased lung eosinophil and neutrophil populations in saline dosed mice.
bleomycin had increased bronchoalveolar lavage fluid inflammatory cell content, increased histopathological lung fibrosis scores and a decline in static compliance indicative of lung stiffening (all p<0.05). Systemic PGE, administration did not appreciably alter airway inflammatory cell content but did result in lower histopathological scores and prevented the decline in static compliance in mice treated with bleomycin (all p<0.05). These data reveal a protective effect of exogenous PGE, on bleomycin-induced lung fibrosis and dysfunction and suggest that targeted modulation of eicosanoid levels may prove beneficial in the treatment of lung fibrosis and associated functional decline.

**924 COMPARATIVE EFFECTS OF DIESEL EXHAUST AND AMBIENT PARTICLES ON CARDIOVASCULAR SYSTEM.**

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Air pollution has been associated with cardiovascular diseases (CVD) by many epidemiology studies. However, the components of air pollution that are responsible for the CVD are unknown and the mechanisms underlying the associations need to be elucidated. The objectives of this study were to investigate 1) whether subchronic inhalation of ambient particulate matter (PM), whole diesel exhaust (WDE), or diesel exhaust gases (DEG), leads to exacerbation of atherosclerosis, alteration of endothelial function, and pulmonary/systemic inflammation; 2) to what extent do WDE, and DEG, contribute to air pollution enhanced CVD; 3) whether there is interaction between DEG and ambient PM in inducing/enhancing cardiovascular effects. ApoE-/- mice were exposed via inhalation for 5 h/d, 4 d/wk, for up to 5 mo and pretreated with different doses of DEG (0.5-5 g/L filtered air, FA), 2) concentrated ambient fine particles PM2.5 (CAPs, av. conc. 105 μg/m3), 3) WDE (436 μg/m3), 4) DEG (same as gases in WDE at 436 μg/m3), and 5) CAPs+DEG (PM: 113 μg/m3; Gae: same as WDE at 436 μg/m3). After 3 mo and 5 mo of exposure. The results of this study show: 1) Subchronical inhalation of CAPs, WDE and DEG all increased the serum VCAM-1 expression and enhanced the PE-induced vascostriction to a similar level; 2) For plaque exacerbation, the effects of CAPs > WDE > DEG > FA, indicating that PM (rather than gases), especially some components of the ambient PM (not present in WDE), were more responsible for the plaque development; 3) There were no interactive effects between CAPs and DEG on plaque exacerbation, vasomotor dysfunction, and pulmonary/systemic inflammation.

**925 COMPARISON OF INHALATION TOXICITY OF GENTAMICIN SOLUTION IN RATS AND DOGS.**

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Nebulized gentamicin solution was administered to rats (nose-only) and dogs (face mask) for 14 days followed by a 14 day recovery period. Mean inhaled doses were 0, 39, 123, and 245 mg/kg for rats over 30, 90, and 180 minutes, respectively, at 2 mg/L, aerosol concentrations (MMAD 1.6-2.9 μm). Because dogs would not tolerate exposure as long as rats, inhaled doses were limited to 0, 7, 14, and 41 mg/kg over 15, 30, and 60 minutes, respectively, at 1.2-1.7 mg/L aerosol concentrations (MMAD 1.9-2.5 μm). Therefore, comparable inhaled doses were achieved only at the low dose for rats and the high dose for dogs. Serum AUCs (4 ± 2 mg/mL*h (mean ± SD)) at 39 mg/kg in rats and 11 ± 7 mg/mL*h at 41 mg/kg in dogs showed comparable exposure between the two species implying similar absorbed doses and hence indicating similar deposited lung doses. Rat exposures resulted in dose-related lung pathology manifest as an irritant reaction in the upper respiratory tract as well as alveolar histiocytosis, inflammation, airway epithelial epithelial metaplasia, and associated lymphomage in the lung tissue. This pathology was associated with high lung tissue gentamicin levels in rats measured 24 hours post dose on Day 14 (575 ± 35 μg/mg at 39 mg/kg). Dose related kidney tubular necrosis, a well-known toxicology was also observed. A No Observed Effect Level (NOEL) was not established in the rat. The NOAEL for gentamicin in dogs was 14 mg/kg due to kidney tubular regeneration observed at 41 mg/kg. There were no test-article related effects on lung histopathology in dogs even at the highest inhaled dose of 41 mg/kg, in contrast to test-article related lung effects in rats at a similar inhaled dose of 39 mg/kg. The absence of any significant lung effects in the dog was associated with much lower lung tissue gentamicin levels 24 hours post dose on Day 14 (42 ± 11 μg/mg at 41 mg/kg) compared to those measured in the rat. These results suggest that increased lung retention of gentamicin in rats vs dogs plays a role in the differential pathological findings.

**926 AN EVALUATION OF THE PERMISSIBLE EXPOSURE LIMIT OF INHALED SYNTROLEUM S-8 SYNTHETIC JET FUEL IN MICE.**

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No current studies have examined pulmonary health effects associated with Syntroleum S-8 synthetic jet fuel. In order to gain an understanding about the cytotoxic effects, varied S-8 concentrations were used to determine the threshold concentration in which lung injury is observed. Methods and procedures were duplicated from a previous JP-8 jet fuel study to evaluate S-8 in contrast to JP-8,

**923 SENSITIZATION OF THE SENSORY IRRITATION RESPONSE BY ADENOSINE.**

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Our previous studies have suggested adenosine signaling pathways may be involved in initiation of the sensory irritation response. This response, characterized by decreased breathing frequency due to bracing in the early phase of expiration, results from increased neuronal activity in the central nervous system. The current study was performed to determine if nasal trigeminal responsiveness to irritants was increased through adenosine receptor-dependent pathways. Toward this end, sensory irritant responsiveness was measured in female C57Bl/6J mice immediately following subcutaneous injection of the adenosine precursor, adenosine 5’-monophosphate (AMP), or vehicle. To quantify the sensory irritation response, the time of respiratory bracing was measured during irritant exposure by non-invasive plethysmography. Sterylene (50 ppm) and capsaicin (0.2 mg/m3) were used as prototype irritants. As assessed by plethysmography, both sterylene and capsaicin induced a mild sensory nerve response (time of bracing approximately 100 msec). For both irritants, the response was increased 2–4 fold in animals pretreated with AMP indicating sensitization of trigeminal nerves occurred. The AMP-sensitization was blocked by pretreatment with the broad acting adenosine receptor antagonist theophylline (20 mg/kg). The AMP-sensitization was also blocked by pretreatment with the adenosine A2 receptor antagonist 5,7-dimethyl-1-propargylxanthine (1 mg/kg). These results suggest adenosine A2 receptor stimulation leads to trigeminal nerve sensitization and enhanced sensory irritant responsiveness in the mouse. Airway mucosal adenosine levels are elevated in allergic airway disease. Adenosine A2 receptor signaling pathways may, therefore, represent a potential mechanism for the enhanced irritant responsiveness associated with allergic airway disease.
C57Bl/6 male mice were nose-only exposed to 5–8 synthetic jet fuel for 1 hr/day for 7 days at average concentrations of 93, 352, and 616 mg/m3. Pulmonary functional and respiratory permeability tests were performed 24 hours after the final exposure. No significant effects were observed at 93 mg/m3 5–8, but significant decreases were detected in expiratory lung resistance and a significant increase was observed with total lung compliance of the 357 mg/m3 group. Morphological examination and morphometric analysis of distal lung tissue revealed cellular damage in alveolar type II epithelial cells and terminal bronchial epithelial cells, with significant increases in volume density of lamellar bodies/vacuoles at 357 and 616 mg/m3. These results indicate increased surfactant production, which implicates damage. Evidence of lung injury was observed at relatively low concentrations, suggesting if synthetic jet fuel is utilized, the current permissible exposure limit (PEL) of 550 mg/m3 for 8-h jet fuel should be substantially evaluated (Supported by AFOSR).

927  PERMEABILITY CHANGES - A SENSITIVE MARKER OF LUNG INJURY MEASURED WITH INHALED HORSERADISH Peroxidase (HRP) IN THE PERFUSED AND VENTILATED RAT LUNG (IPL).

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Macromolecule absorption from the lung is a largely unknown process. The intact alveolar epithelium with tight junctions (TJ) between pulmonary epithelial cells, is the major permeability barrier to passage of macromolecules through the alveolar-capillary membrane. In this study, the pulmonary absorption of the 40 kDa protein HRP was measured in the rat IPL. The DustGun aerosol technology was used for a well-controlled delivery of HRP as respirable dry powder aerosols (mass median aerodynamic diameter 0.7 μm.) to the rat IPL. The lung perfusate was repeatedly sampled until 125 min after the HRP exposure. The amounts of the active HRP that was cleared with the perfusate or retained in the lung tissue was measured with an enzymatic assay. Following inhalation, the penetration of HRP was rapid and the time to peak concentration (Tmax) was 218±39 s (mean±SD, n=13). After this time, the rate of penetration of HRP remained almost constant. After 2 h, 2±0.3 μg HRP (mean±SD, n=5) had cleared to the perfusate. This constitutes only 0.32% of the initially deposited dose of 67±59 μg HRP per exposure. The effect of a transient opening of TJ on HRP absorption was studied by exposing the IPL to a nebulized aerosol of 0.2% poly-L-arginine (PLA) (mean MW 42.5 kDa) in PBS, at between 40 and 45 min after the previously used HRP exposure. The concentration of HRP in the perfusate jumped eightfold from 2±1 ng/mL before to 16±5 ng/mL immediately after the PLA treatment. In the PLA-treated lungs, the total amount of HRP cleared to the perfusate was 16±8 μg or 2.2±1% (mean±SD, n=3) of the total initial dose of 74±14 μg HRP per exposure. Thus, the pulmonary absorption of HRP is very slow and inhalation of respirable HRP aerosols can be used as a sensitive marker of permeability changes induced by toxic insults to the alveolar epithelium. This inhalation technique can also be of great use in studying the pulmonary absorption of biopharmaceuticals in general.

928  SYSTEMIC DELIVERY OF COTININE THROUGH INHALATION EXPOSURE.

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Cotinine, a primary metabolite of nicotine, is a potential pharmacological agent for the treatment of various psychiatric syndroms such as Alzheimer’s disease. The objective of this study is to develop a method for systemic delivery of cotinine using the lung as a portal of entry. Specifically, two cynomolgus monkeys (one male and one female) were exposed to a test atmosphere containing respirable nicotine aerosol (MMAD=3 μm, GSD=1.6) at the concentration of 75 mg nicotine/m3 for two hours. Respiratory function (i.e., tidal volume, breathing rate and minute volume) was measured during the exposure using Fleisch #1 pneumotachograph attached to the mask. Upon exposure termination, blood samples were collected via the femoral artery at twelve discrete time points and analyzed for quantification of nicotine, cotinine, and trans-3-hydroxycotinine concentrations in plasma using high performance liquid chromatography-mass spectrometry-mass spectrometry (LC-MS-MS). Pharmacokinetic parameters (i.e., AU, Cmax, Tmax, elimination half-life, apparent clearance, and apparent volume of distribution) determined using WinNonlin software are reported. The results showed that following inhalation exposure, plasma cotinine level reached a maximum at 1-2 hours post-exposure whereas nicotine level reached a maximum within 0.25 hour. Plasma levels of cotinine decreased at much slower rate compared to nicotine. Compared to nicotine, pharmacokinetic parameters Cmax and AUCl for cotinine were significantly greater and elimination half-life was significantly longer. This suggests that administration of nicotine aerosol via inhalation may be a viable method for maintaining cotinine concentrations at levels suitable for exploring therapeutic opportunities.

929  CIGARETTE SMOKE-INDUCED MOUSE EMPHYSEMA MODEL USING A DIRECT PUMP EXPOSURE SYSTEM.

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Various animal models are evaluated for the chronic obstructive pulmonary disease investigation and among others, mouse emphysema models following chronic cigarette smoke (CS) exposures are most common. In the CS models, different exposure regimens are reported using diluted/continuous exposures or undiluted/intermittent exposures. The latter systems are widely used in research community but operational details are limited in literature. In this study, one of representative direct-pump exposure systems was tested to expose CS mice for 24 hrs and its smoke characteristics and biological endpoints associated with emphysema development were evaluated. Female AKR mice were exposed to mainstream smoke from 24RF reference cigarettes (filter removed), 2.5 mg/L air, 6 d/wk for 24 wks. Smoke was analyzed for WTPM, CO, and nicotine. Blood samples were collected postexposure and analyzed for nicotine and cotinine. There were notable inter- and intra-machine variability in smoke delivery. At Wks 4 and 24, groups of mice were subjected to lung function measurement or sacrificed for lung collection (for histopathology/morphometry; bronchoalveolar lavage (BAL); transcriptomics). Mild emphysema was confirmed morphometrically (increases in the mean Lm and chord length at Wk 24). A minimal increase in quasi-static compliance was observed at Wk 4. Pulmonary inflammation was detected as increased BAL protein, macrophages at Wk 4 and neutrophils at Wk 24, while lung GSH levels were slightly but consistently reduced at Wks 4 and 24. The background lymphoma was observed for both control and exposed groups at Wk 24. There were more differentially expressed genes at Wk 4 compared to Wk 24. In summary, chronic smoke exposure using the undiluted/intermittent direct-pump smoking system induced mild emphysema with chronic inflammation in AKR mice. (Funded by Battelle Biology and Health Science Initiative)

930  HISTOCHEMICAL STUDY OF INTESTINAL MUCINS AFTER ADMINISTRATION OF SILVER NANO PARTICLES IN SPRAGUE-DAWLEY RATS.

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To investigate the effects of silver nanoparticles (NPs) on the histological structure and properties of mucosubstances of the intestinal mucosa, Sprague-Dawley rats were administered to silver NPs (60 nm) at concentration of 1000 mg/kg for 28 days, following OECD test guideline 407 with GLP application. Control sections contained no silver NPs. Experimental samples showed luminal and surface particles and also contained silver NPs within the tissue. Accumulation of silver NPs was observed in increased numbers in the lamina propria both small and large intestine. It did show in the tip of upper villi in the ileum and in the protruding surface of fold in the colon. In the silver NPs-treated rats, numbers of goblet cells which have released their mucus granules were higher than those in controls and there were also large mucus materials in the crypt lumen and ileal lumen. Moreover, shedding of large mucus materials in the crypt lumen and ileal lumen. Furthermore, shedding of cell at the tip of villi was frequent. The amount of neutral and acidic mucus in goblet cells in the silver NPs was decreased. In the proctrics of acidic mucins, amount of sialomucins was increased while that of sulfomucins was decreased. Particularly, in colon, sialylated mucins were detected in the lamina propria, connective tissue under the epithelia, which was not found in the control rats. Therefore, the present results suggest that silver NPs induce the discharge of mucus granule and abnormal mucus composition of goblet cells in the intestines.
into systemic circulation; however this has not been shown. We hypothesized that following a single intratracheal instillation (IT), zinc translocates through the pulmonary circulation, directly reaching the heart. To test this, we used high resolution magnetic sector field inductively coupled plasma mass spectrometry to measure levels of endogenous zinc in whole blood, liver, spleen, and kidney of male Wistar Kyoto rats (13 wks old, 250-300 g), 1, 2, 4, and 48 h after a single IT or oral gavage of saline or 0.7 nmol/rat 70Zn (as ZnSO4), using a solution enriched with 76.6% 70Zn. Natural abundance of 70Zn is 0.6%, making it an easily detectable tracer following exposure. In IT rats, lung 70Zn was highest 1 h post IT and declined by 48 h. Liver endogenous zinc was increased 24 and 48 h post IT. 70Zn was detected in all extra-pulmonary organs, with levels higher following IT than following gavage. In the hearts of IT rats, 70Zn not only was increased, but seemed to accumulate, with cardiac 70Zn highest 48 h post IT. Conversely, following gavage of Zn, Zn cardiac only modestly increased, and did not accumulate. Liver, spleen and kidney 70Zn peaked 4 h following gavage, and 24 h following IT. 70Zn IT exposure elicited changes in copper homeostasis in all tissues. Pulmonary exposure to zinc results in selectively higher cardiac zinc levels relative to oral exposure. Following IT, soluble zinc directly moves from the lungs into circulation and accumulates in the heart, causing changes in copper homeostasis. This provides biological plausibility to the suggestion that soluble zinc possibly mediates cardiovascular effects from exposure to zinc-rich PM. Abstract does not reflect USEPA policy. Funded in part by UNC/EPA CT829471.

PS 932 TOXICITY OF PARTICULATE MATTER GENERATED BY PYROTECHNIC DISPLAYS.

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Particle samplers were used to collect emissions from 14 different pyrotechnic displays in a stainless steel exposure chamber. To address our hypothesis that pyrotechnic displays emit toxic particulates which can affect human health, collected particles were used to treat a human pulmonary microvascular endothelial cell line (HPMEC-ST1.6R) and a human bronchial epithelial cell line (BEAS-2B), which are stably transfected with a NF-kB luciferase reporter gene. Cells were dosed for 24 hr with 10, 50 and 100 μg/ml of PM10 for each pyrotechnical display particle type, as well as size-fractioned particles for selected displays. Cytotoxicity was measured as release of LDH, and no significant toxicity was measured for any dose in either cell line. Reactive oxygen species (ROS) levels were measured by intracellular oxidation of a fluorescent dye. In both cell types, PM10 from less than half of the particle types elicited ROS production in a dose dependent manner. These results indicate that the composition of particles resulting from each display varied and that dose can impact in vitro cellular responses. One display, ”Black Cuckoo” yielded a high response in both cell types and upon further investigation; it was shown to cause a dose-dependent response in both. Examination of the size-fractioned particles show that coarse particulate matter (PM10-2.5) caused the greatest production of ROS in HPMEC-ST1.6R cells and equal or greater production than the fine fraction (PM2.5) in BEAS-2B cells, while ultrafine PM caused the least response in both cell types. Each display particle type also impacted NF-kB levels in BEAS-2B cells, which was measured by luciferase expression. Interestingly, changes in NF-kB levels did not correlate with ROS production, suggesting that an alternative mechanism may be involved. Ongoing studies are aimed at verifying our findings in a murine model, and correlating our findings to specific components of the particulate matter produced by pyrotechnic displays.

PS 933 SUPPRESSION OF WNT/β-CATENIN SIGNALING IN BONE OF FEMALE RATS EXPOSED TO ETHANOL POST-LACTATION.

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The mechanism through which chronic alcohol intake induces bone loss remains unclear but oxidative stress might be the key event in skeletal injury. We chronically infused ethanol (EtOH, 12g/kg/day) into post-lactational female Sprague-Dawley rats for 4 weeks using total enteral nutrition. EtOH decreased bone mineral density compared to control animals during this period of bone rebuilding (P < 0.05). EtOH-induced bone loss was blocked by administration of the antioxidant N-acetylcysteine (NAC) at 1.2 g/kg/d (P < 0.05). NAC was able to reverse down-regulation of the bone formation markers alkaline phosphatase and osteocalcin in serum and gene expression in bone tissue (P < 0.05). RNA isolated from bone tissue and specific wnt real time array analysis revealed that the majority of wnt signaling components were down-regulated by chronic EtOH infusion (P < 0.05). Real-time PCR confirmed down-regulated gene expression in a subset of the wnt signaling components by EtOH, however the wnt antagonist DKK1 was up-regulated by EtOH (P < 0.05). Western blot showed that the key canonical wnt signaling molecule β-catenin protein expression as well as glycogen synthase kinase-3-β were inhibited by EtOH in bone, and these actions of EtOH were blocked by NAC (P < 0.05). Finally, we transfected TCF/LEF-dependent transcription of a luciferase reporter gene TOPFLASH and β-catenin plasmid into osteoblastic UMR-106 cells. We found that EtOH trans-inactivated TCF/LEF gene transcription and blocked β-catenin nuclear translocation in osteoblasts. All of these effects of EtOH in vitro in osteoblasts were also eliminated by NAC pre-treatment. These observations strongly suggest that EtOH can inhibit bone formation through its novel action on suppression of wnt signaling. Supported in Part in R01 AA12928 (M.J.R.).

PS 934 THIOREDOXIN REDUCTASE DEPLETION INHIBITS NRF2 SIGNALING BY A THIOREDOXIN INDEPENDENT MECHANISM.

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Nuclear factor-kappaB (NfkB) is a redox sensitive transcription factor involved in cell survival, proliferation, and inflammation. Certain inhibitors of NRF2 signaling are also known to inhibit thioredoxin reductase (TR1), an enzyme important for processes such as oxidative insult defense and DNA replication. In the present study we tested the hypothesis that TR1 inhibition prevents cellular NfkB signaling through a build-up of oxidized thioredoxin (Trx1), a major substrate of TR1 that has been implicated in the redox maintenance of NfkB. To test this hypothesis, HeLa cells were depleted of TR1 using siRNA. Following a 90% knock-down of TR1 protein and activity and stimulation with TNFα, there was a 40% decrease, compared to controls, in both NfkB activity as measured using reporter constructs and expression of the NfkB target gene i-kappaB-alpha. In contrast, an 80% knock-down of Trx1 did not result in significant inhibition of NfkB activity. Furthermore, TR1 knock-down did not result in oxidation of either cytosolic or nuclear Trx1, suggesting that TR1 is necessary for NfkB signaling and has a role that is independent of Trx1.

PS 935 TRANSCRIPTOMIC REGULATORY RELATIONSHIPS INVOLVING GLUTATHIONE S-TRANSFERASE (GST) AND OXIDATIVE STRESS RELATED GENE PRODUCTS: TARGETS INFLUENCING MELANOTOXICITY OF SUBSTITUTED PHENOLS.

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Substituted phenolic substrates of tyrosinase (the enzyme responsible for melanin synthesis) are selectively toxic to melanocytes in the skin. It is suggested that tyrosinase catalyzed formation of phenol-o-quinone derivatives generates oxidative stress and melanocyte toxicity. We have demonstrated previously that the toxicity of 4-tert-butyphenol (4-TBP, a prototypical substituted phenolic substrate of tyrosinase) in melanoma cells is directly associated with GST activity. In this study, computational techniques were used to determine transcriptional regulatory relationships between GST expression and oxidative stress in melanoma cells. Using the algorithm for the Reconstruction of Accurate Cellular Networks and probes representing genes involved in cellular response to oxidative stress, a transcriptional regulatory network was reverse-engineered from 207 publicly available melanoma microarrays. Combination of toxicity and RT-PCR studies demonstrate that TR-3p (epoxy-3-(p-nitrophenoxy) propane (ENPP) mediated inhibition of GSTT1 sensitizes melanoma cells to 4-TBP induced toxicity compared to other GST isoforms. The expression of GSTT1 was found to be tied to the expressions of 31 other genes including those of GSTP1, NQO1 and Gpx3 which are involved in detoxification of quinones. There are direct statistical dependencies between the expressions of the GSTM isoforms and that of GSTP1 but not GSTT1. However, the expression of GSTA1 is directly tied to expressions of both GSTP1 and GSTT1. Feed-forward loops involving hubs in the network GSTT1, GSTP1 and Gpx3 (whose average expression is higher in primary melanomas than metastatic melanomas), or Gsr (whose average expression is higher in metastatic melanomas) were established. This analysis identifies molecular targets validation of which will provide insights on their ability to influence toxicity of 4-TBP selectively in melanocytic cells.

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936 OXIDATIVE LIPIDOMICS OF MACROPHAGE ACTIVATION AND APOPTOSIS INDUCED BY PHAGOCYTOSIS OF PARTICLES AND PATHOGENS.

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Macrophages are essential to the innate immune system in eliminating invading microorganisms and as major orchestrators of the inflammatory response. As pathogens undergo phagocytosis and intracellular digestion; this mechanism can lead to apoptosis of the phagocyte. However, the mechanisms of apoptosis and its links with phagocytosis are largely unknown. We hypothesized that triggering of phagocytosis and activation of macrophages – including NAPDH oxidase generated derived reactive oxygen species - mediates selective oxidation of phospholipids participating in the execution of apoptotic program. To approach this, zymosan, silicica particles and carbon nanotubes were utilized as different types of pathogen/particles capable of activating macrophage in vitro and/or in vivo. Macrophage cell lines (RAW 264.7 and IC-21) as well as C57Bl/6 mice were treated with one of the above and NAPDH oxidase activation, superoxide generation, cytochrome c release, PS externalization and caspase 3/7 activity were used to confirm the apoptotic cell death. Oxidation of major classes of phospholipids - phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), and cardiolipin (CL) was determined by electro-spray ionization mass spectrometry as well as by fluorescence HPLC/Amplex Red assay. The results demonstrated that robust and selective peroxidation of anionic phospholipids (CL>>PS>PI) accompanies macrophage apoptosis in vitro. Moreover, oxidation of the same species of anionic phospholipids was detected in lungs of mice exposed to single walled carbon nanotubes via pharyngeal inhalation. We conclude that anionic phospholipid-mediated signaling may participate in phagocytosis induced macrophage apoptosis. Supported by NIOSH OH088282, NORA 927000Y, NIH HL70755, ES010859 and the 7th Framework Program of the European Commission.

937 SIRT1 IS POST-TRANSLATIONALLY MODIFIED BY ALDEHYDES AND CIGARETTE SMOKE IN LUNG EPITHELIAL CELLS.

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Rationale: Sirtuin 1 (SIRT1), a class III histone/protein deacetylase, is involved in stress responses and regulation of inflammation, apoptosis and aging. We previously shown that SIRT1 protein levels are decreased in lungs of smokers and patients with COPD leading to increased NF-kB-dependent inflammation. As oxidative modification of proteins can alter both a protein’s function and level, we hypothesized that oxidative modification of SIRT1 by cigarette smoke (CS) and its metabolites leads to down regulation of SIRT1 activity and protein level. Methods: Human bronchial (BEAS-2B) and primary airway epithelial cells were exposed to different concentrations of cigarette smoke extract (CSE 0.5-1%), H2O2 (150 μM), and acrolein (10 and 30 μM) for 6 hours. Lysates were used to measure SIRT1 activity (ColorQ Ly assay, Biomed) and oxidative modifications using biotin switch assay. Results: SIRT1 activity was significantly decreased by CSE, acrolein, and H2O2. However, only CSE and acrolein dose-dependently decrease SIRT1 activity. Pre-treatment with N-acetyl-L-cysteine (2 mM) attenuated CSE depletion of SIRT1 levels and activity. Labeling cell lysates with maleimide-biotin revealed that SIRT1 was modified on cysteine residues in response to CSE, and that NAC could prevent these modifications. Treatment of cells with N-ethylmaleimide (100 μM) decreased SIRT1 activity as well as protein levels further implicating modification of cysteines in SIRT1 regulation. Furthermore, SIRT1 is degraded by proteasome as proteasomal inhibitors (5 μM ALLN and 10 μM MG-132) attenuated CSE-mediated reduction of SIRT1. Conclusion: Post-translational modification of SIRT1 by carbonylation on cysteine residues inactivates the enzyme and renders it for proteasomal degradation. These data may have implications for stress responses and abnormal inflammation in response to environmental stimuli and oxidants.

938 IMPACT OF PEROXIREDOXIN 6 GENE DELETION AND OVER-EXPRESSION ON ETHANOL-MEDIATED LIVER DAMAGE IN MICE.

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Oxidative stress is implicated in the etiology of many diseases including alcoholic liver disease (ALD). Peroxiredoxin 6 is a cytosolic peroxidase that has been demonstrated to protect various tissues, such as skin, lung and cardiac muscle, against acute oxidative insults. Taken together, peroxiredoxin 6 was hypothesized to also protect the liver from oxidative stress generated during the process of chronic ethanol metabolism. To test this, both peroxiredoxin 6 knockout mice (KO) and transgenic, peroxiredoxin 6 over-expressing mice (TG) were fed an ethanol containing diet and various biomarkers of ALD were assessed along with the effects of chronic ethanol consumption on the antioxidant defenses. After 9 weeks of ethanol consumption all backgrounds exhibited increased plasma ALT activity, steatosis, CYP2E1 induction and lipid peroxidation. Differences in antioxidant protein expression and activity were also observed. Significantly induced catalase and glutathione S-transferase activity in ethanol-fed KO and TG mice along with elevated levels of glutathione peroxidase activity were noted. These results could be attributed to either compensatory responses due to the genetic manipulations or ethanol-mediated responses. It can then be concluded that both ethanol-fed KO and ethanol-fed TG mice developed early stage ALD and that oxidative stress and overall pathology were not exacerbated by the absence of peroxiredoxin 6 and not prevented or attenuated by its over-expression.

939 OXIDATIVE STRESS IS THE MECHANISM OF PPAR-INDUCED SKELETAL MYOPATHY IN RATS.

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The PPAR-alpha receptor is activated by fibrates which causes proliferation of peroxisomes, hepatomegaly and hepatocarcinogenesis in rodents. Skeletal muscle is also a major site of PPAR-alpha and delta expression and represents a principal tissue responsible for lipid uptake and utilization. Skeletal myopathy, and rhabdomyolysis have been reported with administration of fibrates. The aim of this study was to determine the mechanism by which PPAR (alpha/delta) agonists induce skeletal myopathy in rats. Sprague Dawley rats were treated with GW617042X, a dual pharmacologic agonist of PPAR (alpha/delta) nuclear receptors, over a time course study with animals sacrificed at 6 hrs, 24 hrs, 2, 4, 6, and 10 days. The soleus muscle, extensor digitorum longus muscle, heart, liver, whole blood and serum were collected at each time point. In addition to standard clinical chemistry and histopathology, samples were analyzed using transcriptomic (Affymetrix) and proteomic (Fourier transform mass spectroscopy) techniques. Minimal skeletal myopathy was first noted in soleus (type 1) muscle of some animals on Day 4, with the lesions becoming progressively more severe and homogeneous over the remainder of the time course. Development of skeletal myopathy was preceded by hepatic peroxisomal proliferation, increased plasmalagens (antioxidant lipids) and lipid peroxides in the serum, and decreased GSH in liver, whole blood and skeletal muscle. The onset of myopathy was also preceded by increased fatty acid beta-oxidation in the soleus, and was coincident with a strong induction of genes associated with oxidative stress. Based on these data, we propose that PPAR-induced skeletal myopathy in rats is the result of a combination of two pharmacological effects; hepatic peroxisome proliferation which results in systemic GSH deficiency, and increased beta-oxidation in skeletal muscle. Together, these effects result in oxidative stress which leads to the observed skeletal myopathy.

940 INDICATORS OF OXIDATIVE STRESS AND APOPTOSIS FOLLOWING EXPOSURE TO STYRENE AND ITS METABOLITES IN MOUSE WHOLE LUNG AND CLARA CELLS.

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Styrene causes both acute and chronic toxicity in humans. In mice, styrene is both hepatotoxic and neurotoxic and causes lung tumors. One possible explanation for the tumorigenicity is oxidative stress/damage. Decreases in glutathione levels, linked to increases in apoptosis, occur in lung homogenates and isolated Clara cells 3 hrs following styrene or styrene oxides (SO) administration. Since low levels of reactive oxygen species (ROS) have been linked with increased apoptosis, ROS levels following administration of styrene and its metabolites were measured in vitro and in vivo. Both in vitro and in vivo measurements showed significant increases in ROS levels after styrene. R-SO, S-SO, and racemic SO were administered intraperitoneally. None were seen with styrene or R-SO at 12 hrs or beyond. Ratios of bax/bcl-2 mRNA expression were determined at time periods from 3 to 240 hrs following exposure to styrene and R-SO. The mRNA expression ratio increased at 12 and 24 hrs following R-SO and at 120 hrs following styrene administration. Since a consequence of oxidative stress can be 8-hydroxydeoxyguanosine (8OHdG) adduction formation, levels were measured in mouse lung homogenates 3 hrs and 12 hrs after the administration of 600 mg/kg styrene or 300 mg/kg R-SO. Significant increases in 8OHdG with both compounds were seen at 3 hrs, but not at 12 hrs. Thus both ROS and...
8OHdG formation increased 3 hrs following treatment with styrene and R-So, with increases in base/oval ratios occurring later. This increased oxidative stress may be responsible for the increased susceptibility to lung tumorigenesis in mice. Additional studies using TUNEL staining for DNA fragmentation and analysis of caspase activity are needed to complement these studies. (Supported in part by a grant from the Stryne Information and Research Center (GPC) and NIH grant (RO1 CA100908; JEk)).

**941 CELLULAR ADAPTIVE RESPONSE TO ENVIRONMENTAL OXIDATIVE STRESS PERTURBS PANCREATIC BETA-CELL FUNCTION.**

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Accumulating evidence indicates that many environmental factors are involved in the incidence of type 2 diabetes (T2D). It is clear that insulin resistance plays an early role in the pathogenesis of T2D, and defects in insulin secretion by pancreatic beta-cells are instrumental in the progression to hyperglycemia. In contrast to what has been a prevailing view that increased oxidative stress play prominent roles in beta-cell dysfunction in T2D, we proposed previously that acute and transient glucose-triggered ROS contribute to glucose-stimulated insulin secretion (GSIS) in beta-cells. In the current study, we test the hypothesis in vitro and in vivo that induction of antioxidant enzymes in response to chronic environmental oxidative stress interferences with these ‘ROS signals’ that are involved in GSIS. Exposure of INS-1(832/13) cells or isolated mouse islets to non-cytotoxic levels of oxidative stressors, including arsenic, HC, high glucose or palmitate led to decreased GSIS in a dose- and time-dependent fashion. The impaired GSIS was associated with increases in a battery of cellular adaptive response genes including antioxidant enzymes. Accordingly, the increased antioxidant activity significantly inhibited glucose-stimulated intracellular H2O2 accumulation, which as already stated is a component of GSIS. To understand the quantitative nature of ‘ROS signalling’, we resorted to an ordinary differential equation-based model which describes the Nrf2-mediated adaptive response to oxidative stress. Our simulation indicates that in the absence of oxidative stress, a stimulus mimicking glucose treatment causes a large spike in intracellular H2O2 concentration. However, when the cell is under continuous oxidative stress, the same glucose-mimicking stimulus triggers a much smaller increase in H2O2. Taken together, these integrated approaches demonstrate that chronic oxidative stress can blunt ‘ROS signalling’ by adaptively upregulating overall antioxidant capacity. (Supported by NIH-R01ES016005 and K01DK076788).

**942 TCDD INDUCES HEPATIC Nqo1 IN MICE: VI/AhR AND Nr2f.**

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2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (TCDD) induces genes via the aryl hydrocarbon receptor (AhR), but whether TCDD also induces genes via nuclear factor erythroid 2-related factor 2 (Nrf2) remains unclear. Despite previous reports of TCDD-induced Nqo1 expression in Hepa1C1c7 cells, we tested the hypothesis that TCDD induced Nqo1 expression. (Supported by NIH grants ES09716, ES07079, ES013714, ES09649, and RR021940).

**943 ROLE OF NF-KB IN LUNG INFLAMMATION INDUCED BY IRON AND SELENIUM.**

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Epithelial cells lining the lung face a constant threat of sustaining damage caused by inhaled atmospheric pollutants. Metals present in ambient particulate matter have been strongly implicated in causing lung inflammatory diseases. We studied the effects of Fe or Se, at environmentally relevant concentrations, on induction of oxidative stress and generation of the chemokine MCP-1 in cultured human lung alveolar epithelial A549 cells. To characterize the underlying mechanisms of induction of the inflammatory response, we investigated the influence of these metals on the activation of the nuclear factor kappa B (NF-kB) intracellular signaling pathway. Exposure to Fe or Se increased cellular ROS levels measured by the fluorescent probe DCFH-DA as early as 30 min after exposure, with enhanced release of MCP-1 at 24h. Immunoblotting of cytosolic and nuclear extracts for p65 (NF-kB dimer) showed that both Fe and Se induced the nuclear translocation of NF-kB. MCP-1 levels in response to Fe were significantly suppressed in the presence of the antioxidant N-acetylcysteine (NAC); similar effects were obtained in response to Se. In the presence of BMS-345541, a specific inhibitor of NF-kB, Fe- and Se-induced release of MCP-1 was significantly decreased, indicating the involvement of NF-kB in the metal-induced chemokine release. Results indicate that both Fe and Se possess the potential for inducing lung inflammation via an oxidative stress pathway, and that the induction of NF-kB activity is, at least in part, responsible for up-regulating genes encoding for inflammatory chemokines. (Supported by: MD DHMH CH605CRT).

**944 INCREASED NRF2 ACTIVATION IN KEAP1-KNOCKDOWN MICE INCREASES HEPATIC CYTOPROTECTIVE GENES THAT DETOXIFY ELECTROPHILES BUT NOT REACTIVE OXYGEN SPECIES.**

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Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor critical for induction against electrophilic and oxidative stress. A newly engineered mouse with knockdown of Keap1 (Keap1-kd mice), the cytosolic repressor of Nrf2, has a 55% decrease in Keap1 mRNA and a 200% increase in Nrf2 protein in liver. Many experiments with Nrf2-null mice demonstrate the effects of a lack of Nrf2. However, little is known about the biological effects of more Nrf2 activation, according to the phenotype of Keap1-kd mice, as well as mRNA expression of detoxifying and antioxidant genes were compared with Nrf2-null and wild-type mice. Messenger RNA quantification by multiple suspension array identified three distinct patterns of gene expression among wild-type, Nrf2-null, and Keap1-kd mice. The first pattern encompassed genes that were lower in Nrf2-null mice and considerably higher in Keap1-kd mice than wild-type mice, and included genes mainly responsible for the detoxification and elimination of electrophiles such as NAD(P)H:quinone oxidoreductase 1, and glutathione-S-transferases (Gst), as well as multidrug resistance-associated proteins. The second pattern encompassed genes that were lower in Nrf2-null mice, not increased in Keap1-kd mice, and included genes such as epoxide hydrolase-1, UDP-glucuronosyltransferases, aldehyde dehydrogenases, as well as genes important in the detoxification of reactive oxygen species, such as superoxide dismutase 1 and 2, catalase, and peroxiredoxin 1. The third pattern encompassed genes that were not different among wild-type, Nrf2-null, and Keap1-kd mice and included genes such as glutathione peroxidase, microsomal Gts, heme oxygenase-1, and uptake transporters. In conclusion, the present study suggests that hepatic Nrf2 is more important for the detoxification and elimination of electrophiles rather than reactive oxygen species. (Supported by NIH grants ES09716, ES07079, ES013714, ES09649, and RR0219400).

**945 EXOGENOUS HYDROGEN PEROXIDE RESCUES HYPEROXIA-INDUCED MACROPHAGE DYSFUNCTION DUE TO BACTERIAL KILLING BY INHIBITION OF HMGB1 RELEASE.**

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Mechanical ventilation with hyperoxia is necessary to treat critically ill patients. However, prolonged exposure to hyperoxia can cause acute inflammatory lung injury and contribute significantly to ventilator-associated pneumonia. Our previous studies show that high-mobility group box 1 (HMGB1) protein, a novel inflammatory cytokine plays a pivotal role in mediating hyperoxia-induced inflammatory lung injury and macrophage dysfunction. In addition, hydrogen peroxide (H2O2) can rescue hyperoxia-induced macrophage impairment in phagocytosis of Pseudomonas aeruginosa. The aim of this study is to determine whether HMGB1 is involved in the mechanisms of H2O2 in rescuing hyperoxia-induced inflammatory lung injury and macrophage dysfunction. Following the exposure of RAW 264.7 cells to 95% oxygen for 24h, the cells were treated with various concentrations of H2O2 ranging from 50μm to 750μM for one hour. Corresponding to the rescuing effect on hyperoxia-impaired macrophage functions, H2O2 at moderate concentrations increased the ability of hyperoxic macrophage to kill the Pseudomonas aeruginosa significantly and reduced markedly the release of HMGB1 into extracellular milieu.
which is required for its proinflammatory function. Analysis of subcellular localization of HMGB1 via immunofluorescence examination indicates that moderate concentrations of H2O2 inhibited HMGB1 cytoplasmic translocation. However, H2O2 at higher concentrations exacerbated hyperoxia-induced cytotoxicity on macrophages resulting in more HMGB1 extracellular translocation and more HMGB1 release, and impairing macrophage’s ability to kill Pseudomonas aeruginosa. The study provides evidence that HMGB1 plays a critical role in the effects of H2O2 in rescuing of macrophage dysfunctions under hypoxic conditions.

946 SERINE 374 C-FOS MEDIATES OXIDANT INDUCED C-FOS PROTEIN STABILIZATION.

E. Sheveleva and Q. M. Chen. Pharmacology, University of Arizona, Tucson, AZ.

The c-Fos protein, a component of the AP-1 transcription factor, is crucial for cellular adaptation to many disease states involving oxidative stress. Previous study from our laboratory found that oxidants induce c-Fos protein phosphorylation downstream of JNK activation. c-Fos protein phosphorylation results in resistance against proteasome mediated c-Fos degradation. Here we test whether Ser 374, a consensus phosphorylation site of MAPKs, mediates oxidant induced c-Fos protein stabilization. When wild type or Ser374 replacement mutant c-Fos was transfected into HEK293 cells, the phospho mutant (374-Asp) of c-Fos has a higher basal level of c-Fos protein, which is further elevated by H2O2 treatment compared with the wild type c-Fos. The phospho eliminating mutant (374-Ala) had a lower basal or H2O2 induced level of c-Fos protein. The c-Fos protein increases in the absence of c-fos mRNA increase, suggesting a role protein stabilization in H2O2 induced c-Fos protein elevation. Inhibiting protein synthetic synthesis with cycloheximide caused a rapid decrease of 374-Ala by 99% within 1 hr of H2O2 treatment, while wild type c-Fos decreased by 50% and 374-Asp mutant decreased only 10%. The proteasomal inhibitor MG132 caused an accumulation of c-Fos protein. Our data suggest that 374 Ser residue plays a critical role in H2O2 induced c-Fos protein stabilization.

947 INHIBITION OF NFκB ATTENUATES HYPEROXIA-INDUCED HMGB1 RELEASE FROM MACROPHAGES.

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Mechanical ventilation and therapy with high concentrations of oxygen (hyperoxia) is a life saving intervention in patients with respiratory insufficiency including sepsis. However, exposure to hyperoxia is associated with pulmonary toxicity. Our previous studies demonstrate that HMGB1 can be released from macrophages exposed to prolonged hyperoxia and contributes to hyperoxia-induced inflammatory lung injury. The objective of this study was to elucidate whether pro-inflammatory transcription factor NFκB mediates the release of HMGB1 under hyperoxic conditions from macrophages using RAW 264.7 cells (a macrophage-like cell line). Bailey 11-7821, a selective and irreversible inhibitor of NFκB, was first used in this study. RAW 264.7 cells were exposed simultaneously to a series of concentrations of Bailey 11-7821 and hyperoxia for 24 hr. No concentration of Bailey 11-7821 and HMGB1 release were determined by immunofluorescence and Western Blotting analyses. The release of HMGB1 under hyperoxic conditions was inhibited by the treatment with Bailey 11-7821 in a dose-dependent manner. To further confirm that inhibition of NFκB was mediating hyperoxic stress-induced HMGB1 release, Ethacrynic acid (EA), a diuretic which inhibits NFκB signaling, was also used. Inhibition of HMGB1 release was also seen with EA (1-2µM). Immunofluorescence analysis showed that the inhibition of HMGB1 release with both Bailey 11-7821 and EA was a result of nuclear localization of HMGB1. Corresponding to the decreased release of HMGB1, Bailey 11-7821 and EA significantly improved macrophage’s ability to migrate under hyperoxia. These results indicate that HMGB1 release from macrophages is at least partly mediated by NFκB, which may be used as a potential therapeutic target to minimize HMGB1 induced pulmonary inflammation and macrophage dysfunction.

948 15-DEOXY-A12, 14 PROSTAGLANDIN J2-INDUCED HEME OXIDASE-1 IN MEGAKARYOCYTES REGULATES THROMBOPOIESIS.

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Platelet production is an intricate process that is poorly understood. Recently, we demonstrated that the natural peroxisome proliferator-activated receptor gamma (PPARγ) ligand, 15-deoxy-A12,14 prostaglandin J2 (15d-PGJ2), augments platelet numbers by increasing platelet release from megakaryocytes through the induction of reactive oxygen species (ROS) (O’Brien, Blood, 2008). 15d-PGJ2 can exert effects independent of PPARγ, such as increasing oxidative stress. Heme oxygenase-1 (HO-1) is a potent antioxidant and may influence platelet production. To further investigate the influence of 15d-PGJ2 on megakaryocytes and to understand whether HO-1 plays a role in platelet production, Meg-01 cells (a primary human megakaryoblastic cell line) and primary human megakaryocytes derived from cord blood were used to examine the effects of 15d-PGJ2 on HO-1 expression in megakaryocytes and their daughter platelets. 15d-PGJ2 induced HO-1 protein expression in Meg-01 cells and primary human megakaryocytes. The platelets produced from these megakaryocytes also expressed elevated levels of HO-1, 15d-PGJ2-induced HO-1 was independent of PPARγ, but could be replicated using other electrophilic prostaglandins, suggesting that the electrophilic properties of 15d-PGJ2 were important for HO-1 induction. Interestingly, inhibiting HO-1 activity enhanced ROS generation and augmented 15d-PGJ2-induced platelet production, which could be attenuated by antioxidants. These new data reveal that HO-1 negatively regulates thrombopoiesis by inhibiting ROS.

949 OXIDATIVE LIPIDOMICS OF ACUTE LUNG INJURY INDUCED BY HYPEROXIA AND GAMMA-IRRADIATION.

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Pulmonary endothelium is the locus of early structural and functional changes in acute lung injury induced by toxins, hyperoxia and irradiation. Apoptosis of pulmonary endothelial cells is a life-saving intervention in patients with respiratory insufficiency involving sepsis. However, exposure to hyperoxia is associated with pulmonary toxicity. Previous study from our laboratory found that oxidants induce c-Fos protein phosphorylation downstream of JNK activation. c-Fos protein phosphorylation results in resistance against proteasome mediated c-Fos degradation. Here we test whether Ser 374, a consensus phosphorylation site of MAPKs, mediates oxidant induced c-Fos protein stabilization. When wild type or Ser374 replacement mutant c-Fos was transfected into HEK293 cells, the phospho mutant (374-Asp) of c-Fos has a higher basal level of c-Fos protein, which is further elevated by H2O2 treatment compared with the wild type c-Fos. The phospho eliminating mutant (374-Ala) had a lower basal or H2O2 induced level of c-Fos protein. The c-Fos protein increases in the absence of c-fos mRNA increase, suggesting a role protein stabilization in H2O2 induced c-Fos protein elevation. Inhibiting protein synthetic synthesis with cycloheximide caused a rapid decrease of 374-Ala by 99% within 1 hr of H2O2 treatment, while wild type c-Fos decreased by 50% and 374-Asp mutant decreased only 10%. The proteasomal inhibitor MG132 caused an accumulation of c-Fos protein. Our data suggest that 374 Ser residue plays a critical role in H2O2 induced c-Fos protein stabilization.

950 DETECTION OF ACETAMINOPHEN PHARMACOKINETICS USING MASS SPECTROMETRY BASED METABONOMICS.


Understanding of a drug’s pharmacokinetics is extremely important for drug discovery and development. Liquid chromatography coupled with mass spectrometry with exact mass accuracy combined with multivariate statistical principal component analysis (PCA) can provide rapid detection of drug metabolites lowering both the time and cost associated with the study drug. In this paper, we describe a method for detecting acetaminophen (APAP) as a model compound shows the capability to detect APAP metabolites in urine samples. The PCA score plots show a clear separation in groups 8 hrs post-dosing with vehicle (control), acute administration (rats treated with acute single dose of 400 mg/kg APAP) and chronic dosing (rats dosed daily with 200, 400 or 800 mg/kg APAP). Using this approach, APAP, APAP-sulfate, APAP-glucuronide, APAP-N-acetyl-L-cysteine (APAP-NAC) APAP-cysteine, APAP-SOCH3 and APAP-S-S-APAP were detected. After an APAP overdose, glutathione (GSH) can deacetylate N-acetyl-benzoquinone imine (NAPQI, reactive intermediate responsible for tissue damage) to form an APAP conjugate (APAP-GSH). The corresponding conjugates including APAP-NAC, APAP-cysteine, APAP-SOCH3 and APAP-S-S-APAP are breakdown products of APAP-GSH. Results show that APAP eliminated in urine as APAP-NAC.
The aim of this study was to compare oral gavage and dietary administration of aspirin in a pharmacokinetic/pharmacodynamic study in rats. The non-steroidal anti-inflammatory drug (NSAID) aspirin, is the subject of intense study as an agent in the prevention of cardiovascular disease and cancer. In animal models, an untested assumption is that exposure and the resultant pharmacological effects are similar for both gavage and dietary administration. However, for another NSAID, Sundilac, the route of administration significantly alters pharmacokinetic and pharmacodynamic profiles. This study examined and compared time-dependent plasma and colon mucosal concentrations of aspirin metabolite salicylate, plasma thromboxane B2 (TXB2), and colon mucosal prostaglandin E2 (PGE2) concentrations following the two different dosing paradigms. Dosage yielded relatively constant plasma and colon salicylate concentration vs. time profiles. Oral gavage dosing led to a rapid peak followed by a fast decline in salicylate concentration in both plasma and colon. Nevertheless, the exposures as measured by the area under the concentration-time curve (AUC0-24h) of salicylate was linearly related to dosage irrespective of the dosing method. Linear relationships were also observed between colon and plasma salicylate AUC0-24h and between colon PGE2 and plasma TXB2 AUC0-24h. This study therefore demonstrated that: 1.) For aspirin, which is rapidly absorbed and metabolized in the rat, the choice of gavage vs. dietary administration does not impact plasma or colon AUCs for the salicylate metabolite, but does it impact the pharmacodynamic marker; and 2.) The more easily accessible plasma salicylate and TXB2 concentrations were representative of the salicylate pharmacodynamic profiles. This study examined and compared time-dependent plasma salicylate and TXB2 concentrations were representative of the salicylate pharmacodynamic profiles. This study examined and compared time-dependent plasma salicylate and TXB2 concentrations were representative of the salicylate pharmacodynamic profiles. This study examined and compared time-dependent plasma salicylate and TXB2 concentrations were representative of the salicylate pharmacodynamic profiles. This study examined and compared time-dependent plasma salicylate and TXB2 concentrations were representative of the salicylate pharmacodynamic profiles. This study examined and compared time-dependent plasma salicylate and TXB2 concentrations were representative of the salicylate pharmacodynamic profiles. This study examined and compared time-dependent plasma salicylate and TXB2 concentrations were representative of the salicylate pharmacodynamic profiles. This study examined and compared time-dependent plasma salicylate and TXB2 concentrations were representative of the salicylate pharmacodynamic profiles. This study examined and compared time-dependent plasma salicylate and TXB2 concentrations were representative of the salicylate pharmacodynamic profiles.

Perfluorononanoic acid (PFNA) is a fluorinated organic chemical found at low levels in the environment, but is detectable in humans and wildlife. This study compared the pharmacokinetic properties of PFNA in two laboratory rodent species. Male and female Sprague-Dawley rats (n = 5) were given PFNA by oral gavage at 1, 3, or 10 mg/kg, and blood was collected from tail vein at 1, 2, 3, 4, 7, 16, 21, 28, 35, 42 and 50 days after treatment. CD-1 mice were given a single oral dose of PFNA at 1 or 10 mg/kg, and 4 males and 4 females were killed at similar time intervals; trunk blood and liver were collected. Serum and liver concentrations of PFNA were determined by HPLC-CMS-MS. Serum elimination of PFNA was faster in the group administered a toxic dose on day 2, which is consistent with histopathology data that showed tissue damage only in the high dosed group. This abstract does not necessarily reflect U.S. EPA policy.

The elevation of 3H and 14C in the spleen is indicative of the role of the immune response consistent with lipid participation in the endotoxic response during infection.

The US Air Force is transitioning from petroleum-based Jet Propellant-8 (JP-8) to a blend of JP-8 and synthetic jet fuel (S-8) produced from gas or coal using the Fischer-Tropsch process. The composition of S-8 fuel differs from JP-8 fuel primarily by a complete lack of aromatic hydrocarbons and a substantial increase in the amount of branched alkanes. Limited toxicity information is available for S-8 fuel. The present research evaluates hydrocarbons found in tissues and excreta of male F344 rats exposed in a nose-only chamber for 4 hours to 1000 mg m-3 of aerosolized S-8 fuel. A portion of the animals were placed in metabolism cages for 48 hours after exposure while the others were sacrificed and blood and tissues (liver, brain, lungs and fat) collected two hours into the exposure and up to one-hour post exposure. Several metabolites associated with the fuel exposure were identified in tissues and excreta. The burden of metabolites appeared to be greatest in the liver compared to other tissues. Identifications of hydrocarbon structures suggest that the hepatic cytochrome P450 system is involved in the metabolism of these chemicals. In separate inhalation exposures, n-alkane metabolites were found in liver and lung of rats exposed to a mixture of n-alkanes (n-C10 to n-C35). This study is part of a larger ongoing study to develop a hydrocarbon mixture Physiologically Based Pharmacokinetic (PBPK) model for jet fuel. This abstract is first to report on the metabolism of S-8 fuel. (This research was supported by AFSOR Grant FA9550-07-1-0132).

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could be metabolized to reactive species. ARB has been reported to be easily hydroyzed by dilute acid; however, this contradicts evidence that ARB is stable at pH of 2 and is excerted unchanged in the urine of gavaged rats. The fate of ARB in mice has not been reported. The present studies were designed to investigate the stability of the glycosidic bond of ARB in aqueous acid and to determine the chemica's in vivo fate in male rats (F344 and Wistar Han) and mice (B6C3F1). Results of the present work indicated that little or no hydrolysis of ARB occurred in aqueous acid at pH as low as 0.1. In the in vivo studies, a 100 mg/kg orally administered dose of 14C-labeled ARB was rapidly absorbed from the gut, with greater than 95% of the recovered dose (96 ± 6, 105 ± 2, and 87 ± 9% for F344 rats, Wistar Han rats, and B6C3F1 mice, respectively) excreted in the urine within 24 h post-dosing. The major tissues, including blood, contained little or no 14C at the timepoint. Results were similar for rats and mice receiving 0.1 mg/kg. Over 90% of the 14C excreted in 0.4 hr urine of F344 or Wistar Han rats (75 ± 5 and 84 ± 10% total dose, respectively) consisted of a peak eluting at the retention time of parent ARB in the HPLC system. The peak, accounting for greater than 75% of the 14C excreted in mouse urine, was isolated and confirmed as ARB by LC/MS analysis. In conclusion, the present studies demonstrated the stability of the glycosidic bond of ARB in aqueous acid well below gastric pH and in vivo in the rat and mouse. Humans may in fact be more sensitive to the effects of ARB than rats and mice; therefore, ARB-treated rodents are probably poor models for predicting effects of exposure to the chemical in humans.

**956 IN VITRO METABOLISM AND GENDER SPECIFIC PHARMACOKINETICS OF K777, A NOVEL CYSTEINE PROTEASE INHIBITOR.**

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K777 (N-methyl-piperazine-Phe-homo-Phe-lysyl-lysyl-phenyl) is a novel cysteine protease inhibitor under investigation as a therapeutic candidate for Chagas Disease. Metabolism and pharmacokinetic profiles of K777 were evaluated in this study. Human and rat liver microsomes (0.2 mg/ml) were incubated with K777 (0 to 100 μM), MgCl2 (5.3 mM) and NADPH (2.5 mM) in a phosphate buffer (pH=7.4) medium. The major metabolites of K777 identified include N-desmethyl and N-oxide metabolites with apparent Kₘ values of about 20 and 18 μM (human) and 8 and 5 μM (rat), respectively. A hydroxyl metabolite of K777 was also formed by human liver microsomes (Kₘ of 10μM). The bioavailability and pharmacokinetics of K777 was determined in male and female rats after oral (20 and 100 mg/kg) and iv (20 mg/kg) administration. Plasma exposure of K777 was 2 to 8-fold higher in females than males, as shown by AUC values; iv group (20 mg/kg): 5758 (males) and 6924 (females); oral groups (20 and 100 mg/kg): 101 and 6594 (males), 799 and 12852 (females) μg.hr/ml. Oral bioavailability was higher in females (12 and 37%) than in males (2 and 22%) at K777 doses of 20 and 100 mg/kg. Plasma concentration time profiles in rats of both genders showed the presence of secondary peaks indicative of enterohepatic circulation. K777 was also studied in a toxicity study after 7 days of oral administration at 30 mg/kg body weight dose on days 1 and 7 indicated 2.5 to 3-fold higher levels of K777 in females than in males. In addition, there were marked decreases (1 to 3-fold) in plasma concentrations of K777 on day 7 versus day 1 in both genders. This indicated an increased clearance of K777 with multiple oral doses, which may be due to a possible autoinduction of its metabolism after chronic administration. Additional studies are in progress to further evaluate the gender differences in K777 disposition, and mechanisms of induction of own its metabolism after chronic administration. Funded by NIAID Contract No1AI-60011.

**957 IDENTIFICATION OF TRANSPORTERS INVOLVED IN RENAL ELIMINATION OF PERFLUOROCARBOXYLATES IN RATS.**

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Perfluorocarboxylates (PFCAs) are globally distributed environmental chemicals that are widely used in many industrial processes. Readily absorbed through contaminated water, they can affect lipid metabolism in animal models. Both hepatic accumulation and renal clearance of PFCAs are influenced by chain length and gender in rats. PFCAs with shorter chain length are in general excreted in urine while the longer ones accumulate in liver. We wanted to test the hypothesis that this renal elimination and liver accumulation are caused by tissue and gender specific expression of candidate transporters. We transiently transfected HEK 293 cells with Oat1 and Oat3. For Oatp1a1, we used a stable CHO cell line. We measured uptake of the model substrates p-aminophenol (Oat1), estrone-3-sulfate (Oat3) and estradiol-17β-glucuronide (Oatp1a1) in the absence or presence of 10 μM of PFCAs with chain lengths from 2-18 carbons. To determine direct transport of PFCAs, we used LC-MS/MS analysis. Oat1-mediated transport was inhibited strongest by perfluorohexanoic acid (PFHA), followed by perfluorooctanoic acid (PFOA) and perfluorooctanoic acid (PFNA). PFOA and PFNA were the strongest inhibitors for Oat3-mediated estrone-3-sulfate transport. Oatp1a1-mediated transport of estradiol-17β-glucuronide was inhibited strongest by perfluorodecanoic acid (PFDA), followed by perfluorooctanoic acid (PFUDA) and PFNA. With respect to transport, uptake into Oat1 expressing cells was highest for PFHA and PFNA, followed by Oatp1a1. Uptake into Oat3 expressing cells was highest for PFOA and PFNA, followed by PFNA. In Oatp1a1 expressing cells, uptake of PFDA was highest followed by PFNA and PFOA. In summary, for the three tested transport proteins, the strongest inhibitors are also the best substrates. Based on their cellular localization, we conclude that Oat1 and Oat3 may be responsible for active renal secretion of PFHA, PFOA and PFNA while Oatp1a1 may be responsible for reabsorption of PFNA, PFDA, and PFOA.

**958 IDENTIFICATION OF HUMAN GENES THAT COMPLEMENT A GLUTATHIONE TRANSPORT DEFICIENCY IN YEST.**

S. Shi, S. Noreenbo, and N. Ballatori, Environmental Medicine, University of Rochester, Rochester, NY.

Reduced glutathione (GSH; γ-glutamyl-l-cysteinyiglycine) is a major intracellular thiol, and disturbances in its homeostasis are associated with a number of human diseases. GSH export from cells is one of the two major mechanisms involved in regulating GSH levels, although relatively little is known about the proteins responsible for GSH export. To examine whether unidentified GSH transport mechanisms exist in the human genome, a human mammary gland cDNA library was screened using a yeast (S. cerevisiae) functional complementation strategy. Unlike mammalian cells, yeast cells are able to uptake GSH from the extracellular space utilizing hgp1p, a high affinity GSH uptake transporter. The deletion of the HGT1 gene (hgp1Δ strain) leads to a deficiency in GSH uptake and the inability to grow in sulfur-deficient media in which GSH is the only sulfur source. This screening strategy initially identified more than 300 colonies that grew faster in sulfur-deficient medium, and from this pool five human genes were identified, namely: THFEP1, transmembrane, prostate androgen induced RNA; LATM4, lysosomal-associated protein transmembrane 4 alpha; SLC25A1, solute carrier family 25, member 1; LITAF, lipopolysaccharide-induced TNF factor; and CYR1, cysteine/tyrosine-rich-1. All five genes are predicted to encode for small membrane proteins. The expression of these five genes was able to rescue growth of hgp1Δ cells and to increase intracellular glutathione levels when cells were cultured in sulfur-deficient medium supplemented with 750 μM GSH. [†]GSH uptake was measured in whole cells, and the result confirmed that GSH uptake abilities in yeast cells transformed with these five human genes was increased. Altered expression of THFEP1, SLC25A1, LITAF, and CYR1 has been reported in a number of human diseases, but little is known about the normal biological functions of these five genes. The present results suggest that they may be involved in plasma membrane transport events and/or the regulation of GSH homeostasis.

**959 THE EFFECTS OF TEMPERATURE AND TIME ON THE GENERATION OF ALCOHOL IN STORED BLOOD.**

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The present study was conducted to investigate whether and, if so, under what conditions spontaneous alcohol generation would occur in blood stored over extended periods of time in sterile collection tubes. Fresh blood was collected from our blood bank and aliquots of blood were transferred into red top Vacutainer tubes containing no added preservative or anticoagulant. The tubes were divided into two groups for storage and maintained for up to 1 week after collection. One group was placed in a refrigerator where the temperature range was between 7-9°C. The other group was kept on a bench top, where the median room temperature was maintained at 25°C. Samples of blood (100μl) were drawn from each tube at time zero, 48, 72, 96, 120 and 144 hours after initiation of storage. The specimens were analyzed for alcohol content on a Shimadzu 14A-GA Gas Chromatograph, equipped with a Supelco 5% Carboxaw packed column. The refrigerated specimens developed no significant levels of ethanol within the entire time frame while those stored at room temperature began to produce slight but detectable levels of ethanol by 120 hours. By the 144 hour time point, all blood stored at room temperature showed detectable levels.
of alcohol, whereas blood stored at 7.9°C produced no evidence of alcohol production. This study indicates that both temperature and duration of storage are factors, which may result in the generation of blood alcohol in such specimens used for toxicological assessment, even when maintained in sterile blood tubes.

**PS 960 INHALATION PHARMACOKINETICS AND METABOLISM OF 8:2 FLUOROTELEROMER ALCOHOL IN RATS AND EXTRAPOLATION TO HUMAN EXPOSURE.**


This study investigated the profile of 8:2 fluorotelomer alcohol (8:2 FTOH, F(CF2)8CH2CH2OH) and its metabolites in plasma under controlled inhalation exposure as the basis for comparison with potential environmental exposure. Male and female rats were exposed to 8:2 FTOH at 3 or 30 mg/m3 for 6 h. Blood was collected at 1, 3 & 6 h during nose-only exposure and at 6 & 18 h after the end of exposure. Plasma samples were analyzed by LC/MS/MS for the alcohols (8:2 FTOH, 8:2F(CF2)8CH2CH2OH) and its metabolites in plasma under controlled inhalation exposure as the basis for comparison with potential environmental exposure. Male and female rats were exposed to 8:2 FTOH at 3 or 30 mg/m3 for 6 h. Blood was collected at 1, 3 & 6 h during nose-only exposure and at 6 & 18 h after the end of exposure.

**PS 961 PHARMACOKINETIC MODELING OF PERFLUOROOCTANOIC ACID DURING GESTATION AND LACTATION IN MICE.**

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Perfluorooctanoic acid (PFOA) is used industrially as a processing aid in the polymerization of commercially valuable fluoropolymers. Its widespread environmental distribution, presence in human serum, and adverse effects in animal toxicity studies have triggered attention to its potential adverse effects to humans. PFOA is not metabolized and exhibits dramatically different plasma half-lives across species. Estimated plasma half-lives for humans, monkeys, mice, and female rats are 3-5 years, 20-30 days, 12-20 days, and 2-4 hours, respectively. Developmental toxicity is one of the most sensitive adverse effects associated with PFOA exposure in rodents. In mice, developmental delays and reduced postnatal growth and survival occurred at much lower administered doses than in rats. Risk assessment of PFOA is currently hampered by the lack of understanding of its cross-species pharmacokinetics in animal toxicity studies. To address this uncertainty, a biologically-supported dynamic model was developed whereby a two-compartment system linked via placental blood flow described gestation and milk production linked the lactating dam to a growing pup litter compartment. The model incorporated changes in body weight, placental blood flow, milk yield, and PFOA-specific pharmacokinetic information. Postnatal serum levels of PFOA for 129S1/SvImJ mice at doses of 1 mg/kg or less were reasonably simulated while prenatal and postnatal measurements for CD-1 mice at doses of 1 mg/kg or greater were simulated via addition of a biologically-based renal resorption description. Our results suggest that a linear model may suffice for describing the pharmacokinetics of PFOA at low doses while a more complex model may be needed to describe non-linear behavior at higher doses. Based on internal dose estimates, the mouse achieves much higher levels as compared to rats which may help explain the observed differences in developmental toxicity in these two species. (This work was reviewed by EPA and approved for publication but does not necessarily reflect official agency policy).

**PS 962 IN VITRO TO IN VIVO EXTRAPOLATION WITH A PBTK MODEL FOR 2-CHLORO-1, 3-BUTADIENE.**

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Beta-chloroprene (2-chloro-1,3-butadiene) (CD) is a volatile, flammable liquid monomer used for the production of synthetic rubber and latexes. Although human cancer epidemiology studies have been inconclusive, animal studies have demonstrated carcinogenic effects of CD in B6C3F1 mice and Fischer rats. In this study, in vitro kinetic data for CD metabolism in liver and lung were used to predict in vivo kinetics in female mice after single and repeated inhalation exposures. For parameterization of CD metabolism, a 2-compartment kinetic model was used to describe the distribution and metabolism of CD in an in vivo closed chest system. The ratios for liver Vmax/Km (mL/hr/mg) were 112 and 86 in the mouse and rat, respectively. Metabolism in the lung was slower (Vmax/Km = 9.1 for mice and 0.9 for rats). The Vmax values were scaled for use in the PBTK model. In vivo exposure of female mice to 13, 32, or 90 ppm CD for 6 hrs resulted in end exposure blood concentrations that were non-linearly related to exposure concentration and required addition of metabolism in the upper respiratory tract and kidney (as 5% and 10% of the liver Vmax, respectively) for the model to fit the reduced blood concentrations at days 5 and 15, relative to day 1. These additions partially accounted for the reduced blood concentrations and a 50% reduction in the ventilation rate from the 1-day exposure was needed for the model to reproduce the observed data. Ventilation was measured experimentally on day 1 and corresponded to a QPC = 34 L/hr/kg⁰.⁷５. The 1-day minute volumes were not affected by increasing CD exposure concentration, suggesting physiological adaptation with repeated exposure. With the revised PBTK model structure and the in vitro metabolite parameters, the PBTK model successfully predicted the time-course of CD in the blood after single and repeated exposures. This in vitro to in vivo extrapolation (IVIVE) approach could be used with human metabolism data to predict human kinetics.

**PS 963 ANALYTICAL METHOD VALIDATION OF 2-METHOXY-4-NITROANILINE IN NTP-2000 AND NIH-07 RODENT DIETS.**


2-Methoxy-4-nitroaniline (MONA), selected for toxicological evaluation by the National Toxicology Program (NTP), is used as a textile dye, in the printing industry, and in the synthesis of azo dyes with applications in tattoo inks, emulsion paints, and toy enamels. An analytical method was developed and validated for MONA in both NTP-2000 and NIH-07 rodent diets to cover a dose formulation range of 750 µg/kg to 30,000 µg/kg/MOA. MONA was extracted from feed with acetonitrile and evaporated to a final concentration of 5 min, 0.999), accurate (from -11.9 to 2.1% relative error), and precise (% RSD ≤ 0.5). Following method validation, a 42-day dose formulation stability study and a 7-day simulated dosing study were performed at 1,500 µg/g MONA in both diets. The 42-day dose formulations were performed under ambient (25°C), refrigerated (5°C), and freeze–thaw (20°C) conditions. When compared to Day 0, the results indicated losses up to 39.6% under ambient conditions, up to 9.7% under refrigerated conditions, and up to 2.4% under freeze–thaw conditions. The 7-day simulated dosing formulations were stored in stainless steel hoppers under ambient (25°C) temperature with 50% relative humidity and 12-hr daily light cycles. When compared to Day 0, the results indicated losses up to 12.2%.

**PS 964 EFFECT OF NARINGENIN ON DISPOSITIONS OF DEOXYVINALENOL IN PIGLETS.**

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Naringenin (NAR) is a naturally occurring flavonoid glycoside obtained from grapefruit, which is expressed on a diuretic effect. The aim of this present research is to evaluate the effect of NAR on dispositions of deoxynivalenol (DON) in piglets.
The 42-day dose formulations were stored under ambient (25°C), refrigerated (5°C), and freeze (−20°C) conditions. When compared to Day 0, the results indicated losses up to 10.8% under ambient conditions, up to 5.9% under refrigerated conditions, and up to 5.8% under freezer conditions. The 7-day simulated dosing formulations were stored in stainless steel hoppers under ambient (25°C) temperatures with 50% relative humidity and 12-hr daily light cycles. When compared to Day 0, the results indicated losses up to 7.1%.

**965 VALIDATION OF PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODEL PREDICTIONS OF PRESYSTEMIC ELIMINATION OF TRACE ORAL DOSAGES OF TRICHLOROETHYLENE (TCE).**

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TCE is a common contaminant of groundwater and drinking water in the U.S. Concentrations range from ppt to a few ppm. Ingestion of such water should not pose a non-cancer or cancer risk of injury to extrahepatic organs, if all of the TCE is removed from the venous blood by the liver and lungs (i.e., pre-systemic elimination) before it reaches the arterial circulation. Unanesthetized male Sprague-Dawley rats were given TCE iv and by gavage in doses of 0.001, 0.01, 0.1, 1.2, 5 and 10 mg/kg. Serial blood samples from the cannulated rats were analyzed by head-space solid phase microextraction-gas chromatography with negative chemical ionization mass spectrometry. The method's LOQ was 0.025 ng/ml. Blood time-course data for collection of heart rate. Plasma atropine concentrations were measured using an LC/MS method. Single intramuscular injections of saline (control), atropine (0.2 mg/kg), and/or MB-4 (160 mg/kg) or 2-PAM (60 mg/kg) were administered. PK parameters used to assess atropine absorption (ka, Cmax, Tmax, AUC) were determined from the time-course data and simulated first-pass uptake by the gut, liver and lungs. Supported in part by U.S. DOE Coop. DE-FG09-02CH11109.

**966 ANALYTICAL METHOD VALIDATION OF TRIS(CHLOROPROPYL) PHOSPHATE, MIXTURE IN NTP-2000 AND NIHO-07 RODENT DIETS.**


Trischloropropyl phosphate (TCP; mixture of four isomers), selected for toxicological evaluation by the National Toxicology Program (NTP), is a flame retardant added to flexible polyurethane foam used in home furnishings. In this work, an analytical method was developed and validated for the analysis of TCP in both NTP-2000 and NIHO-07 rodent diets to cover a dose formulation range of 1,500 μg/kg to 75,000 μg/kg. TCP was extracted from feed with methanol using a wrist-action shaker for 15 min followed by centrifugation for 5 min at 2,200 rpm. Afterwards an aliquot was combined with DON at the same internal standard (IS), and analyzed using gas chromatography (GC) with flame ionization detection (FID) with a J&W Scientific DB-5 (30 m x 0.53 mm I.D., 1.5-μm film thickness) column. The oven was maintained at 160°C (5 min hold) to 180°C (5 min hold) at 1.0°C/minute. The retention times for the three major isomers of TCP were 13.9 min, 14.5 min, and 15.1 min with 25.6 min for IS. Both method validations were linear (r² > 0.9999), accurate (from -7.0 to 1.3% relative error), and precise (% RSD ≤ 2.4). Following method validation, a 42-day dose formulation stability study and a 7-day simulated dosing study were performed at 3,000 μg/kg TCP in both diets.

**967 PK/PD EFFECTS OF VARIOUS MB-4 DOSING REGIMENS ON ATROPINE BIOAVAILABILITY IN RATS.**

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Concomitant administration of atropine with pralidoxime (2-PAM) is standard treatment following organophosphorous poisoning. Human studies demonstrated a delay in atropine absorption when injected with 2-PAM (Sudell, 1974, C. Pcd. Ther.; Fried et al., 1989, J. Pharm. Sci.). This delay was attributed to the osmolality of the 2-PAM. The (4-[(hydroxyimino)methyl]pyridinium) dimethanesulfonate (MB-4), a potential new oxime therapeutic for organophosphorous poisoning, will be co-administered with atropine if approved. This study determined what the impact of co-administered MB-4 would be on atropine absorption, i.e. could these two compounds be co-administered or would they require separate administration. Adult male Sprague-Dawley rats (3 animals/group) were cannulated (femoral vein) for automated serial blood sample collection (Cakes) and implanted with telemetry devices for collection of heart rate. Plasma atropine concentrations were measured using an LC/MS method. Single intramuscular injections of saline (control), atropine (0.2 mg/kg), and/or MB-4 (160 mg/kg) or 2-PAM (60 mg/kg) were administered. PK parameters used to assess atropine absorption (ka, Cmax, Tmax, AUC) were similar for all treatment groups. The maximum heart rate response was similar for all treatment groups. Initial results indicate that the time to maximum heart rate response occurred earliest when atropine and MB-4 were co-mixed but the temporal difference compared to the separate injections was not considered biologically relevant. Co-mixed atropine and 2-PAM resulted in a 1.5 to 3-fold delay in the time to maximum heart rate response compared to the atropine and MB-4 groups. The results for this study suggest that co-mixing atropine and MB-4 does not reduce atropine absorption in rats. [Supported by CBRNIAC contract #590700.00-D-3180]

**968 CHARACTERIZATION OF THE TRANSPORT AND INHIBITORY EFFECTS OF NBuPy-Cl AND ITS STRUCTURALLY RELATED IONIC LIQUIDS BY HCOT2.**

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N-butyrylpyridinium chloride (NBuPy-Cl) is known as an ionic liquid (IL) with solvent properties. In vivo studies in rats revealed that the systemically available NBuPy-Cl was eliminated in urine as the parent compound. Since its plasma clearance exceeded glomerular filtration rate, NBuPy-Cl appears to be secreted by organic cation transporters (OCTs) in the kidney. Here, we determined the transport and inhibitory effects of NBuPy-Cl and other structurally related ILs on human OCT2 (hcOT2) expressed in Chinese hamster ovary cells. NBuPy-Cl was effectively transported by hcOT2 (k=18±3×10⁻⁶ μM) and was also a potent inhibitor of hcOT2 mediated transport of tetraethyl ammonium (TEA, IC₅₀=1.7±0.1 μM) and metformin (IC₅₀=0.14±0.01 μM). Another butyl substituted IL, 1-butyly-3-methylimidazolium chloride, had similar inhibitory characteristics on hcOT2 as NBuPy-Cl, but 1-butyly-3-methylpyridinium chloride was a more potent inhibitor of hcOT2 mediated transport of TEA (IC₅₀=0.5±0.1 μM) and metformin (IC₅₀=0.14±0.01 μM). Results of SAR studies showed that the inhibitory effects of alkyl substituted pyridinium IIs decreased dramatically as the length of the side chain decreased. The IC₅₀ values were 0.3, 2.3, 37, 790 μM (C₂H₅₃-, C₄H₉₃-, C₆H₁₃₅-, H-pyridinium chloride) and 0.1, 0.7, 20, 555 μM (same order) for hcOT2 mediated transport of TEA and metformin, respectively. These data demonstrate the transport of the tested IIs by hcOT2 and their inhibitory effects on hcOT2 are greatly dependent on the chemical structure and size. This research was supported by the NIEHS NTP Grant No. N01-ES-45529 and NIEHS-sponsored Southwest Environmental Health Science Center Grant Number P50-ES-066094.

following intravenous (i.v.) administration. Piglets were then divided into two groups; three piglets (group 1) were pretreated orally with NAR at a dosage of 25 mg/kg body weight (bw), once a day for 3 consecutive days, followed by a single i.v. administration of DON at a dosage of 1 mg/kg bw. The other three piglets (group 2) were i.v. administered with DON at the same dosage. The level of DON in plasma and various tissues were determined using liquid chromatography/random mass spectrometry (LC/MS/MS). Plasma levels of DON were measurable up to 20 h in both groups after administration of DON. However, DON level in piglets pretreated with NAR was lower than that of piglets untreated with NAR. The level of DON in plasma with respect to time was analyzed with a non-compartmental pharmacokinetic model. The elimination half-life was longer in piglets untreated with NAR than in piglets pretreated with NAR. The values of peak concentration at initial time, area under the curve and mean residence time were also higher in piglets pretreated with NAR than in piglets pretreated with NAR. The tissue deple-
**969 DRIED BLOOD SPOT METHODOLOGY EVALUATION STUDY IN RATS AND DOGS GIVEN A SINGLE ORAL (CAPSULE) DOSE OF ACETAMINOPHEN OR DEXAMETHASONE.**


Analysis of blood spots dried on specialized filter paper has been reported for therapeutic drug monitoring of certain immunosuppressive drugs and antiretroviral compounds in patients, as well as in screening newborns for metabolic disorders. The small 15 µl volume blood sample required for this analysis can provide a number of advantages over traditional sample volumes collected from laboratory animals for toxicokinetic evaluations in preclinical safety assessment studies. The advantages include eliminating or reducing the need for subgroups in rodent toxicity/toxicokinetic studies, resulting in a significant reduction in animals required for these studies, potentially higher quality rodent toxicokinetic data as a result of serial sample collection from individual animals for better correlations of exposure to toxicity, simplified handling, storage, matrix preparation and transfer, and less invasive sampling. To determine the utility of the dried blood spot (DBS) methodology in a standard safety assessment setting, two commonly used laboratory animal species (rat and dog), were given a single oral (capsule) dose of acetaminophen (150 mg/kg rat; 50 mg/kg dog) or dexamethasone (100 mg/kg rat; 2.5 mg/kg dog). Blood samples were collected using either the DBS methodology or traditional blood and plasma preparation to compare the toxicokinetic profile for each species using the two techniques. Samples from either dried blood spots or plasma will be analyzed by validated LC-MS/MS methodologies. Use of the DBS technique has the promise of providing high quality toxicokinetic data with much smaller sample collection volumes while also enhancing one of the principle goals of a reduction of animal use on preclinical safety assessment studies.

**970 BAYESIAN ANALYSIS OF PHYSIOLOGICAL-BASED PHARMACOKINETIC (PBPK) MODELING OF PERCHLOROETHYLENE (PCE) IN HUMANS.**

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PCE is widely distributed in the environment and the primary chemical used in dry-cleaning. A human PBPK model developed by Covington et al. (2007) was modified and used to predict target tissue (e.g., brain and liver) doses of PCE and its major metabolite, Trichloroacetic acid (TCA), during and following inhalation exposures. A Bayesian approach using Markov chain Monte Carlo (MCMC) analysis was employed to combine information from prior distributions of model parameters and data on individuals from 5 previous human kinetic studies of PCE. Interindividual variability can thereby be captured and estimated more readily. Use of posterior results to more accurate PBPK levels, blood concentrations of PCE and TCA, and urinary excretion of TCA under different exposure conditions. The 95th percentile for the fraction of PCE metabolized upon continuous inhalation of 1 ppm was forecast to be just 1.89%. Values as high as 58 and 79% have been adopted by regulatory agencies in estimating cancer risks for low-level PCE exposures.

**971 DISPOSITION OF C_{60} FULLERENE AFTER INTRAVENOUS INJECTION, INTRACHANAL Instillation, OR INHALATION IN MALE F344 RATS.**

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Fullerene-C_{60} nanoscale materials have been selected by the National Toxicology Program (NTP) to represent “nanoparticles” for the evaluation of nanoscale material disposition and toxicity. LC-MS and HPLC-UV (350 nm) methods were developed to measure C_{60} fullerene. Male F344 rats were treated with C_{60} in PBS/1% Tween through intravenous (IV) injection or intrachannel (IT) instillation, or aerosol inhalation, and the animals were sacrificed at different time points up to 168 h. Blood, liver, lung, spleen, and kidneys were harvested and lyophilized, and the C_{60} in these tissues was extracted with toluene. Jujugar vein-cannulated rats receiving an IV dose of C_{60} (11.6 mg/kg) through the cannula showed that C_{60} was removed from the blood within 1 h, and most of the dose was taken up by the liver. IT instillation of C_{60} (1 and 5 mg/kg) in normal rats showed that C_{60} did not distribute from lung to any other surveyed tissues at 0.5, 2, 6, 24, and 168 h post-dosing. Normal rats exposed to C_{60} (amorphous particles of approximately 20 nm) aerosol at a concentration of 1 mg/m³ for 6 h showed no distribution of C_{60} from lung to any other surveyed tissues at 0.5, 1, 2, 4, 60, and 168 h post-dosing, except trace amounts were detected in kidneys. The lung half-life of C_{60} nanoparticles in the inhalation study was estimated to be 22 days, although how C_{60} was cleared remains unknown. In conclusion, C_{60} was mostly taken up by liver from IV administration, and remained in lung after IT or inhalation exposure. [Supported by NIEHS Contract N01-ES-75562 (HHSN29120077562C)]

**972 ABSORPTION, DISTRIBUTION, AND EXCRETION OF (CARBONYL-C13)PERFLUOROHYDROXYACID IN RATS AND MICE.**

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To study the pharmacokinetics of perfluorohexaoxate (PFHx, F(CF2)2COO), male and female rats and mice were dosed with either 2 or 100 mg/kg of the sodium salt of 13C-PFHx. A plot of plasma concentration (C) versus time shows the rapid elimination with half-lives of approximately 2 hrs in male and 0.5 hrs in female rats. Absorption was rapid and complete as evidenced by a short time to maximum concentration (C_{max}) of 50 min (male) and 15 min (female) and the nearly complete elimination in urine by 24 hrs for C_{max} was approximately in both sexes (6 to 7 mg equiv/g at 2 mg/kg; 250 to 750 mg equiv/g at 4 mg/kg). The area-under-the-curve (AUC) integrated from zero to infinity was 3 and 2 times higher in mice than in females (2 and 200 mg/kg dose, respectively). As with rats, absorption in the mice was rapid with a T_{max} achieved 0.5 hrs after dosing. The plasma concentration shows biphasic elimination that precludes facile calculation of half-life using a noncompartmental model. Absorption appears to be nearly complete. There is a sex difference in elimination rate in mice, but the C_{max} was similar to that observed in rats. The AUC integrated from 0 to the last time point with a quantifiable concentration was 3 and 2 times higher in male than in female mice (2 and 100 mg/kg, respectively). Material balance was determined in both rats and mice. Nearly 100% of the dose was excreted in the urine in both sexes, both species, and both doses. Rat hepatocyte data shows that PFHx is not metabolized. This result implies that bioavailability was close to 100%. The majority of the dose was eliminated within the first 24 hrs in both sexes of rats and mice. Additional groups of rats and mice were dosed with 13C-PFHx and sacrificed at T_{max} to determine if the dose and tissues were then analyzed for total radioactivity. The rates and extent of elimination from tissues were consistent with the observation that the dose was rapidly and completely eliminated through the urine.

**973 METHYLTRIETHYRAMINE (MTHF) BRAIN CONCENTRATIONS FOLLOWING A SINGLE INTRAVENOUS OR GAVAGE ADMINISTRATION OF MTHF TO F344 RATS AND B6C3F1 MICE.**

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A TK study of MTHF, a component of alternative fuels (P-series fuels), was conducted in Fischer 344/N rats and B6C3F1 mice to correlate toxicokinetic and toxicodynamic availability and to improve the usefulness of toxicity study results in risk assessment. Rats and mice (12 to 13 weeks old) were given a single IV administration at dosages of 2.5, 10, or 40 mg/kg for rats, and 10 (females only), 20, 40, or 80 (males only) mg/kg for the mice and a single gavage administration at dosages of 10, 30, 100, or 400 mg/kg for rats and 20, 100, or 400 mg/kg for mice. Milli-Q water was the vehicle. Brain MTHF concentrations were measured using a validated headspace capillary GC method with mass selective detection (MSD) for the low range and with flame ionization detection (FID) for the high range. MTHF brain concentrations were measured within 2 to 5 min after IV or gavage administration. Tmax and AUC increased with increasing dose. IV group rats had a larger than proportional increase from 10 to 40 mg/kg (not biologically relevant) and IV group mice exhibited proportional increases with dose. Gavage group rats had a greater than proportional increase from 10 to 100 mg/kg but not from 100 to 400 mg/kg; however, there was a substantial increase in the half-life for the 400 mg/kg group. For the mice, a larger than proportional increase was observed at 100 and 400 mg/kg, respectively. These results suggest that dosages ≥ 400 mg/kg for rats and 100 mg/kg for mice produce brain MTHF concentrations greater than what would be expected based on the increase in the dose. IV and gavage group brain:plasma ratios tended to be below 1 for rats and mice at all dose levels, thereby indicating that MTHF was not sequestered in the brain. Although there were no overt differences in the MTHF brain/plasma ratio between the sexes of either species, males generally had a slightly higher ratio than the females. [This work was supported by NIH, N01-ES-55551.]
DIMETHYLAMINOLANOLINE, DMAE, IS A HIGH VOLUME CHEMICAL USED IN INDUSTRIAL AND CONSUMER PRODUCTS, INCLUDING DIETARY SUPPLEMENTS. DMAE IS SIMILAR TO THE ESSENTIAL NUTRIENT, CHOLINE, AND MAY INTERFERE WITH ITS DISPOSITION IN MALE RATS.

However, the fate of DMAE and its interactions with choline have not been well characterized. Therefore, the present studies were designed to determine the metabolism and disposition of 500 mg/kg [14C]DMAE administered by gavage to male Wistar Han rats and female B6C3F1 mice and to investigate the effects of single oral 1 h-pretreatment of DMAE on the disposition of [14C] choline chloride in male rats. Within 24 h, male rats excreted 62% of the [14C]DMAE dose in urine, 0.2% in feces, 4.8% as CO, and 0.2% as volatile organics. Female mice excreted 43% in urine, 0.6% in feces, 12% as CO, and 1.5% as volatile organics. Ca. 24 h and 58% of the dose remained in the tissues of rats and mice, respectively. Liver, muscle, skin, small intestine, adipose, blood (rats only), kidney (mice only), and stomach (mice only) contained more than 1% of the total dose. Parent DMAE and DMAE oxide were identified by HPLC and MS analysis in urine of [14C]DMAE-treated animals. Formation of choline by methylation of DMAE was not confirmed due to co-elution of DMAE and choline upon HPLC analysis and presence of endogenous choline in urine. Rats receiving [14C]choline (160 mg/kg) by gavage, excreted 33% of the dose in urine, 22% in feces, 13% as CO, and 0.1% as volatile organics within 24 h. Tissues contained 2% total dose. The disposition of choline was not affected by pretreatment with 100 mg/kg unlabeled DMAE; in contrast, a 500 mg/kg DMAE dose delayed choline-derived CO excretion. In conclusion, DMAE was well absorbed following oral dosing in male rats and female mice, and DMAE administration may affect the disposition of choline in male rats. [Supported by NEIHS Contract N01-ES-75562 (HHSN291200775562C)]

Ginsenoside Rg1, one of the active components of Panax ginseng, is known to have various immune-modulating effects such as increased activity of T helper cells. In the present study, a sensitive method was applied for the determination of ginsenoside Rg1 in rat serum following oral and intravenous administration, together with the pharmacokinetic feature of ginsenoside Rg1. A sensitive liquid chromatography-electrospray tandem mass spectrometry (LC/ESI-MS) was used to determine ginsenoside Rg1 following oral administration at 100 mg/kg and an iv administration at 10 mg/kg. The pharmacokinetics of Rg1 in rat serum could be described by a non-compartmental model. Oral Tmax and Cmax of Rg1 were 0.4 ± 0.0 h and 1154 ± 257 ng/ml. Two phase of Rg1 decline was observed from concentration-time profile after oral administration. The half-life of the phase was 0.17 ± 0.07 h and that of the β-phase was 2.13 ± 0.86 h. Rg1 in serum was not quantifiable beyond 4 h after administration. The half-life of β-phase in an iv administration was 0.28 ± 0.09 h and AUC was 5.05 ± 0.31 mg h/L. The absolute bioavailability of ginsenoside Rg1 in rat serum was estimated at 1.5%.

Sulfuryl fluoride (SO2F2) is a structural and post-harvest fumigant used to control a wide variety of insect pests. SO2F2 is a key alternative to methyl bromide, a stratospheric ozone depleting substance scheduled for phaseout by the Montreal Protocol. A physiologically based pharmacokinetic (PBPK) model was recently developed which includes a quantitative description of the processes that regulate the kinetic disposition of SO2F2 in blood and target tissues in the adult rat after inhalation exposure. Our previous in vitro studies of hydrolysis rates in various tissues and cell fractions showed that blood was the primary tissue responsible for the hydrolysis of SO2F2. There was a greater than 80% decrease in SO2F2 levels that occurred in the presence of adult rat blood. In the current study, we show that in vitro hydrolysis of SO2F2 in adult rat blood leads to a stoichiometric increase in the metabolites fluoroiodosulfuryl fluoride (FSO2F) and fluoride. The secondary hydrolysis of FSO2F to fluoride was not found to occur in vitro with blood, liver or lung tissue from adult rats or human donors, at various substrate and protein concentrations when FSO2F was added directly to these tissues. This finding is consistent with the relatively slow in vivo hydrolysis of iv-dosed FSO2F. In vivo kinetic data indicate that the absorbed SO2F2 is rapidly hydrolyzed at the point of contact with respiratory tract tissues. The results of this in vitro study suggest that blood within the local respiratory tissues may be important for hydrolysis because no parent SO2F2 has been detected systemically following in vivo exposures up to 500 ppm. These in vitro and in vivo studies...
taken together, increase the understanding of the sites of hydrolysis of SO₂F₂ and help parameterize the PBPK model that will be used to calculate internal dosimetry of SO₂F₂, FSO₂, and fluoride at environmentally relevant concentrations.

**979 SINGLE INTRAVENOUS ADMINISTRATION TOXICOKINETIC STUDY OF Bis(2-Chloroethoxy)Methane Using Fischer 344 Rats and B6C3F1 Mice.**


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Bis-(2-chloroethoxy)methane (CEM), a colorless liquid halocarbon, is used as a starting compound in manufacturing processes to produce polyurethane elastomers. CEM does not occur naturally in the environment but can be released by volatilization during manufacturing and translocates through plants and animals. CEM is found in industrial waste of metal finishing, plastics, chemical manufacturing, and steam electric power industries. Groups of rats (12 to 14 weeks old) and mice (11 to 13 weeks old) were given a single IV administration of CEM in cremophor/ethanol/water (1:1:8 v/v/v) at a dosage of 20 or 40 mg/kg (rats) and 50 or 100 mg/kg (mice). Plasma CEM concentrations were measured using a validated gas chromatography method with selected ion monitoring. Plasma CEM concentrations were characterized using a two-compartment pharmacokinetic model. Cmax and AUC values increased in a dose proportional manner, from 12.4 to 24.1 μg/mL and 357 to 882 μg·h/mL in rats and from 38.2 to 65.1 μg/mL and 282 to 638 μg·h/mL in mice, respectively. CEM was rapidly eliminated without any dose dependence. The elimination half-life ranged from 16.3 to 25.4 minutes in rats, and 4.86 to 7.01 minutes in mice. There were no gender- or dose-related effects on clearance or volume of distribution. Clearance ranged from 45.4 to 64.1 mL/min/kg in rats and from 157 to 234 mL/min/kg in mice. The volume of distribution of the central compartment ranged from 1510 to 1660 mL/kg in rats and from 1310 to 1640 mL/kg in mice. The only species difference occurred in elimination of CEM where mice had a shorter elimination half-life and faster clearance than rats. There were no gender differences in either the rats or mice. The results of this study provided TK parameters for CEM in rats and mice to correlate toxicokinetic studies and to improve the usefulness of toxicity study results in risk assessment. [Supported by NIH, N01-ES-55551.]

**980 ANALYTICAL METHOD DEVELOPMENT FOR THE EXTRACTION AND ANALYSIS OF TRIS(CHLOROPROPYL)PHOSPHATE, MIXTURE, 1-CHLOROACETONE AND N-ACETYL-L-CYSTEINE.**


Midwest Research Institute, Kansas City, MO and National Toxicology Program, NIEHS, Research Triangle Park, NC.

Tris[chloropropyl]phosphate (TCP), a mixture of four isomers, is used in construction as a flame retardant in rigid polyurethane foam for flame retardant furnishings as a substitute for TCPP (a precursor in the synthesis of glutathione and may be affected by TCPP). The pres- ence of TCPP is also used in flame retardant furnishings. Midwest Research Institute is currently conducting analytical support to the National Toxicology Program on a study of TCP and 1-chloroacetone (a potential metabolite) in Wistar Han rats. Also being studied is N-acetyl-L-cysteine (NAC) which can be used by the body as a precursor in the synthesis of glutathione and may be affected by TCP. The present work includes the analytical method developed to extract and analyze for TCP, NAC and 1-chloroacetone from Wistar Han rat blood using Solid Phase Extraction. During the extraction, NAC is separated from TCP and 1-chloroace- tone. The eluent containing TCP and 1-chloroacetone was analyzed using gas chromatography/mass spectrometry (GC/MS). NAC analysis indicated that it formed disulfide bonds which needed to be reduced prior to analysis. The NAC eluent was analyzed using liquid chromatography/mass spectrometry. Because of its volatility, 1-chloroacetone required extensive method development to improve recovery. To facilitate this, in vivo studies were performed in saline solution. Recoveries for TCP were greater than 85 percent while recoveries for 1-chloroacetone were inconsistent. Multiple methods were examined to improve the recovery of 1-chloroacetone. The lower limit of detection was explored for each compound and the method was suit- able for the analysis of the analytes.

**981 PHARMACOKINETIC AND TISSUE DISTRIBUTION STUDY OF 14C-FLUASTERONE IN MALE BEAGLE DOGS.**


Toxicology Research Laboratory, Chicago, IL and National Cancer Institute, Bethesda, MD and Temple University, Philadelphia, PA and University of Maryland, Baltimore, Baltimore, MD.

The purpose of this work was to compare the pharmacokinetics (PK) and tissue distribution of 14C-fluasterone following intravenous (IV), subcutaneous (SC) and oral (PO) administration to male Beagle dogs. Fluasterone is a product of the syn- thesis of a derivative of didehydroepiandrosterone (DHEA). The route of administration may have a profound effect on the efficacy and side effects of a drug. Two groups of animal (6/dose) received an IV dose of 14C-fluasterone in Phase I of the study. In Phase II of the study, Group 1 received a single SC dose while Group 2 re- ceived a single PO dose. The 14C-fluasterone dose was followed by 25 days of non- labeled fluasterone administration (Group 1: SC; Group 2: PO). The PO 14C-flu- asterone formulation had a lower bioavailability (47%) compared to the SC formulation (84%). PO and SC administration resulted in similar times of maxi- mum concentrations (Timax); however, the Cmax following SC dose was less than half of the PO dose. The elimination half-life of the subcutaneous dose was nearly double that of the oral route (SC=41.38 hr; PO=24.38 hr), indicating a more sustain- able systemic level with the SC dose, which is also evident in the overall sys- temic exposure as seen in the AUC0-∞. Tissue distribution analysis 2 hours post in- travenous dosing showed that excretory organs (gall and urinary bladder, liver, kidney, and ureter) and adipose tissue accumulated high levels of 14C-fluasterone-derived radioactivity. The mean percentage of the total administered dose of 14C- fluasterone-derived radioactivity measured in the body was equal to ~81%. In con- clusion, the SC route of administration had greater exposure and more sustainable levels than the PO and IV routes, which is evident in the differences in systemic ex- posure, as well as rates of absorption and elimination. This was also evident in weight loss observed in the SC group, possibly as a result of an anti-glucocorticoid effect of the test article.

**982 BILIARY CLEARANCE AND ENTEROPHATIC CYCLING OF MYCOPHENOLIC ACID IN RATS.**


Toxicology, UNC-Chapel Hill, Chapel Hill, NC and School of Pharmacy, UNC-Chapel Hill, Chapel Hill, NC.

Mycofenolic acid (MPA) and its prodrug mycophenolate mofetil (MMF) are both used in the clinic as immunosuppressants and for prophylaxis of organ rejec- tion in transplant patients. Although MPA is generally well tolerated, 20% of treated patients experience delayed onset diarrhea resulting in cessation of MPA therapy leading to potential life threatening complications. MPM undergoes es- terase hydrolysis and subsequent metabolism through the actions of UGT enzymes within the liver to form the phenolic (MPAG) and acyl glucuronide conjugates (acMPAG) which are effluxed into the biliary tract or sinusoid. Hepatic efflux of these conjugates can result in either irreversible elimination through the urine or feces or bile flow within the intestinal lumen. Glucuronide cleavage and resorption of MPA back into the liver through enteroplastic cycling (EHC) can affect both the efficacy and toxicity of MPA. Male rats were dosed with 50 mg/kg MPM followed by bile cannulation and blood collection over 8 hours. ACN and suprofen internal standard were added to plasma samples followed by centrifugation, then the supernatant was dried and reconstituted. Bile aliquots were diluted and cen- trifuged followed by addition of suprofen internal standard. HPLC analysis was employed on a RP C18 column with UV detection at 250nm. MPA and MPAG levels were quantified and then analyzed to examine total and biliary clearance of MPA. Biliary clearance was not statistically different between PO (0.81 mL/min) and IV (1.10 mL/min) treated rats. Relative MPAG exposure levels (AUCMPAG(0-∞))/AUCMPA(0-∞) were lower for male rats compared to humans (ratio of 0.52 in rats and 4.76 in humans). Rats also extensively eliminate MPAG through the biliary tract (rat Feibel 0.64) and 18% of MPA exposure is derived through EHC in rats. The data obtained indicate that rats have similar metabolism, signifi- cant biliary excretion and EHC of MPA, as occurs in humans, thus the rat model is appropriate to employ for studying MPA toxicity. Supported in part by NIH training grant T32 ES007126.

**983 ZEBRAFISH: A PREDICTIVE MODEL FOR ASSESSING P-GLYCOPROTEIN INHIBITORS.**


Phylonix Pharmaceuticals, Inc., Cambridge, MA.

Major central nervous system disorders are refractory to small molecules (Pardridge 2007) and some drugs delivered by direct injection or by an implantable devices are pumped out by transporters such as Pgp. Inhibition of Pgp-dependent efflux is one
strategy for enhancing drug retention in the brain. Currently, there is no efficient in vivo model for assessing compound effect on Pgp-dependent efflux from the brain. Conventional methods including HPLC and microprobe/liquid chromatography, mouse brain uptake and rat microdialysis assays are time consuming and expensive (Wang et al., 1995; Fuss et al., 2003). Zebrafish has been shown to exhibit drug metabolism that is comparable to mammals (McGrath et al., 2008) and we recently developed a novel zebrafish bioassay to identify potential Pgp inhibitors. Since zebrafish brain structure is transparent during development, drug effects on dye retention can be assessed without complicating in vivo histology. In this study, we determined that Pgp is expressed at 5 days post fertilization (dpf) and is fully functional by 7 dpf. In order to monitor Pgp-dependent dye clearance kinetics, we injected rhodamine conjugated horseshadish peroxidase into the midbrain of zebrafish and quantified the fluorescence intensity using confocal microscopy. Our results demonstrate that zebrafish exhibit Pgp-dependent efflux transport machinery and effectively transfer xenobiotics from the brain to the blood. To validate this zebrafish model, we then assessed effects of 7 compounds shown to inhibit Pgp-dependent efflux in mammals. We confirmed that 6 positive control drugs (Verapamil, Phenytion, Loperamide, Cyclosporine, RU486, Quinidine, inhibited Pgp-dependent efflux in zebrafish brain; results for Alanine were inconclusive). Caffeine, a negative control drug, did not exhibit significant inhibitory effects. >95% correct prediction is considered excellent by ECVAM. The transparent zebrafish brain can serve as an excellent animal model for studying drug effects on ABC efflux transporters.

985 PHARMACOKINETIC INTERACTIONS BETWEEN BISPHENOL A AND NAPROXEN IN HUMANS.

S. Haddad, E. Blanchette, I. Rheault and M. Verner, sciences biologiques, TOKEN, Université du Québec à Montréal, Montréal, QC, Canada. Bisphenol A (BPA) was shown to disrupt endocrine signals in animals and human cell lines. The toxicity of this compound is limited by its rapid elimination through glucuronidation, a metabolic pathway leading to the production of an inactive metabolite. The pharmacokinetics of BPA is susceptible of being altered when co-exposure with pharmaceuticals occurs. Considering that the high inhibitory potential of naproxen on BPA metabolism in rat hepatocytes and hepatic microsomes, we aimed to (i) determine if BPA binding to human serum albumin (HSA) is altered by the presence of naproxen or other drugs (naproxen, salicylic acid, carbamazepine and valproic acid) at a concentration 10-fold higher than the reported maximal blood concentration (Cmax) in humans, and (ii) characterize metabolic interactions between BPA and naproxen in human microsomes. Displacement assays of BPA-HSA using equilibrium dialysis showed that naproxen, salicylic acid and salicylic acid can significantly lower BPA binding to HSA. Further assessment of naproxen inhibition of BPA-HSA binding showed that the reported Cmax of naproxen in humans (374 μM) yielded a 20% increase in BPA free fraction. Human microsomal assays were carried out to determine the type of inhibition and related constants for naproxen inhibition potential on BPA glucuronidation. Naproxen was shown to inhibit BPA glucuronidation non-competitively with a Ki of 293 μM, a concentration below the reported Cmax in humans. The results of this study suggest that administration of drugs such as naproxen might interfere with BPA glucuronidation and increase its free fraction in blood. These findings promote further investigation to (i) characterize the displacement constant of naproxen on BPA binding to human albumin, and (ii) assess the impact of observed interactions on the risk assessment of mediated population through physiologically-based pharmacokinetic (PBPK) modeling of BPA in human. Supported by NSERC.

986 PHARMACOKINETIC (PK) ANALYSIS AND OPTIMAL SAMPLING STRATEGY (OSS) FOR A RECOMBINANT PROTEIN (ENB-0040) IN ADULT AND JUVENILE RATS.

M. Beliveau1, J. Lemire2, M. Reimer1, J. Marier1 and P. Leonard1. 1Pharsight Corporation, Montreal, QC, Canada and 2Northern Biomedical Research, Muskegon, MI. The pharmacokinetics of ISIS 335611, a 2’-O-methoxyethyl phosphorothioate (2’-MOE) antisense oligonucleotide to human SOF-1, was evaluated in Rhesus monkeys after continuous intravenous (i.v.) infusion and in rats showing that OA blocks CAR upregulation of cytochrome P450 2B in rodent liver. In mouse brain capillaries, p-gp, Mrp2 and BCRP, are selective components of this barrier. ATP-binding cassette (ABC) efflux transporters play a fundamental role in the delivery of CNS-active drugs and thus to treatment of brain diseases. ATP-binding cassette (ABC) efflux transporters play a fundamental role in the delivery of CNS-active drugs and thus to treatment of brain diseases. ATP-binding cassette (ABC) efflux transporters play a fundamental role in the delivery of CNS-active drugs and thus to treatment of brain diseases. ATP-binding cassette (ABC) efflux transporters play a fundamental role in the delivery of CNS-active drugs and thus to treatment of brain diseases. ATP-binding cassette (ABC) efflux transporters play a fundamental role in the delivery of CNS-active drugs and thus to treatment of brain diseases. ATP-binding cassette (ABC) efflux transporters play a fundamental role in the delivery of CNS-active drugs and thus to treatment of brain diseases. ATP-binding cassette (ABC) efflux transporters play a fundamental role in the delivery of CNS-active drugs and thus to treatment of brain diseases. ATP-binding cassette (ABC) efflux transporters play a fundamental role in the delivery of CNS-active drugs and thus to treatment of brain diseases. ATP-binding cassette (ABC) efflux transporters play a fundamental role in the delivery of CNS-active drugs and thus to treatment of brain diseases. The primary objective of this study was to derive a quantitative method for determining the minimum number and timing of blood draws to assess exposure (Cmax and AUC) of ENB-0040 in juvenile rats in a toxicology study. Using knowledge of PK parameters derived from prior rat studies, a two-compartment population model with a maturation function as a covariate (to account for changes in body weight over time) was developed using NONMEM® (v6.2). Following model development, an OSS was derived that took into consideration: 1) the body weight of juvenile rats, 2) a maximum of 1 blood draw for PK assessment on Day 1 of the planned study, and 3) additional blood draws beginning at 4 weeks for the more mature rats. Using partial derivatives from PK model equations developed in WinNonlin® (v5.2), the 0.08-, 0.5-, 1- and 5-hour time points were identified as potential optimal sampling times. The predictive performance of the PK model was then validated by comparing the PK of ENB-0040 evaluated from a rich simulated dataset to a sparse simulated dataset for a number of candidate scenarios (e.g. 9 rats all sampled at the same time point (0.08, 0.5, 1, or 5 h post dose) or 3 rats sampled at 3 time points (0.5, 1 and 5 h post dose or 0.08, 0.5 and 1 h post dose) on Day 1). Precision and bias on Cmax, AUC and effective half-life (t1/2eff) were calculated to determine the robustness of the model according to the proposed OSS after single dose or multiple doses. The best overall precision and bias resulted from sampling at 0.5 h (~9%, %MPE and %RMSE for AUC was 2% and 25%, respectively) and 0.08, 0.5 and 1 h post dose (n=3, %MPE and %RMSE for AUC was 4% and 26%, respectively). This study provides a quantitative approach for deriving optimal sampling strategies in technically challenging and sample-limited toxicology studies.

987 REGULATION OF EFFLUX TRANSPORTERS AT THE BLOOD-BRAIN BARRIER (BBB) BY XENOBIOTICS.

X. Wang, D. Sykes and D. S. Miller, Laboratory of Pharmacology and Chemistry, National Institute of Environmental Health Sciences, Research Triangle Park, NC. The BBB, which resides within the brain capillary endothelium, is a major obstacle to the delivery of CNS-active drugs and thus to treatment of brain diseases. ATP-binding cassette (ABC) efflux transporters, such as p-glycoprotein (p-gp), play critical roles in determining the systemic distribution of drugs after their absorption. The resistance-associated protein 2 (Mrp2) and breast cancer resistance protein (BCRP), are selective components of this barrier. In other barrier and excretory tissues efflux transporter expression is under control of xenobiotic-activated nuclear receptors, e.g., pregnane X receptor (PXR) and constitutive androstane receptor (CAR). Here we identify CAR as a regulator of p-gp, Mrp2 and BCRP expression in rat and mouse BBB. Freshly isolated brain capillaries were used to investigate CAR-mediated modulation of drug efflux transporter function. Capillaries were incubated with fluorescent substrates specific for each transporter, imaged with confocal microscopy and luminal substrate accumulation quantitated by digital image analysis. Three hour exposure rat brain capillaries to the CAR ligand, phenobarbital (PB), increased p-gp-mediated transport. This induction was abolished by the protein phosphatase 2A inhibitor, okadaic acid (OA at 2nM), a finding consistent with previous reports showing that OA blocks CAR upregulation of cytochrome P450 2B2 in rodent liver. Western blot analysis confirmed increased expression of p-gp, Mrp2 and BCRP in rat brain capillaries treated with PB. In mouse brain capillaries, p-g. Mrp2 and BCRP transport activity was increased by in vitro exposure to the specific CAR agonist TCPOBOP (50-500nM). No such increases were found in capillaries from CAR knockout mice. Finally, TCPOBOP dosing of mice (0.3mg/kg) increased p-gp, Mrp2 and BCRP mediated transport in brain capillaries assayed ex vivo. Thus, CAR ligands increase transport activity and expression of p-gp, Mrp2 and BCRP. This is another a molecular signaling pathway through which the selective BBB is tightened. Exposure to xenobiotics that activate CAR (e.g. antiepileptic drugs) could contribute to pharmacoresistance in clinic.
IS THERE A FUTURE FOR ANIMAL MODELS IN THE INVESTIGATION OF IDIOSYNCRATIC DILI IN HUMANS?

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Idiosyncratic drug induced liver injury (DILI) remains a concern for drug development and patient safety, and continues to be the leading cause for regulatory action including withdrawal of drugs from the market, restrictions in indications, and warnings to health care providers and patients. Idiosyncratic DILI is typically unpredictable, and is largely undetectable, in preclinical animal studies. Accordingly, it is only in large scale clinical trials or, more often, post-marketing, that such toxicities are revealed. To date there is no universally accepted animal model for investigation or prediction of idiosyncratic DILI in humans. A lofty goal for preclinical scientists and physicians is to, not only develop animal models that detect idiosyncratic DILI, but to translate such knowledge into the clinic and allow identification of patients who would be susceptible or tolerant to a particular drug. Much effort has been expended in the study of improved/enhanced animal models that better predict untoward human idiosyncratic drug reactions. In some instances, the data imply that we can in fact identify drug candidates that may be predisposed to DILI. On the other hand, the multiple etiologies potentially underlying idiosyncratic DILI may render it difficult to routinely evaluate for, and predict, these events. A case example will be presented to illustrate this point. The complexities of using non-standard animal models and endpoints in regulatory safety assessment studies also must be considered. These discussions are designed to highlight the current thinking on animal models for idiosyncratic DILI, and focus attention on the future direction of such research.

NATIONAL CHILDREN'S STUDY: OPPORTUNITIES AND CHALLENGES FOR TOXICOLOGISTS.

S. P. Darney, ORD, U.S. EPA, Research Triangle Park, NC.

The National Children’s Study (NCS) is the first-of-its-kind U.S. study tracking children’s health from womb to adulthood. Involving 100,000 children across the country, the NCS will be the largest long-term study of children’s health and development ever conducted in the U.S.. Initiated in response to the Children's Health Act of 2000, the NCS is led by a consortium of agencies which include the U.S. Department of Health and Human Services, including the National Institute of Child Health and Human Development, the National Institute of Environmental Health Sciences at NIH, and the Centers for Disease Control and Prevention, and the U.S. EPA. The study seeks to address questions that can only be answered through a longitudinal study of this size and scope. The study’s hypotheses incorporate the following main outcomes — pregnancy outcomes, neurodevelopment and behavior, asthma, obesity and growth, injury, and reproductive development. NCS will ultimately reduce the public health burden of chronic childhood diseases and disorders, including not only pain and suffering, but also missed school days, health care expenses, and other costs to children, their families, and society at large. It will also be large enough to assess factors related to health disparities and differences in disease occurrence between groups of people. What does this study mean for toxicologists? The time is ideal to obtain input from toxicologists, as this landmark study is beginning to recruit families, to apply lessons learned from ongoing children’s studies, including the application and interpretation of new biomarkers, and to develop exposure and dose models for pregnant women and infants. Its design and innovative approaches will provide unique opportunities to collect and correlate early biomarkers of exposure and effect to disease outcomes later in development, and it will help the U.S. tease apart the complex interplay between environmental factors and genetic influences that impact health.

SETTING A SAFE STARTING DOSE IN INITIAL CLINICAL TRIALS WITH BIOTHERAPEUTICS: DO I USE THE NOAEL OR THE MABEL?


Years ago, establishment of safe exposures to new therapeutics utilized the determination of NOEL/NOAELs derived from routine toxicology studies followed by some form of dose adjustment prior to human exposure, an approach that evolved into the NOAEL and is the most commonly used to date. More recently, approaches to clinical trial design have become more specific to the TA and nation of NOEL/NOAELs derived from routine toxicology studies followed by an evaluation that depends on a combined assessment of the nature of the target, pharmacology, toxicology, and the intended patient population. Accurate estimation of the human efficacious dose is also critical and the complexity surrounding this question can have significant impact on the testing strategy. Low and slow provides maximum safety but can also result in unnecessary time delays evaluating doses where little information may be gained, and in at least some instances may make it impossible to recruit patients who may have no benefit for weeks.

THE FUTURE OF ENVIRONMENTAL HEALTH SCIENCE: FEATURING NIH FUNDED EARLY CAREER INVESTIGATORS.

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An essential element of the mission of the NIH is the support and career promotion of the future generation of exceptionally talented and creative new scientists who will push forward research in understanding the impact of environmental exposures and human health. Support through critical transitional stages has been identified as being particularly important in developing a cadre of talented early career scientists. In response, the NIH and the NIEHS has started the Outstanding New Environmental Scientist (ONES) Award which is one of many initiatives that it has taken to provide the funding for the research and career enhancement of scientists during the transition from postdoctoral to faculty positions, and to allow selected outstanding junior faculty to flourish. The ONES scientists are expected to make a long-term commitment to environmental health sciences, and to bring innovative, ground-breaking research thinking to bear on understanding how environmental exposures affect human biology, human pathophysiology, and human disease. In the first three years, 21 awards have been made and the NIH’s ONES program has become an important showcase for the future leaders in environmental health sciences. Research. Three ONES awardees have been chosen to present who have had innovative publications in the first year of the award and who display a broad spectrum of research in the environmental health sciences. These exceptional scientists, who will present cutting edge science at the interface of toxicology and environmental health sciences, are a model for junior faculty attendees who are considering applying for these competitive but highly rewarding grants.

NITROGEN-CONTAINING BISPHOSPHONATES INHIBIT FARNESYL DIPHOSPHATE (FPP) SYNTHASE IN THE KIDNEY: IMPLICATIONS FOR RENAL SAFETY.


Bisphosphonates are potent inhibitors of osteoclast-mediated bone resorption and play an important role in the treatment of osteoporosis, metastatic bone disease, and Paget disease. nephrotoxicity has been reported with some bisphosphonates. Nitrogen-containing bisphosphonates directly inhibit farnesyl diphosphate (FPP) synthase activity (mevalonate pathway) and reduce protein precipitation leading to osteoclast cell death. The aim here was to elucidate if this inhibition also occurs in kidney cells and may directly account for nephrotoxicity. In an exploratory study in rats receiving zoledronate or ibandronate an approximate 2-fold increase in FPP synthase mRNA levels was observed in the kidney. The involvement of the mevalonate pathway was confirmed in subsequent in vitro studies with zoledronate, ibandronate, and pamidronate, using the non-nitrogen-containing bisphosphate clodronate as a comparator. In vitro changes in FPP synthase mRNA expression correlated with high protein precipitation levels of prenylated proteins. Using two cell lines (a rat normal kidney cell line, NRK-52E, and a human kidney proximal tubule cell line, HK-2), ibandronate and zoledronate were identified as most cytotoxic (EC50: 23 μM >1000 μM and 16 μM, respectively) and as the most potent inhibitors of FPP synthase (Ki >1.0 μM and 0.5/0.7 μM, respectively). In both cell lines, inhibition of FPP synthase activity occurred prior to a decrease in levels of prenylated proteins followed by cytotoxicity. This further supports that the mechanism responsible for osteoclast inhibition (therapeutic effect) might also underlie the mechanism of nephrotoxicity.
TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is a highly toxic, widespread environmental contaminant. The goal of this project is to use functional genomic and bioinformatic methods to identify molecular biomarkers as indicators of the impact of TCDD exposure. Specifically we investigated the effects of chronic TCDD exposure on developing rainbow trout global gene expression analyzed using microarrays. Juvenile rainbow trout were fed diet containing TCDD at 0, 0.1, 1, 10 and 100ppb, and fish were sampled from each group after 1, 2, 4, and 6 weeks. Gene expression analysis was performed using the cGRASP 16k cDNA microarray. At the end of six weeks, significant mortality was not seen in treatment groups below 10 ppb TCDD, but the 100 ppb diet was 100% lethal by day 40. Condition factor was not affected by the TCDD exposure. A number of genes were dysregulated by dietary TCDD that function in cell division, cell cycle progression, neuronal function and transmission, retinal phototransduction, steroid metabolism, skeletal development, ion binding and homeostasis, stress response, transcriptional regulation and proteolysis. Histological analysis revealed that by 6 and 12 weeks significant lesions were found in the liver, kidney, gills, and heart, and caused a delay in the development of the skull, ovary, and brain. TCDD has dramatic effects in juvenile rainbow trout well below lethal levels by disrupting molecular pathways involved in the growth and development of multiple organ systems. These dysregulated genes may be useful as biomarkers for TCDD exposure in aquatic environment to assess the potential impacts of low-level environmental TCDD in the food chain and on the health of humans and animals.

1H-NMR and LC/MS based metabonomics were applied to urine samples from a toxicology project employing rats. For early detection of toxicity and improved mechanistic understanding, GC/MS, 1H-NMR and LC/MS data discriminated controls from animals dosed with 210 μg/kg bw OTA as early as 14 days and animals dosed with 70 μg/kg bw 28 days after start of treatment, correlating with mild histopathological changes in the kidney. Integration of histopathology scores as discriminators in OPLS-DA models resulted in better model prediction and facilitated marker identification. Decreased 2-oxoglutarate and citrate and increased glucose, pseudouridine, 5-oxoproline and myo-inositol excretion was detected with GC/MS. Decreased 2-oxoglutarate and citrate and increased taurine excretion was found with NMR. Some markers are well-established markers of kidney damage (glucose and creatinine) and indicate disturbances in mitochondrial energy production (citrate and 2-oxoglutarate). Others are associated with cell proliferation (pseudouridine), changes in renal osmolyte handling (myo-inositol and taurine), and oxidative stress (5-oxoproline), established mechanisms of OTA-toxicity. LC/MS was also able to discriminate controls and treated animals but contained more noise and marker annotation was often speculative due to lack of reference databases. Use of multiple analytical platforms for metabonomics analysis may result in a more comprehensive metabolic coverage and may be applied to obtain mechanistic information from conventional rodent toxicity studies.
rats) are similar to those of other rat strains fed with this compound (Han:Rc/Wist, Crl:CD® and F344/Crl). The results indicate that (1) the patterns are sufficiently similar to identify the MCPA treatments for rats of all four strains as the most likely treatment in a data base containing studies with more than 400 other compounds and (2) that the greatest similarity is between the rats of both Wistar strains (Crl:Wt(Han) and Han:Rc/Wist) compared to the others. In conclusion, metabolomics is a new and promising tool for the (early) identification and characterization of toxicological modes of action and can be applied to develop early markers of (chemically induced) disease.

A novel extension of the pathway activity methodology was applied to microarray data to develop a method of identifying pathways affected after toxic chemical exposure over time rather than first identifying differentially expressed genes (DEGs). This approach was tested within the context of identifying pathways affected after in utero exposure to Di-n-buty phthalate (DBP). Transcriptional studies in rats after in utero exposure to DBP lead to a repression of genes involved in cholesterol transport, steroid synthesis, in turn leading to a decrease in fetal testicular testosterone. The analysis of microarray data from the rat testis was applied on individual genes whose expression changed statistically.

We developed an alternative approach focusing on the genes by analyzing the microarray data for pathways that are significantly altered. The pathway activity level (PAL) analysis entails a singular value decomposition (SVD) of the expression data of the genes constituting a given pathway. The PAL is therefore the weighted sum of gene expression of individual genes in the given pathway and represents the change of all gene expressions in a given pathway regardless of specifying up or down regulation. The microarray study has both control and treated profiles over time which enables us to include the temporal changes of control profiles into the analysis. This novel methodology led to the identification of pathways that were consistent with the published literature and pathways that were not identified in the published literature. We were able to monitor the temporal evaluation of pathway behaviors, which allowed for the development of a regulatory network model for DBP. 

### TEMPORAL PATHWAY ACTIVITY ANALYSIS OF TRANSCRIPTIONAL PROFILING OF IN UTERO EXPOSURE TO D-N-BUTYL PHthalate (DBP).

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A new extension of the pathway activity methodology was applied to microarray data to develop a method of identifying pathways affected after toxic chemical exposure over time rather than first identifying differentially expressed genes (DEGs). This approach was tested within the context of identifying pathways affected after in utero exposure to Di-n-buty phthalate (DBP). Transcriptional studies in rats after in utero exposure to DBP lead to a repression of genes involved in cholesterol transport, steroid synthesis, in turn leading to a decrease in fetal testicular testosterone.
1002 DEVELOPMENT OF A DNA MICROARRAY TO EVALUATE GENE EXPRESSION PROFILES OF DRUG-METABOLIZING GENES IN THE CYMONOLUS MONKEY.

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Drug-metabolizing enzymes impact the efficacy and adverse effects of drugs under development. Currently, there is limited information on the molecular mechanisms of drug metabolism in the cymonolus monkey (Macaca fascicularis) which is utilized extensively in preclinical studies. To address this issue, we produced a DNA microarray that includes 43 probes specific for metabolic enzymes in the cymonolus monkey. We evaluated the ability of the DNA microarray to detect tissue-specific metabolic enzyme gene expression and compared microarray results for five key metabolic enzymes with real-time RT-PCR results. We measured gene expression profiles of the liver, intestinal tract, and kidney for adult five male cymonolus monkeys. In the liver, CYP2C76, 2E1, 2A23, 3A8, and 2C20 were highly expressed. In the intestinal tract, we found that although the genes expressed were similar, the expression level of these genes was greater in the jejunum than the ileum. In the kidney, expression profiles were similar in the cortex and medulla. Thus, the DNA microarray successfully detected tissue-specific gene expression. Results from DNA microarray and real-time RT-PCR produced similar patterns, indicating the detection of CYP2C24, 2C75, 2D17, 3A45, 3A48: all genes with human homologs that frequently influence xenobiotic metabolism. Overall, we have shown that this DNA microarray reliably measures metabolic enzyme genes expression in the cymonolus monkey. Future work with this DNA microarray could focus on identifying metabolic enzyme expression patterns for species-specific drug metabolism. An enhanced understanding of metabolic enzyme activity in cymonolus monkeys can help to determine the extent to which animal toxicity data can be used to predict drug toxicity in humans. In conclusion, we developed a reliable DNA microarray that can contribute to a more thorough evaluation of efficacy and safety in new drug development.

1003 ANALYSIS OF SECRETED PROTEINS AS AN IN VITRO MODEL FOR DISCOVERY OF LIVER TOXICITY MARKERS.

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Despite the wealth of sequence data and new technologies that can probe large portions of the transcriptome or proteome in a single experiment, attempts to identify human biomarkers of toxicity have met with limited success. We have developed an in vitro system using the hepatoma-derived cell line, HepG2/C3A, to model protein secretion by hepatocytes in response to toxic insult. Using quantitative proteomics we are able to characterize qualitative and quantitative changes in the abundance of secreted proteins that are likely to be detectable in blood samples of exposed animals. In a proof of concept experiment, conditioned medium from cells exposed to ethanol, after immunodepletion of abundant proteins, was subjected to quantitative mass spectral analysis. Nearly 250 proteins were identified with almost half changing in abundance. A large portion of these was only identified in the highest dose and is presumably a result of tissue leakage due to disruption of the cell membrane upon cell death. However, a small number was also identified which represent known effects of ethanol toxicity or alcoholism. This approach shows promise as a targeted system for identification of human biomarkers for liver damage. Disclaimer: Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army. The research described herein was sponsored by the U.S. Army Medical Research and Materiel Command, Military Operational Medicine Research Program.

1004 PREDICTION OF LIVER TOXICITY – AN IN VITRO TOXICONGENOMICS APPROACH.


Prediction of organ toxicity early in the compound selection process would help to decrease attrition in later development. Therefore, it is necessary to develop tools to predict liver toxicity in rat using genomics data obtained from cultured rat hepatocytes. Method: Proprietary and non-proprietary compounds (56) were selected to build a database. The compounds were well-balanced distributed to represent rat liver toxins (divided into phenotypic subgroups termed “steatosis”, “cholestasis”, “direct acting” and “PPAR agonists”) and compounds showing no liver toxicity in rats in vivo. Primary rat hepatocytes (batch size: 5) were treated with test compound concentrations 2 to 4 times below the determined cytotoxic concentration. After exposure for 24 hours microarray analysis was performed. Gene expression changes relative to vehicle treatment form the same series were calculated and used to train classifiers based on a support vector machine (SVM) algorithm. The obtained SVM models were evaluated using a compound drop cross validation. Results: Treatment with PPAR ligands could be identified with almost perfect accuracy. For the other compound classes, a 3-class model distinguishing between the classes “non-toxic”, “direct acting” and “cholestatic or steatoctic” compounds performed best (overall accuracy: 69%). Differentiation of the “cholestatic/steatoctic” compounds from the rest worked better than differentiation of either the “direct acting” or “non-toxic” compounds from the rest. We suggest two main reasons that influence the performance of the presented approach: 1) The heterogeneity of toxicity mechanism of the compounds in the “direct acting” group and 2) the concentration selection approach, which - on purpose - disregarded the pharmacologically- or toxicologically-relevant in vivo plasma compound concentrations. Conclusion: Prediction of in vivo toxicity from in vitro gene expression data is possible for compounds of distinct phenotypic subgroups or with well defined modes of action.

1005 ESTABLISHING A PROFILE OF GENE EXPRESSION IN AVIAN SPECIES EXPOSED TO PCBs.

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Genotoxic effects of exposure to endocrine disruptors are a hazard of environmental pollution. Issues of concern include changes in gene expression associated with developmental, reproductive, and endocrine system, and other alterations. We are endeavoring to establish a gene expression profile in birds in response to PCBs using microarray technology. With the power to measure the expression levels of an entire genome at once, microarray technology presents itself as an excellent candidate for detecting patterns of gene expression. Using Japanese quail (Coturnix coturnix) as a lab model, we injected eggs on the third day of incubation with a vehicle control or a PCB mixture (6, 12, and 49 μg/g egg) and sampled organs at hatch. RNA was extracted from hatching livers and applied to the Del_Mar Chicken Integrated Systems Microarray. Validation showed therefore a 90% correspondence between quail liver samples and the chicken microarray, 75% correspondence in quail hypothalamus, as well as 60-85% correspondence in turkey and duck livers and hypothalamis, and tree swallow and American kestral livers. Further application of quail samples to the microarray revealed a number of candidate genes, including cytochrome P450 1A5 (CYP1A5), which was expressed greater than control in 6 μg/g and 49 μg/g-injected eggs; and mitochondrial ribosomal protein S16, which was expressed 3-fold under control in 6 μg/g injected eggs, but only 1.24-fold under control in 12 μg/g-injected eggs. Other genes that have shown differential expression in the microarray experiments include ornithine decarboxylase antizyme, signal transducer and activator of transcription (STAT) 6, and NADH Dehydrogenase (Ubiquinone) Flavoprotein 1, among others. These genes may be used as toxicogenomic markers for hatching birds exposed to PCBs. Further examination of the microarray study reveals several biological processes for further genotoxicological screening, including the immune system, pathways relating to oxidative stress, as well as regulatory pathways for regulation of gene expression.

1006 TRANSCRIPTOMIC COMPARISON OF TCDD-ELICITED GLOBAL GENE EXPRESSION RESPONSES IN HUMAN PRIMARY HEPATOCYTES.

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a ubiquitous environmental contaminant that causes physiological and toxic effects in a species-specific manner. In order to further elucidate these differences, a comparative genomic approach was utilized to examine species-specific differential gene expression elicited by TCDD. Primary human hepatocytes isolated from non-smoking Caucasian donors were used to examine TCDD-elicited temporal gene expression responses. Gene expression changes were measured using Agilent whole genome oligonucleotide microarrays following treatment with 10 nM TCDD or vehicle control (DMSO) for 1, 2, 4, 12 and 48 hrs. Using Empirical Bayes p(1)
values (>0.80) and fold change (>1.5). 447 differentially expressed genes were identified with responses ranging from +40.1- to -5.5-fold. This included the induction of CYP1A1 and CYP1A2, while other notable TCDD responsive genes, such as ALDH3A1 and NQO1 were not statistically significant due to the inherent variability between human donors. Hepatocyte responses were compared to the top 500 ranked genes from in vitro human HepG2 and mouse Hepa1c17 gene expression data sets, as well as to the top ranked hepatic responses from immature ovariectomized female C57BL/6 mice treated with 30 μg/kg TCDD. These comparisons identified 35, 21 and 15 orthologous gene responses that were common between the HepG2, Hepa1c17 and C57BL/6 hepatic tissue, respectively. Therefore, despite the conservation of the AhR signaling pathway, there are significant differences in the subsequent gene expression profiles between species, which likely contribute to the species-specific toxic effects of TCDD. Supported by SBRP P42ES094911.

1007 ANALYSIS OF PHASES 1 AND 2 METABOLIZING ENZYMES IN HUMAN SKIN SUGGESTS IMPORTANT ROLE OF PHASE 2 ENZYMES IN THE DETOXIFICATION.

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Skin plays a role of physical barrier and protects the deeper tissues from injury, excess water loss and invasion by foreign substances and organisms. It also expresses a certain number of detoxification enzymes to help dealing with environmental chemicals. To gain a better knowledge about these chemical protection enzymes in the skin, we quantified mRNA expression levels of multiple enzymes involved in phases 1 and 2 metabolism using Affymetrix GeneChip and RealTime PCR in total skin and epidermis. There is a good correlation between these two methods of quantification. Among phase 1 enzymes, alcohol dehydrogenase 1B (ADH1B) is expressed at very high levels (approximately 400 000 copies/µg total RNA), while CYP450s are expressed at approximately 10-fold lower. The latter represent mostly isozymes involved in hydroxylation of endogenous compounds such as retinoids (CYP26B1), prostanoids (CYP4F12, arachidonic acid (CYP2J2) and cholesterol (CYP51A1). On the other hand, most of phase 2 enzymes, namely, glutathione-s-transferase P1 (GSTP1), catechol-O-methyl transferase (COMT), steroid sulfotransferase (SUSTR2B1) are highly expressed with an expression level varying from 200 000 to 1 000 000 copies/µg total RNA. It is noteworthy that 10 000 - 20 000 copies/µg total RNA represent 1 copie/cell. Our data suggest that, in contrast with detoxification in the liver which involves many CYP450 isofoms, in the skin, detoxifying process uses mostly phase 2 enzymes, while CYP450s are rather involved in the elimination of endogenous compounds.

1008 LOW-DOSE MODULATION OF EMBRYONIC GENE EXPRESSION NETWORKS BY INORGANIC ARSENIC.

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Inorganic arsenic (arsenite, As3+) is an environmentally ubiquitous contaminant that presents a serious health risk to populations exposed primarily via drinking water. Associated risks include, increased incidence of certain cancers, diabetes, and high blood pressure. The biochemical processes triggered during arsenic detoxification are well characterized yet the effects of arsenic on development, modulation of gene expression, disease origin and the potential for long-term trans-generational effects are poorly understood. A large-scale microarray analysis of zebrafish embryos exposed to very low (10 ppb) and moderately high (100 ppb) As3+ concentrations during the early-phase (24 hours post-fertilization; hpf) and late-phase periods of development (48 hpf) was performed in order to determine the effect low-level arsenic exposure has on the expression profiles of embryonically active gene networks. In addition to the occurrence of pleiotropic developmental abnormalities concentrated in the area of anterior-posterior axis development, arsenic elicited global changes in gene expression. Non-parametric statistical analysis of the microarray data revealed 5484 genes with significantly affected expression by a different mechanism compared to higher concentrations—one wherein stochasticity, and not necessarily dose, determines whether an embryo will undergo observable changes in gene expression. Several arsenic-perturbed networks were identified and expression changes were validated by quantitative RT-PCR. The results of this study may have uncovered a novel mechanism by which low levels of arsenic could lead to potentially detrimental effects during embryonic development.

1009 AN INTEGRATIVE STATISTICAL FRAMEWORK FOR THE METABOLIC ANALYSIS OF NON-GENOTOXIC HEPATOCARCINOGENESIS IN THE RAT.

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Metabolomics makes use of high throughput global profiling technologies, including nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS), to profile the metabolic compliment of a cell, tissue or biofluid. The informatic tools for the integration of data from such global methods significantly lags behind the rapid progress made in analytical techniques. We have applied modern statistical and signal processing techniques in order to improve information recovery from large heterogeneous datasets. Data was obtained from male F344 rats continuously exposed to ten compounds for up to 91 days. The chemicals were selected from the National Toxicology Program database to provide examples of both genotoxic and non-genotoxic carcinogens and hepatotoxic non-carcinogens. Metabolomics was performed on liver, blood plasma and urine along with transcriptomics and histopathology of liver tissue. Applying Bayesian statistics and Markov Chain Monte Carlo (MCMC) methods for the automated processing of NMR signals, datasets were produced from the NMR spectra that represented individual resor- nance fragments inclusive of noise. This approach significantly improved classification according to the treatment group and aided interpretation of the biological changes. An ANOVA based approach was applied to the resultant metabolomic data to cross-correlate the information with transcriptomics, Gas Chromatography MS based metabolomics, clinical chemistry and histopathology. This approach has highlighted key mechanisms associated with the mode of action of the hepatocarcino- gens used in the study. While the data set was dominated by the effects of PPAR agonists on fatty acid metabolism, classification embedded non-genotoxic and non-genotoxic carcinogens, demonstrating the potential of distinct mechanisms of response between these two classes of compounds.

1010 FUNCTIONAL ROLE FOR CLASS A SCAVENGER RECEPTORS IN THE RECOGNITION OF ENGINEERED NANOPARTICLES BY MACROPHAGES.


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The initial mechanism of cell entry is a potentially important parameter that determines how cells respond to engineered nanomaterials. Previous studies have implicated class A scavenger receptors in mediating some macrophage effects induced by larger (micron size) environmental particulates, including metal oxides. However, little is known about the role these receptors play in mediating uptake and responses to nano-scale (<100 nm) materials. We hypothesize that anionic nanoparticles serve as ligands for scavenger receptor A (SR-A) and contribute to the inflammatory signaling in macrophages. Stable RAW 264.7 cell mutants where expression of the major class A receptors, SR-A/II, was silenced were developed using lentiviral siRNA transduction. Flow cytometry analysis demonstrated that SR-A/II was functionally inhibited by siRNA, as indicated by reduced endocytosis of fluorescent acetylated low density lipoprotein. Cellular uptake of 20 nm fluorescent polystyrene nanoparticles was also reduced in SR-A/II mutants compared to wildtype cells. In contrast, gain of function experiments in human cells devoid of endoge- nous SR-A/II protein demonstrated uptake of 20nm nanoparticles was signific- antly augmented when SR-AI expression was introduced. Using live cell microscopy, fluorescent silica nanoparticles were found in vesicular structures and co-localized with transferrin receptor, a marker of clathrin-dependent endocytosis. Protein microarray assays also showed that the pattern of cytokines secreted in response to amorphous silica nanoparticle treatment was altered in the SR-AI siRNA mutants compared to wildtype RAW 264.7 cells, indicating a functional role for SR-A in macrophage signaling. These results provide direct evidence that SR-AI constitutes at least one pathway for the internalization of anionic nanoparticles in macrophages. A molecular understanding of these interactions will aid in future design of safe nanomaterials. Supported by the Laboratory Research and Development Program at PNNL and R01 ES016212 to BDT.

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1011 ABSENCE OF AN ENDOTHELIAL CELL TRANSCRIPTOMIC SIGNATURE IN THE PERIPHERAL BLOOD AFTER ADMINISTRATION OF THREE ONCOLOGY TUBULIN BINDERS.

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Transcriptomic analysis, histopathology and immunohistochemistry identified acute endothelial damage and apoptosis in the heart of rats following intra-venous administration of oncology tubulin binders. Therefore, the transcriptomic profile of the peripheral blood may transiently be enriched in endothelial cell markers subsequent to the administration of tubulin binders. In this study, the transcriptomic profile of the whole peripheral blood collected in PaxgeneTM tubes was evaluated in rats 6 and 24 hours after intravenous dosing with the 3 tubulin binders vincristine, vinblastine and colchicine. All three compounds induced an increase in apoptotic cells and a mitotic arrest in the heart, as well as hematologic changes, including a decrease in platelet, lymphocyte, monocyte and eosinophil counts with an increase in neutrophil counts. There was no enrichment of endothelial cell-associated genes in the whole peripheral blood. Possible causes for this result include inadequate collection times, dilution of the transcriptomic signature of endothelial cell-specific transcripts, decreased expression of endothelial cell-specific genes in apoptotic endothelial cells, or rapid clearance of damaged of endothelial cells by the reticulo-endothelial system. The transcriptomic profile correlated well with the hematologic changes, except for the decrease in eosinophil counts, possibly because of the relative scarcity of these cells in the peripheral blood. Protein- and cell-based technologies may provide better inroads for the identification of novel biomarkers of endothelial damage.

1012 COMBINED METABONOMIC/TRANSCRIPTOMIC EVALUATION OF THE EFFECTS OF OVERNIGHT (16HR) FASTING ON SD RATS.

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The overnight fast prior to necropsy or blood collection is a routine procedure in many toxicology studies. With ‘omics’ evaluations becoming more common in these routine studies, it became apparent that the effect of fasting on transcript and metabolite profiles, relative to ad lib feeding prior to sample collection, required evaluation. Male and female Sprague-Dawley rats (5/group) were fasted for 16 h hours and effects of fasting on the liver transcriptome, urine and serum metabolic profiles and clinical pathology parameters were determined relative to rats maintained on an ad lib diet. Fasted rats lost -9% and 7% bodyweight in males and females, respectively while their ad lib counterparts gained -3 and 4%, respectively. Urine volumes and serum phosphorus were significantly increased in fasted animals while urine osmolality and protein and serum, TRIG, GLU, AP ALT were all decreased. The number of transcriptional changes in the liver significantly affected by fasting (> 1500) exceeded effects typically seen in preclinical toxicology studies, even compared to compounds associated with hepatotoxicity. Transcript changes included genes with roles in fatty acid, glucose, cholesterol and bile acid metabolism. About 7% of transcripts in males and 10% in females changed. Urine metabolomic profiles revealed that the metabolic response to fasting was similar in males and females. Fasted animals had decreased urinary citrate, oxoglutarate, taurine, glucose, hippurate, and N-methyl nicotinic acid. While increases were noted in N-acetylglutamate, creatinine, lactate, circulating M/S chain fatty acids, and acacetate. No changes in urinary ketone bodies (acetoacetate, acetoacetate, 3-hydroxybutyric acid) were noted. While some changes were anticipated, the number and magnitude of omic changes suggests that significant attention needs to be paid to fasting state when such data are to be generated in toxicological studies.

1013 MIXTURE TOXICOCENOGENICS: THE CASE OF HAIR DYES.


There is an increasing policy need to provide novel scientific tools for improving the health risk assessment of chemical mixtures in consumer products such as cosmetics and in particular hair dyes (HD). Genomic biomarkers of exposure and effects related to chronic exposure to typical low doses of commercially relevant hair dye preparations could be identified with gene expression micro-arrays. Our study comprised in vitro analysis on two human cell lines and in vivo analysis on selected rat tissue for the determination of gene expression patterns in target organs. The chemicals considered were representative of commercial hair dyes. Comparison was made among formulations with commonly found agents like p-phenyldiamine (PPD). Two commercially relevant formulations were tested including hair dyes using nanoparticle-bound PPD for better drying and duration effects (HD nano).

The in vitro studies were dose-response driven. Gene expression, cytotoxicity and endothelial disruption were considered. Gene expression results were corroborated with quantitative RT-PCR using micro-fluidic cards for specific gene families and the related biological processes. Weak cytotoxicity was induced in all cell lines used. The results were confirmed by the very weak gene expression modulation observed in vitro. Hair dye preparations showed extrageogenic activity as suggested by the E-screen assay. Toxicogenomics analysis on adrenal glands in rats treated intra-tracheally and epidermically confirmed that in female rats genes related to estrogenic activity and endocrine equilibrium were strongly modulated. In the lungs of male and female rats exposed to HD, HD-nano and PPD, gene networks related to immunological responses were modulated; yet the number of genes affected in the males was much higher than in the females. Toxicogenomics analysis of urinary bladder showed a significant difference between male and female rats both in terms of total genes modulated and in terms of the effects on specific biological processes induced by exposure to hair dyes.

1014 GENE EXPRESSION EFFECTS OF DIETARY FAT AND METASTASIS IN MICE PREDISPOSED TO OBESITY AND MAMMARY CANCER.

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The prevalence of obesity is growing rapidly, and this has been attributed in part to diets high in fat and to oral exposures to some environmental chemicals. Obese diets and metastatic load play a significant role in breast cancer prognosis. In order to extensively examine the mechanisms underlying the interaction of obesity with breast cancer and its metastasis, we developed an obese mouse model of breast cancer metastasis. Among a F2 mouse population co-segregating obesity and the MMTV-PyMT transgene, those mice fed high fat diet had decreased mammary cancer latency, and increased pulmonary metastases compared to those fed normal fat diet. Genome-wide SNP analyses demonstrated 8 modifier loci for pulmonary metastasis, where diet significantly interacted with novel QTL at all of these loci. Here, gene expression microarray was performed on mammary tumors from the same F2 population, and analyzed for functional significance using Ingenuity software. Despite the substantial impact of diet on metastasis and its interaction with QTL, diet only altered mammary tumor gene expression of five genes, pten and tnf are central nodes of this network of diet-induced differential gene expression. Metastatic load was modulated by substantial alterations in the gene expression of mammary tumors, including many genes known to be involved in endocrine cancers, cell morphology, cell movement, angiogenesis and invasion. The top metastasis network forms central nodes at sth, mark, ink, and rat. Tight junction signaling was the only canonical pathway associated with central nodes molecules of both the differential diet- and metastasis- analyses. In conclusion, aberrant tight junction signaling may be a unique biological mode of metastatic action among obese animals with mammary cancer.

1015 A COMPARATIVE 'OMICS APPROACH TO UNDERSTANDING TUMOR SUPPRESSION BY P19ARF DURING SKIN CANCER PROGRESSION.

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Tumor progression involves genomic, proteomic and chromosomal changes within cells as they transform from a normal state to benign hyperplasias, malignant carcinomas and finally to a metastatic phenotype. Our laboratory has previously found that p19ARF suppresses DMBA/TPA-induced skin cancer in vivo by inhibiting the growth, progression and metastasis of Hras-driven carcinomas. To determine the specific mechanisms of p19ARF activity during tumor progression, we have examined gene expression patterns in papillomas and carcinomas from wild type and p19ARF-null mice by microarray analysis. Profiles for papillomas and carcinomas were significantly enriched in genes related to cell proliferation (including cell cycle progression, transformation, ribogenesis, ubiquitination and sumoylation) and cellular movement (including adhesion, invasion, migration and metastasis), respectively. While activating mutations in Hras were detected in 100% of tumors from
both genotypes. Hras overexpression was only observed in papillomas and carcinomas from p19Arf-null mice. Changes in gene expression are supported by array-CGH structural data showing over representation (gain) of chromosome 7, which contains Hras, in p19Arf-null tumors. Further, LTQ MS/MS analysis of plasma from tumor bearing p19Arf deficient mice also identified specific protein changes to cell cycle, cell migration and localization, and cell death. Overall, these data indicate (1) p19Arf suppresses tumorigenesis through distinct mechanisms that are dependent on tumor stage, (2) loss of p19Arf facilitates an increase in Ras levels, which may be a major driving force throughout progression of DMBA-induced cancer, and (3) comparative ‘omic analysis is a useful tool for identification of candidate biomarkers and processes important for disease progression.

1016 USE OF MICRORNA EXPRESSION ADDS A REGULATORY COMPONENT TO MICROARRAY DATA: A CASE STUDY.

Microarrays have been used to correlate gene expression with physiological endpoints such as liver toxicity to create biomarkers for specific clinical or pathological conditions and have enabled toxicologists to generate mechanistic hypotheses. MicroRNAs (miRNA) are small non-coding RNA transcripts expressed in a tissue-specific manner and are responsible for regulation of gene expression. Development of anti-miRNA drugs have demonstrated successful down-regulation of transcripts responsible for pathological conditions. To investigate the utility of using miRNA expression in toxicology assessments, rats were dosed with 100mg/kg Atorvastatin or vehicle for 4 days. RNA was collected from livers and used for Affymetrix microarray and TaqMan® miRNA analysis. Little is currently known and widely accepted regarding the regulatory roles of miRNA in many specific pathological and physiological conditions as this is a rapidly expanding field, however, our analysis of livers from rats treated with Atorvastatin revealed 25 up-regulated and 20 down-regulated miRNAs and microarray expression analysis revealed that many genes known to be regulated by these miRNAs were down- and up-regulated, respectively. These included genes associated with lipid metabolism, fatty acid biosynthesis, molecular transport, amino acid metabolism, carbohydrate metabolism, biosynthesis of steroids and cell signaling pathways. The targets of the regulated miRNAs were regulatory components of the genes identified in the Affymetrix microarray. The coupling of miRNA with microarray expression analysis has the potential to add a strong regulatory component to clearly identify signaling pathways which may be acting synergistically and therefore provide insight into mechanisms more relevant to pathological/toxicological endpoints than microarray expression alone.

1017 EFFECTS OF VARIOUS HEPATOTOXICANTS ON HEPATIC GENE EXPRESSION IN RATS AND HUMERIC PXB-MICE® WITH HIGHLY HUMANIZED LIVER.
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Drug-induced liver injury (DILI) is one of the leading causes of withdrawal of a new drug. In order to find out the early marker, toxicogenomic approaches have been employed using in vivo (experimental animals, mainly rats) or in vitro (human hepatocytes or hepatoma cells) systems. Recently, chimeric PXB-mice®, in which more than 70% of hepatic parenchymal cells are replaced by human hepatocytes, are attracting much attention as a unique animal model to mimic human type drug metabolism. This animal model has a potential to bridge the gap between rodent-type and human-type livers and to explain the differences of in vivo and in vitro response of human hepatocytes against hepatotoxicants. We have analyzed changes in hepatic gene expression in rats and PXB-mice® treated with 20 different hepatotoxicants (acetaminophen, amiodarone, diclofenac, d-penicillamine, fluoxetine, erythromycin, valproate, sulindac, indomethacin, perhexiline, amantadine, tamofoxen, acetylsalicylic acid, methotrexate, demeclocycline, hydrazine, hdroxyurea, imipramine, orotic acid) and six non-hepatotoxicants. These drugs were orally administered to rats and PXB-mice® three-times daily at high doses (ca. 20% of reported LD50), followed by hepatic total RNA preparation and gene expression analyses using oligo- and TaqMan® microarray chips. Several marker gene candidates, which specifically responded to the hepatotoxicanents, were extracted and further evaluated by using a score method in which DILI prediction score for each drug is calculated from marker gene expression levels and weight factors optimized by least-squares fitting. One-by-one omit analyses revealed that PXB-mice® gave better predictability of hepatotoxicant as compared with rats. Differences of gene expression in rats and PXB-mice® will also be discussed in terms of biological pathways related to liver functions.

1018 INVESTIGATING THE MECHANISM OF 2-BROMOETHANAMINE-INDUCED GLUTARIC ACIDURIA.
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2-Bromoethanamine (BEA) causes renal papillary necrosis (RPN) after acute administration. This compound has been used for studying RPN as the pathophysiological course parallels the morphological and functional changes as associated with excessive use of nonsteroidal anti-inflammatory drugs. While the mechanism of BEA-induced RPN has not been established, urinary metabolic evaluations have demonstrated marked increases in glutaric acid in rats at BEA doses a 25 mg/kg. Functional loss of glutaryl-CoA dehydrogenase (GCDH) causes glutaric aciduria in humans and animal models suggesting that BEA may inhibit GCDH. BEA is a known mechanism-based inhibitor of enzymes such as semicarbazide-sensitive amine oxidase. Therefore, the purpose of this investigation was to determine if BEA or a major metabolite, aziridine is a mechanism-based inhibitor of GCDH. Incubations containing purified GCDH and BEA or aziridine at 50 and 500 μM were prepared and aliquots were removed at various times and added to a complete enzymatic reaction consisting of the intermediate (electron transfer flavoprotein) and terminal (2,6-dichlorophenolindophenol) electron acceptors as well as the glutaryl-CoA substrate. Compared to a control enzyme reaction, no GCDH inhibition was observed with either BEA or aziridine up to 500 μM. Since substrates for GCDH are CoA-containing moieties, additional experiments were conducted to determine whether BEA could form a covalent adduct with glutaryl-CoA. When incubated at equimolar concentrations, BEA formed two unique adducts with glutaryl-CoA (molecular weight 924.2) in the presence of sodium bicarbonate. These results indicate that BEA covalently adds glutaryl-CoA and may directly inhibit GCDH to cause glutaric aciduria.

1019 TEMPORAL PATTERNS IN THE TRANSCRIPTOMIC RESPONSE OF RAINBOW TROUT, ONCORHYNCHUS MYKISS, TO CRUDE OIL.
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Time is often not characterized as a variable in toxicogenomic studies. In this study, the temporal kinetics in gene expression were determined during exposure to crude oil and subsequent recovery. Juvenile rainbow trout, Oncorhynchus mykiss, were exposed for 96 hours to the water accommodated fractions of either 0.4, 2 or 10 mg/L crude oil loadings. Following 96 h of exposure, fish were transferred to recovery tanks for 96 h. Gill and liver samples were collected after 24 and 96 h of exposure, and after 96 h of recovery for RNA extraction and microarray analysis. Fluorescently labeled cDNA was hybridized against matched controls, using salmonid cDNA arrays. Genes that were a minimum 1.5 fold changed relative to controls and significant (p <0.05) by t-test were considered up or downregulated. The expression profile of cytochrome P450 1A, a biomarker for oil exposure, did not predict the majority of gene expression profiles in any tissue or dose. More genes responded to crude oil in the gill than in liver. The Gene Ontology terms associated with predominant (p <0.05) gene sets were coordinated downregulated. The expression profile of cytochrome P450 1A, a biomarker for oil exposure, did not predict the majority of gene expression profiles in any tissue or dose. More genes responded to crude oil in the gill than in liver. The Gene Ontology terms associated with predominant (p <0.05) gene sets were coordinated downregulated. The expression profile of cytochrome P450 1A, a biomarker for oil exposure, did not predict the majority of gene expression profiles in any tissue or dose. More genes responded to crude oil in the gill than in liver. The Gene Ontology terms associated with predominant (p <0.05) gene sets were coordinated downregulated. The expression profile of cytochrome P450 1A, a biomarker for oil exposure, did not predict the majority of gene expression profiles in any tissue or dose. More genes responded to crude oil in the gill than in liver. The Gene Ontology terms associated with predominant (p <0.05) gene sets were coordinated downregulated. The expression profile of cytochrome P450 1A, a biomarker for oil exposure, did not predict the majority of gene expression profiles in any tissue or dose. More genes responded to crude oil in the gill than in liver. The Gene Ontology terms associated with predominant (p <0.05) gene sets were coordinated downregulated. The expression profile of cytochrome P450 1A, a biomarker for oil exposure, did not predict the majority of gene expression profiles in any tissue or dose. More genes responded to crude oil in the gill than in liver. The Gene Ontology terms associated with predominant (p <0.05) gene sets were coordinated downregulated. The expression profile of cytochrome P450 1A, a biomarker for oil exposure, did not predict the majority of gene expression profiles in any tissue or dose. More genes responded to crude oil in the gill than in liver. The Gene Ontology terms associated with predominant (p <0.05) gene sets were coordinated downregulated.

1020 TOWARDS AN INTEGRATED UNDERSTANDING OF GENES CONTROLLING SEXUAL MATURATION IN SALMONIDS.
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As the use of high density gene arrays based on fully or largely sequenced genomes increases in fish toxicological studies, several trends are emerging that limit the interpretation of the results. Many genes successfully identified have no obvious link
to the presumed toxic mode of action and are either ignored or considered a non-specific stress response. Many genes altered in expression, although considered statistically significant, are still only marginally affected (e.g. <2-3 fold) and the biological significance within the context of a gene's normal range of expression is unknown. These problems have been especially apparent in toxicogenomic studies of endocrine disruptors in fish. Therefore, to improve our knowledge regarding gene expression and sexual maturation in fish, we characterized the natural changes in gene expression in tissues comprising the brain-pituitary-ovary-liver axis (BPOL) in female trout over a 12 month reproductive cycle. At 18 different time points during the cycle, groups of fish were euthanized, BPOL tissues removed, gene expression characterized using a 16K salmonid cDNA microarray (GRASP consortium). Changes in gene expression during the cycle were compared against levels occurring immediately after spawning. A second group of female trout was placed under an accelerated photoperiod regime, which was designed to compress the normal 12 month cycle to 7 months and thus force many of the biological variables involved in maturation to reach their respective maximum / minimum values in a more pronounced manner. Initial results from the microarray analysis indicate the ovaries undergo the most dynamic changes among BPOL tissues during the normal reproductive cycle. The photo-compressed trout were not able to fully complete their reproductive cycle, which may also have contributed to the difference.

1021 EXPRESSION QUANTITATIVE Trait LOCI MAPPING IDENTIFIES NEW GENETIC MODELS OF GLUTATHIONE-S-TRANSFERASE VARIATION.

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Expression Quantitative Trait Loci (eQTL) mapping can be utilized to identify the genetic variations that underlie inherited differences in gene transcription. Combining whole genome transcriptional data from the hypothalami of thirty three strains of inbred mice with a detailed haplotype map of those same strains revealed 16,655 unique associations and 31 cis associations between genotype and gene expression that reached statistical significance. One of the cis associations was found to be driven by strain-specific variation in the expression of Glutathione-S-transferase mu 5 (GSTM5). GSTm5 is one of seven members of the glutathione-S-transferase mu family. The glutathione-S-transferase protein binding, phosphate metabolism, vacuolar/lysosomal transport, protein targeting, sorting, and translocation, cell growth/morphogenesis, cell polarity and filament formation and etc. Our studies have potential implications for understanding toxicity and carcinogenesis of arsenic-induced human diseases, such as cancer and aging.

1022 EVALUATION OF FOOD AND DRUG INTERACTIONS ON DNA MICROARRAY ANALYSIS IN CAENORHABDITIS ELEGANS.

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It is important to clarify the interaction between foods and therapeutic drugs because these interactions may enhance the side effects and reduce the efficacy of drugs, or may suppress the effect of therapeutic drugs due to the interference of biological compounds. In C. elegans, the genomic mutation of each identified gene is used as a universal model for human diseases, and it enables our study to prove that the gene is effective in vivo. We need to clarify the gene expression pattern of each identified gene to clear the interaction between food and drugs. We have identified 2000 genes that are sensitive or resistant to environmental chemicals, and we investigated the effect of these chemicals on cell population and gene expression. For example, we have found that these results were shown as acute effect of SJW. In conclusion, it was suggested that the CYP genes of C. elegans are valuable for investigation as an alternative technique of animal examination.

1023 GENOME-WIDE RESPONSES TO ARSENITE IN SACCHAROMYCIES CEREVISIAE.

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We have used Saccharomyces cerevisiae to identify toxicologically important proteins and pathways involved in arsenic-induced toxicity and carcinogenicity in humans. We performed a systemic screen of the complete set of 4,733 haploid S. cerevisiae single gene deletion mutants that affect growth upon exposure to sodium arsenite (NaAsO2). We identified 203 arsenite-sensitive mutants and 61 arsenite-resistant mutants that have human homologues. IC50 values for all sensitive and resistant mutants were determined in another validation screen and the most highly sensitive or resistant mutants upon arsenite exposure were identified. We categorized the identified sensitive mutants based on various functions including protein-binding, phosphate metabolism, vacuolar/lysosomal transport, protein targeting, sorting, and translocation, cell growth/morphogenesis, cell polarity and filament formation and etc. Our studies have potential implications for understanding toxicity and carcinogenesis of arsenic-induced human diseases, such as cancer and aging.

1024 GROWTH FACTORS AND EXTRACELLULAR MATRICES INFLUENCE THE EXPRESSION OF GENETIC MARKERS DURING THE DIFFERENTIATION OF MOUSE STEM CELLS.

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Mouse embryonic stem cells (mESC) (ES-D3, ATCC) are pluripotent cells derived from the inner cell mass of mouse blastocysts. The cells are capable of forming multicellular aggregates (MAs) as an intermediate stage of differentiation. We examined the effects of three growth factors (GF), (EGF, KGF, and LIF) in conjunction with two different ECM components (collagen type IV or fibronectin), known to influence gene expression in mESC. Five genes (Afp, Oct-4, Egfr, Tgfβ2/3 and Arnt) were used as markers to monitor the differentiated state and gene expression was detected using RT-PCR from mRNA purified from confluent monolayers cultured up to 18 days on culture inserts. Concurrently, transmembrane electrical resistance (TMER) was measured as an indicator of tight junction formation. The expression of a specific marker for endodermal cell differentiation, tetraspanin 5 (TSPAN5), showed a 5-fold increase when grown in the presence of EGF on Coll inserts. Arnt and Tgfβ2/3, expressed in MA and immortal cells respectively, were decreased in the presence of LIF when grown on other ECM. This is consistent with the ability of LIF to maintain mESC in the undifferentiated state. Expression of Egfr, a marker for differentiated cells, was decreased regardless of which GF or ECM was used. Cytotoxicity experiments showed that when mES were exposed to arsenic (As) on day 1, TMER was suppressed. Furthermore, after an initial rise for 7 days without As, TMER declined to near baseline when the chemical was introduced at day 7. Concurrently, the expression of Oct 4, a marker for mESC maintenance, was reduced upon introduction of As at day 7. Interestingly, ECM did not influence Oct 4 expression with As. Thus, the culture insert system represents a model for inducing biological membrane formation in drug screening and cytotoxicity testing, and it is also capable of detecting the effects of chemicals on cell differentiation during the early stages of embryogenesis. (Supported in part by grants from Alternatives Research and Development Foundation & International Foundation for Ethical Research).

1025 CLONING, EXPRESSION, AND PHARMACOCOLOGICAL CHARACTERIZATION OF THE ZEBRAFISH DOPAMINE TRANSPORTER.

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The use of the zebrafish model system is receiving increased attention for its potential for developing new disease models and for the development of drugs. However, for the zebrafish model to be validated for such studies, more information is needed.
on the specific interactions of drugs with their molecular target and how the zebrafish target compares with the human target. The dopamine transporter (DAT) is the molecular target for drugs of abuse such as cocaine and methamphetamine and a target for therapeutic drugs such as methylphenidate. In order to assess the potential use of zebrafish as a model for testing drugs that interact with the DAT, we cloned the zebrafish DAT (zDAT) and inserted it into a vector expressing green fluorescent protein (pIRES-EGFP). Following transient transfection into SK-N-AS neuroblastoma cells, zDAT demonstrated strong expression with a punctuate expression at the plasma membrane. zDAT expression resulted in functional 3H-dopamine uptake that was abolished by the DAT inhibitor nomifensine (10 μM) and was similar to mock-transfected cells and cells transfected with empty vector demonstrating the specificity and dependence of dopamine uptake on the expression of zDAT. To assess the specificity of drug interactions with the zDAT, we measured dopamine uptake in the presence of various concentrations of inhibitors specific for the DAT (methylphenidate and nomifensine), the serotonin transporter (fluoxetine) and the norepinephrine transporter (desipramine). Both norepinephrine and nomifensine inhibited zDAT-mediated dopamine uptake in the μM range while fluoxetine and desipramine had no effect until concentrations reached 10 μM. Taken in concert, these data suggest that zebrafish may represent a good model for testing drugs and toxicants that interact with the DAT.

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1026 HISTOPATHOLOGICAL AND TOXICOGENOMIC ANALYSES IN THE LIVER OF HETEROZOYGOS SOD2 KNOCKOUT MICE TREATED WITH TROGLITAZONE OR ACETAMINOPHEN.

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1, 2 Medicinal Safety Research Labs., Daiichi Sankyo Co., Ltd., 3, 4 Fujisawa, 5, 6 Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Aichi, Japan. 1027 REALIZING THE GENOMIC POTENTIAL THROUGH COLLABORATION TO DERIVE AND EVALUATE GEN EXPRESSION SIGNATURES FOR CARCINOGENICITY PREDICATION AND RISK ASSESSMENT.

M. Felden1, R. Dunn2, T. Davison3, J. Sherlock4, E. Walker5, W. Mattes6, P. Lord7, 8 and B. Catt1. 1, 2 Bechtel Palo Alto, Palo Alto, CA. 3, 4, 5, 6, 7 Aukersen, Austin, TX. 8 Critical Path Institute, Tucson, AZ. 9 Bristol-Myers Squibb, Princeton, NJ and 10 Johnson and Johnson, Raritan, NJ.

The Carcinogenicity Working Group of the Predictive Safety Testing Consortium aims to collaboratively advance our ability to predict and mechanistically understand non-genotoxic hepatocarcinogens to facilitate the early risk assessment of chemicals. Retrospective evaluation of published microarray signatures for predicting non-genotoxic carcinogens indicated that hepatic gene expression data can predict the likelihood of chemical-induced hepatic tumorigenicity with reasonable accuracy, however, variability in measurement platform and treatment protocols across laboratories rendered the signatures unsuitable as is. This prompted the consortium to re-derive the signature on a higher throughput and cost-effective measurement platform and begin to standardize the treatment protocol to enable robust predictions across laboratories. Through refinement of the signature, we identified a subset of 26 unique genes from the original signatures that showed potential for classification of carcinogens. To standardize the assay platform, we re-derived the signature on a quantitative real-time PCR platform using liver RNA samples from rats treated with 70 non-genotoxic carcinogens and non-carcinogens. Comparison of the QPCR data with the original microarray data generated on the same RNA indicated good correlation (r=0.7) between platforms for most genes, suggesting that we could re-establish the signature on the QPCR platform. This facilitated wider evaluation of historical samples to further define the accuracy and study parameters important for robust classification of potential carcinogens. Further sample sharing and collaboration with technology companies allowed us to realize the practical utility of our genomic knowledge-base to make early risk assessment for carcinogenic compounds possible.


Sulfur mustard (SM) is a potent vesicant that targets epithelial cells and tissues. Most vesicant research has been performed using bona fide SM; however, some studies have used simulants, most notably half mustard (2-chloroethyl ethylsulfide; CEES) and nitrogen mustard (methylchloramine; NM). Although CEES and NM have similarities to SM and can cause vesication, there are distinct differences in the chemical structures and physical properties of these compounds that may impact their toxic effects. Microarray analysis of cultured primary human keratinocytes (HEK) exposed to each of these vesicants was performed to directly compare the transcriptional responses induced by these vesicants. HEK were exposed to triplicate to concentrations ranging from 0-1000 μM for SM and NM and 0-4000 μM for CEES. Cells were harvested at 1, 2, 4, 8, 16, and 24 h and the RNA isolated for microarray analysis. Transcriptional responses were phenotypically anchored to cell morphology. The dataset was filtered by exposure and timepoint, and an analysis of variance was performed using dose as the factor. The top 500 genes ranked by p-value were analyzed using gene ontology algorithms to identify biological pathways significantly affected by each vesicant. At 2 h post-exposure, p53 signaling, EGF signaling, Erk/MAPK signaling and PDGF signaling were significantly affected by all three vesicants. At 4 h post-exposure, only p53 signaling and glucocorticoid receptor signaling were significantly affected by all three vesicants. At 8 h post-exposure, p53 signaling and glucocorticoid receptor signaling were significantly affected by all three vesicants.
post-exposure, there were no significant pathways commonly affected by all three vesicants. These results suggest that, although there are similarities in the transcriptional responses to each of these vesicants, the transcriptional responses appear to differ over time. Thus, extrapolation of results obtained with one vesicant to other vesicants may be complex and may have important implications for the development of vesicant therapeutics.

**1030 DNA ADDUCTS AND GENE EXPRESSION PROFILES IN VENOUS AND CORD BLOOD AND PLACENTA OF MOTHERS IN PRAGUE.**

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In context of the molecular epidemiology study dealing with the biological effects of ambient air pollution in humans, 32P-postlabelling method was employed to analyze total bulky DNA adducts in venous and umbilical cord blood and placenta of 79 mothers giving birth to living babies in the University Hospital in Prague (Czech Republic). Benzo[a]pyrene specific DNA adducts were analyzed in placenta by ELISA. Simultaneously, gene expression profiles were determined in all 3 tissues using Illumina Human Ref 8 Expression BeadChips. Among mothers, 67 was classified as non-smokers and 12 as passive or active smokers (plasma cotinine levels were expected to induce necrosis to hepatocytes and this was confirmed through histochemical analysis (DA)). In targeted profilings, endogenous metabolites of allantoin, citrate, hippurate, and benzoate were selected as biomarkers. In targeted profilings, endogenous metabolites of allantoin, citrate, hippurate, and benzoate were selected as biomarkers. In targeted profilings, endogenous metabolites of allantoin, citrate, hippurate, and benzoate were selected as biomarkers. In targeted profilings, endogenous metabolites of allantoin, citrate, hippurate, and benzoate were selected as biomarkers. In targeted profilings, endogenous metabolites of allantoin, citrate, hippurate, and benzoate were selected as biomarkers.

1,2-Naphthoquinone (1,2-NQ) is an environmental electrophile that reacts readily with thiolate anions in proteins to yield a stable thioether adduct. Thiols are more reactive in proteins with basic amino acids in close proximity because the latter reduce the thiol pKa. SAMP response element-binding protein (CREB) is a transcription factor and plays an important role in mammalian cells. CREB has a basic leucine-zipper (bZIP) domain that governs binding efficiency to its cognate promoter element CRE, a consensus sequence. bZIP transcription factors contains a highly conserved cysteine residue at regions, which have nearby basic amino acid in their DNA binding domain. Based these findings, we hypothesized that 1,2-NQ could bind to a CREB, resulting in suppression of DNA binding activity, transcriptional activity and thus altered expression of CREB-regulated proteins. To test above hypothesis, we conducted immunoprecipitation, western blot analysis and EMSA in bovine aortic endothelial cells (BAECs) and cell-free system. Exposure of BAECs to 1,2-NQ resulted in a covalent binding to CREB. Under these conditions, DNA binding activity of CREB was dramatically inhibited by 1,2-NQ. 1,2-NQ also suppressed CRE-dependent gene transcription and expression of CREB-regulated gene product, Bel-2. The modification of CREB and inhibition of DNA binding activity of CREB during 1,2-NQ exposure were effectively blocked by DTTP, suggesting that a 1,2-NQ formed covalent bounds with CREB through reactive thiol groups. To identify the modification sites in 1,2-NQ, MALDI-TOF/MS analysis was performed. Results indicate that Cys286, Lys290 and Lys319 were covalently modified by 1,2-NQ. Mutation experiments revealed that CREB disruption was, at least in part, due to covalent attachment of the 1,2-NQ to Cys286 at DNA binding domain. These results suggest that CREB is a molecular target for 1,2-NQ and that such an irreversible binding of 1,2-NQ to Cys286, causes functional loss of this transcription factor.

**1031 BIOMARKER DISCOVERY : NMR SPECTRAL DATA OF URINE AND PLASMA AND HEPATOTOXICITY BY CARBOXYTETRACHLORIDE, ACETAMINOPHEN, AND D-GALACTOSAMINE IN RATS.**

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The primary objective of this study was to discover biomarkers which are correlated with hepatotoxicity induced by chemicals using 1H NMR spectral data of urine and plasma. A procedure of nuclear magnetic resonance (NMR) urinalysis using specific adducts was applied to discover biomarkers which are correlated with hepatotoxicity induced by chemicals. Using 1H NMR spectral data of urine and plasma, a procedure of nuclear magnetic resonance (NMR) urinalysis using specific adducts was applied to discover biomarkers which are correlated with hepatotoxicity induced by chemicals. Using 1H NMR spectral data of urine and plasma, a procedure of nuclear magnetic resonance (NMR) urinalysis using specific adducts was applied to discover biomarkers which are correlated with hepatotoxicity induced by chemicals. Using 1H NMR spectral data of urine and plasma, a procedure of nuclear magnetic resonance (NMR) urinalysis using specific adducts was applied to discover biomarkers which are correlated with hepatotoxicity induced by chemicals.

**1032 1,2-NAPHTHOQUINONE DISRUPTS CREB TRANSCRIPTIONAL ACTIVITY THROUGH MODIFICATION OF CYS286.**

A. Endo, D. Sumi, N. Iwamoto and Y. Kurnagai. Graduate school of comprehensive human sciences, University of Tsukuba, Tsukuba, Japan. Sponsor: A. Nagajuma.

1,2-Naphthoquinone (1,2-NQ) is an environmental electrophile that reacts readily with thiolate anions in proteins to yield a stable thioether adduct. Thiols are more reactive in proteins with basic amino acids in close proximity because the latter reduce the thiol pKa. SAMP response element-binding protein (CREB) is a transcription factor and plays an important role in mammalian cells. CREB has a basic leucine-zipper (bZIP) domain that governs binding efficiency to its cognate promoter element CRE, a consensus sequence. bZIP transcription factors contains a highly conserved cysteine residue at regions, which have nearby basic amino acid in their DNA binding domain. Based these findings, we hypothesized that 1,2-NQ could bind to a CREB, resulting in suppression of DNA binding activity, transcriptional activity and thus altered expression of CREB-regulated proteins. To test above hypothesis, we conducted immunoprecipitation, western blot analysis and EMSA in bovine aortic endothelial cells (BAECs) and cell-free system. Exposure of BAECs to 1,2-NQ resulted in a covalent binding to CREB. Under these conditions, DNA binding activity of CREB was dramatically inhibited by 1,2-NQ. 1,2-NQ also suppressed CRE-dependent gene transcription and expression of CREB-regulated gene product, Bel-2. The modification of CREB and inhibition of DNA binding activity of CREB during 1,2-NQ exposure were effectively blocked by DTTP, suggesting that a 1,2-NQ formed covalent bounds with CREB through reactive thiol groups. To identify the modification sites in 1,2-NQ, MALDI-TOF/MS analysis was performed. Results indicate that Cys286, Lys290 and Lys319 were covalently modified by 1,2-NQ. Mutation experiments revealed that CREB disruption was, at least in part, due to covalent attachment of the 1,2-NQ to Cys286 at DNA binding domain. These results suggest that CREB is a molecular target for 1,2-NQ and that such an irreversible binding of 1,2-NQ to Cys286, causes functional loss of this transcription factor.

**1033 PHYTOESTROGEN PUERARIN PROTECTS AGAINST TERT-BUTYL HYDROPEROXIDE-INDUCED CELL DEATH THROUGH THE ESTROGEN RECEPTOR DEPENDENT PI3K/AKT/HO-1 PATHWAY.**


Phytoestrogens are polyphenolic non-steroidal plant compounds with estrogen-like biological activity. The phytoestrogen puerarin, the main isoflavone glycoside found in the root of Pueraria lobata, has been used for various medicinal purposes in traditional Chinese medicines for thousands of years. Recent studies have indicated that the estrogen receptor (ER), through interaction with p85, regulates phosphoinositide 3-kinase (PI3K) activity, revealing a physiologic, non-nuclear function of ER that may be relevant in cytoprotection. In this study, we demonstrate that the phytoestrogen puerarin inhibits tert-butyl hydroperoxide (t-BHP)-induced oxidative stress via an ER-dependent G6PD/P3K/Akt and heme oxygenase-1 (HO-1) pathway. Pretreatment of Hepa1c1c7 and HepG2 cells with puerarin significantly reduced t-BHP-induced caspase-3 activation and subsequent cell death. Also, puerarin up-regulated HO-1 expression and this expression conferred cytoprotection against oxidative in-jury induced by t-BHP. Moreover, puerarin in-duced nuclear translocation, which is upstream of puerarin-induced HO-1 expression, and P3K activation, a pathway that is involved in induced Nrf2 nuclear translocation, is downstream of puerarin-induced HO-1 expression, and P3K activation.

**1034 PROTHYMOSIN-ALPHA MEDIATES NUCLEAR IMPORT OF INRF2/CUL3-RBX1 COMPLEX TO DEGRADE NUCLEAR NRF2.**

S. K. Niture and A. K. Jaiswal. Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, MD. Sponsor: B. Moomph. Nuclear factor erythroid 2-Related Factor 2 (Nrf2) is a transcription factor that regulates expression of antioxidant genes. It is involved in oxidative stress response, inflammation and defense against ROS. Nrf2 activity is regulated by its location in the nucleus. In the cytoplasm, Nrf2 is sequestered by the inhibitor Kelch-like ECH-associated protein 1 (Keap1). In response to stimuli, Nrf2 is translocated to the nucleus where it binds to antioxidant responsive elements (AREs) in the promoter of genes involved in antioxidant defense. Prothymosin-alpha (PTMA) is a small protein that interacts with Nrf2 and is involved in cancer cell growth. In this study, we investigated the role of PTMA in regulating Nrf2 activity. We observed that PTMA overexpression increased Nrf2 protein levels in HepG2 cells. Furthermore, we found that PTMA overexpression enhanced the nuclear localization of Nrf2. These results suggest that PTMA may be a potential therapeutic target for cancer treatment.
and A. K. Jaiswal. SOT09_Abstracts2  2/25/09  4:19 PM  Page 215

brane vesicle termed the autophagosome. Subsequent fusion of autophagosomes with lysosomes results in the degradation of the encased components. In this study we asked if lysosomal non-esterified cholesterol content influenced autophagosome-lysosome fusion. We used the cationic amphiphilic drug U18666A to induce the accumulation of non-esterified cholesterol in lysosomes, and fluorescence microscopy to monitor non-esterified cholesterol staining with filipin. Exposure of murine hepatoma 1ce7 cells to 0.1-1.0 μM U18666A resulted in a concentration-dependent increase in filipin staining, which co-localized with Lamp-1 positive (i.e., lysosomes) organelles. Elevated filipin staining was observed within 18 h of exposure, and was persistent as long as U18666A was present. Increased filipin staining was paralleled by the accumulation of autophagosomes. Autophagosome formation was monitored by Western blot analyses of the conversion of LC3-I to LC3-II, and the aggregation of a stably expressed GFP-LC3 fusion protein into very fluorescent punctate structures. Fluorescence microscopy indicated minimal co-localization of GFP-LC3 and Lamp-1 punctate structures in U18666A-treated cultures. In contrast, a high degree of co-localization of GFP-LC3 and Lamp-1 were observed in drug-free cultures following the induction of autophagy by leucine deprivation. Filipin staining in U18666A-treated cultures returned to normal levels within 72 h of drug removal. In filipin staining were paralleled by a transient co-localization of GFP-LC3 and Lamp-1, and the subsequent loss of autophagosomes. These findings suggest that lysosomal cholesterol content, which is elevated by numerous therapeutic drugs, and as a consequence of several diseases, influences autophagosome-lysosome fusion. Hence, lysosomal cholesterol content could be a modifier of the autophagic process.

1035 AN AUTO-REGULATORY LOOP BETWEEN NRF2 AND CUL3-RBX1 CONTROLS THEIR CELLULAR ABUNDANCE.

I. W. Kang and A. K. Jaiswal. Pharmacology and Experimental Therapeutics, University of Maryland, Baltimore, MD.

Nrf2/Nrf2 acts as a sensor for oxidative stress. When a cell encounters any form of stress Nrf2 dissociates from IκBδ and translocates into the nucleus. In the nucleus, Nrf2 activates a myriad of antioxidant genes that protect cells. Nrf2 is then exported out and degraded. Nrf2 serves as a substrate adaptor to link Nrf2 to the Cul3-Rbx1 complex. Cul3-Rbx1 makes up the ubiquitin ligase complex that is responsible for the ubiquitination and degradation of Nrf2. Previously we have shown a feedback auto-regulatory loop between Nrf2 and IκBδ indicating that Nrf2 regulates IκBδ by controlling its transcription. Here we are extending this research by demonstrating the presence of another feedback auto-regulatory loop between Cul3-Rbx1 and Nrf2. Western blotting and Real Time PCR experiments using various cell lines indicate that Nrf2 controls its own degradation by regulating expression and induction of the Cul3-Rbx1 genes. Treatment with the antioxidant tert-Butylhydroquinone (tBHQ) leads to induction of Cul3-Rbx1 genes. Mutagenesis and transfection identified an antioxidant response element in the Cul3-Rbx1 promoters that binds to Nrf2 and regulates expression and antioxidant induction of Cul3-Rbx1 genes. In conclusion, the data suggests that Nrf2 regulates Cul3-Rbx1 by controlling the transcription, and Cul3-Rbx1 control Nrf2 by ubiquitination and degradation of Nrf2.

1036 CHARACTERIZATION OF NRF2-INDEPENDENT NUCLEAR IMPORT Activity OF KEAP1 DURING ANTIOXIDANT RESPONSES.

Z. Sun, T. Wu, C. M. Birch, W. Chen and D. Zhang, University of Arizona, Tucson, AZ.

The transcription factor Nrf2 has emerged as a master regulator of cellular redox homeostasis. Nrf2 binds to the antioxidant responsive element (ARE) and regulates the transcription of many antioxidants, phase II detoxification enzymes, and transporters. Keap1 negatively regulates Nrf2 by both mediating redox-sensitive ubiquitination/ degradation of Nrf2 in the cytoplasm and targeting Nrf2 for nuclear export in the nucleus. However, it is not clear how Keap1 gets into the nucleus. In this study, we have characterized the nuclear import activity of Keap1. We found that Keap1 travels into the nucleus independently of Nrf2 or Nrf1. The amino acid sequence responsible for Keap1 nuclear import was mapped to the Kelch domain. Site-directed mutagenesis and fluorescence labeling by GFP showed that a hydrophobic cluster within the Kelch domain of Keap1 is required and sufficient in mediating the Nrf2-independent nuclear import of Keap1. These findings suggest that Keap1 may contain a non-classical nuclear import sequence (NLS) that plays an important role in the repression of Nrf2 under basal redox balanced conditions. [NIEHS 1RO1ES015010-01, ACS RSG-07-154-01]

1037 LYSOSOMAL NON-ESTERIFIED CHOLESTEROL CONTENT MODULATES THE FUSION OF AUTOPHAGOSOMES WITH LYSOSOMES.

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Macroautophagy (hereafter referred to as autophagy) is a normal physiological process whereby cytosol and entire organelles are encapsulated in a double membrane vesicle termed the autophagosome. Subsequent fusion of autophagosomes with lysosomes results in the degradation of the encased components. In this study we asked if lysosomal non-esterified cholesterol content influenced autophagosome-lysosome fusion. We used the cationic amphiphilic drug U18666A to induce the accumulation of non-esterified cholesterol in lysosomes, and fluorescence microscopy to monitor non-esterified cholesterol staining with filipin. Exposure of murine hepatoma 1ce7 cells to 0.1-1.0 μM U18666A resulted in a concentration-dependent increase in filipin staining, which co-localized with Lamp-1 positive (i.e., lysosomes) organelles. Elevated filipin staining was observed within 18 h of exposure, and was persistent as long as U18666A was present. Increased filipin staining was paralleled by the accumulation of autophagosomes. Autophagosome formation was monitored by Western blot analyses of the conversion of LC3-I to LC3-II, and the aggregation of a stably expressed GFP-LC3 fusion protein into very fluorescent punctate structures. Fluorescence microscopy indicated minimal co-localization of GFP-LC3 and Lamp-1 punctate structures in U18666A-treated cultures. In contrast, a high degree of co-localization of GFP-LC3 and Lamp-1 were observed in drug-free cultures following the induction of autophagy by leucine deprivation. Filipin staining in U18666A-treated cultures returned to normal levels within 72 h of drug removal. In filipin staining were paralleled by a transient co-localization of GFP-LC3 and Lamp-1, and the subsequent loss of autophagosomes. These findings suggest that lysosomal cholesterol content, which is elevated by numerous therapeutic drugs, and as a consequence of several diseases, influences autophagosome-lysosome fusion. Hence, lysosomal cholesterol content could be a modifier of the autophagic process.

1038 CGP-OLIGODEOXYNUCLEOTIDES INHIBITS THE TUMOR NECROSIS FACTOR-ALPHA-INDUCED EXPRESSION OF CELL ADHESION MOLECULES IN HUMAN Endothelial cells by SUPPRESSING NF-KB ACTIVATION.

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Expression of cell adhesion molecules (CAM) responsible for leukocyte-endothelium interactions plays a crucial role in inflammation and atherosclerosis. Up-regulation of vascular CAM-1 (VCAM-1), intracellular CAM-1 (ICAM-1), and E-selectin expression promotes monocyte recruitment to sites of injury and is considered to be a critical step in atherosclerotic plaque development. Bacterial unmethylated cytosine-phosphosphate-guanine (CpG)-rich oligodeoxynucleotides (CpG ODNs) elicit immunomodulatory effects similar to that of bacterial DNA. This study examined the effects of CpG-ODN on the cytokine-induced monocyte/human endothelial cell interaction, which is a crucial early event in atherogenesis. CpG-ODN inhibited the adhesion of tumor necrosis factor-alpha (TNF-alpha)-induced monocytes to endothelial cells and suppressed the TNF-alpha-induced protein and mRNA expression of the cell adhesion molecules, VCAM-1 and ICAM-1. Furthermore, CpG-ODN inhibited the TNF-alpha-induced NF-kb activation pathway in these cells. Overall, CpG-ODN has anti-atherosclerotic activities, which occurs partly by down-regulating the pathway that affects the expression and interaction of the cell adhesion molecules on endothelial cells.

1039 TRANSCRIPTOMICS ANALYSIS IN MOUSE LUNG EXPOSED TO MAINSTREAM SMOKE FROM TWO DIFFERENT SMOKING REGIMENS.

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The purpose of this study was to compare the changes of gene expression profiles in the lungs of mice exposed to mainstream smoke of 2R4F reference cigarettes from two different smoking regimens: 1) diluted continuous smoking per ISO at 250 μl/g, WTPM, 5 h/d, 5 d/wk for 3 weeks and 2) undilated sporadic smoking (4 cigarettes [filter removed]/d, 6 d/wk for 4 weeks). AKR/J mice were exposed to regimen 1 using an automated smoking machine, while mice for regimen 2 were exposed using a direct-pump smoking machine. Gene expression profiles were defined in lungs of smoke-exposed mice and the concurrent control mice at the end of exposure using microarray analysis. The number of differentially expressed genes (with a fold change of ≥ 2-fold, and a P value of ≤ 0.05 compared to the concurrent control mice) were 255 in the regimen 1 mice, and 220 for the regimen 2 mice, respectively. The overall response in mice exposed to regimen 2 was minor when compared to mice exposed to regimen 1. This was reflected by smaller fold changes for individual genes and lower significance levels of top biological processes in mice exposed to regimen 2. Top regulated genes in mice exposed to regimen 1 included Mmp12, Nos3, Saa3, Cad5, Hspa1b, etc. Top regulated genes in mice exposed to regimen 2 included Mirt, Cyp1a1, Mmp8, etc. Significant biological processes associated with changed genes in regimen 1 mice included response to heat, response to protein folding, immune and inflammatory response, chemotaxis, and DNA repair, etc., while chemotaxis, proteolysis, and cell cycle regulation, etc., were the top
biological processes in the regimen 2 mice. The observed differences in transcriptomics analysis between the two regimens could be partially related to differences in smoke dosimetry between the two smoking regimens. (Funded by Battelle Biology and Health Science Initiative).

**1040 CHROMIUM (VI) INHIBITS MOUSE METALLOTHIONEIN-I GENE TRANSCRIPTION BY PREVENTING THE ZINC-DEPENDENT FORMATION OF AN MTF-1–P300 COMPLEX.**

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Mouse metallothionein-I (MT-I) transcription is regulated by metal response element-binding transcription factor-1 (MTF-1) which is recruited to the promoter in response to zinc. Chromium(VI) [Cr(VI)] pretreatment blocks zinc-activation of the endogenous MT-I gene and attenuates zinc-activation of MT-I promoter-driven luciferase reporter genes in transient transfection assays. Chromatin immunoprecipitation assays revealed that Cr(VI) only modestly reduces recruitment of MTF-1 to the MT-I promoter in response to zinc, but drastically reduces the recruitment of RNA polymerase II. These results suggest that Cr(VI) inhibits the ability of MTF-1 to transactivate this gene in response to zinc. Zinc has been recently shown to induce the formation of a co-activator complex containing MTF-1 and the histone acetyltransferase p300 which plays an essential role in the activation of MT-I transcription. Herein, co-immunoprecipitation assays demonstrated that Cr(VI) pretreatment blocks the zinc-induced formation of this co-activator complex. Thus, Cr(VI) inhibits mouse MT-I gene expression in response to zinc by interfering with the ability of MTF-1 to form a co-activator complex containing p300 and recruit RNA polymerase II to the promoter. (Supported by KAKENHI grant 17790104 and NIH grant ES05704)

**1041 ZINC-INDUCED EPIGENETIC CHANGES IN THE MOUSE METALLOTHIONEIN-I PROMOTER CHROMATIN ARE MEDIATED BY THE ZINC-SENSING TRANSCRIPTION FACTOR MTF-1.**

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Mouse metallothionein-I (MT-I) transcription is regulated by metal response element-binding transcription factor-1 (MTF-1) which is recruited to the promoter in response to zinc. Here, we show that MTF-1 is essential for zinc-induced epigenetic changes in the mouse MT-I promoter. Chromatin immunoprecipitation (ChIP) assays demonstrated that zinc-treatment lead to a decrease of Lys4-acetylated histone H3 and total H3 in the MT-I promoter. Microcosmic nuclease sensitivity of the MT-I promoter was increased in response to zinc. These results suggest that chromatin structure is locally disrupted in response to zinc. In the absence of MTF-1, these changes were not observed. A deletion mutant of MTF-1, which recruits to the MT-I promoter in response to zinc but fails to recruit the co-activator p300 and activate MT-I transcription, also did not change the amount of histone H3 in MT-I promoter in response to zinc. Furthermore, interference with the ability of MTF-1 to form a co-activator complex containing p300 and recruit RNA polymerase II to the promoter. (Supported by KAKENHI grant 17790104 and NIH grant ES05704)

**1042 CONSEQUENCES OF ALTERED CYTOCHROME P450 251 EXPRESSION IN LUNG CELLS.**

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Cytochrome P450 251 (CYP251) exhibits a unique expression profile among cytochrome P450s. It is one of the few P450 metabolic enzymes that display both selective expression in extrapulmonary tissues and constitutive expression throughout development. Elevated CYP251 expression is observed in the hyperproliferative disorders psoriasis and cancer. Increased CYP251 expression in colorectal cancer and ovarian cancer correlates with poor prognosis and metastasis, respectively. These data suggest an important role for CYP251-mediated metabolism in extracellular cells. To determine whether CYP251 expression was necessary for pulmonary cell physiologic, we performed RNA interference (RNAi) experiments including cancer, immortalized, and primary cell lines. CYP251 knockdown, using a small interfering RNA directed toward the 3’UTR produced a significant reduction in CYP251 gene expression as demonstrated using quantitative PCR. CYP251 protein was also reduced by approximately 50%. Initial phenotypic observations suggest that CYP251 may influence aspects of pulmonary cell growth. This research was supported by NIH grant 1HL60143; NRSA Postdoctoral Fellowship (#32HL087636) from the National Heart, Lung, and Blood Institute; and the Colgate-Palmolive Postdoctoral Fellowship in In Vitro Toxicology through the Society of Toxicology.

**1043 QUANTITATIVE ASSESSMENT OF WHOLE BLOOD RNA PROFILING AS AN EARLY TEMPORAL MARKER OF TOXICOLOGICAL EFFECTS.**

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Systemic molecular profiling as an early temporal screen for toxicity could markedly improve timelines, costs, animal use and the late attrition in the development of drugs. Our aim was to quantitatively assess, at the whole genome level, whether global alterations in whole blood gene expression can provide a statistically robust early correlate to toxicological events in rats. A 14-day oral toxicity study was conducted looking at the effects of acetaminophen (0, 100, 600, 1200 mg/kg/day). Groups of rats (6M) were sacrificed at 24h, 3 and 14 days, clinical pathology measurements taken, and histopathology conducted on tissues. Blood samples were collected at sacrifice and RNA extracted, labeled and applied to Affymetrix rat whole genome arrays for gene expression profiling. Integration of histopathology data with the genes that were significantly differentially expressed enabled candidate markers of early toxicity to be identified. Acetaminophen (1200 mg/kg) caused an acute necrosis and inflammation of the liver in animals at 24h and 3 days. Transaminase levels associated with liver function were markedly increased. Changes were also evident in the kidney, salivary gland, spleen, thymus, bone marrow, testes, submandibular lymph nodes and the small intestine at 24h and 3 days. Alterations were observed in the gene expression profiling data, disclosing a specific systemic genomic signature of toxicity, detectable within the first 24h of the toxic response. Specific innate immune signaling and T-cell marker responses were observed, predictive of an immune infiltrate in the liver. RNA biomarkers extracted via a non-invasive blood sampling procedure and analyzed using microarray-based analyses can provide a statistically robust platform to reproducibly monitor drug responses in whole blood, supporting its potential use as an early stage toxicity screen. The potential of an immune-correlate to hepatotoxicity has also been identified.

**1044 EVIDENCE FOR THE INVOLVEMENT OF XENOBIOTIC-RESPONSIVE NUCLEAR RECEPTORS IN TRANSCRIPTIONAL EFFECTS UPON PERFLUOROALKYL ACID EXPOSURE IN DIVERSE SPECIES.**

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Humans and other species have detectable body burdens of a number of perfluorinated alkyl acids (PFAA) including perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). We compared the profiles to those produced by exposure to prototypical xeno- biotic metabolizing enzymes (XME). To determine the potential role of constitutive activated receptor (CAR) and pregnane X receptor (PXR) that regulate xenobiotic metabolizing enzymes (XME). To determine the potential role of CAR/PXR in mediating effects of PFAAs in rat liver, we performed a reanalysis of transcript profiles from published studies in which rats were exposed to PFOA or PFOS. We compared the profiles to those produced by exposure to prototypical activators of CAR, (phenobarbital (PB)), PXR (pregnenolone 16 alpha-carbonitrile (PCN)) or PPARa (WY-14,643 (WY)). As expected, PFOA and PFOS elicited...
transcript profile signatures that included many known PPARα target genes. Numerous XME genes were also altered by FFOA and FPOS but not VY. These genes exhibited expression changes shared with PB or PCN. Reexamination of the transcript profiles from chicken exposed to PFAAs indicated that although the expression of some XMEs was altered, there was no evidence that PFAAs altered the expression of typical PPARα target genes. Our results indicate that PFAAs activate PPARα, CAR and PXR in rats but PPARα does not appear to be activated in exposed chickens. Lastly, we provide evidence that human populations with higher CAR expression have lower body burdens of PFAAs. (This abstract does not necessarily reflect US EPA policy.)

1045 IDENTIFICATION OF TRANSCRIPTIONAL NETWORKS INVOLVED IN PEROXISOME PROLIFERATOR CHEMICAL-INDUCED HEPATOCYTE Proliferation.

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Peroxisome proliferator chemical (PPC) exposure leads to increases in rodent liver tumors through a non-genotoxic mode of action (MOA). The PPC MOA includes increased oxidative stress, hepatocyte proliferation and decreased apoptosis. We investigated the putative genetic regulatory events leading to hepatocellular proliferation following short-term exposure to the PPC: cro-2-ethylhelyphthalate (DEHP). Male B6C3F1 mice were exposed to DEHP via oral gavage at a single concentration of 1150 mg/kg/day and sacrificed after 2, 8, 24 and 72 hours. Liver samples were analyzed using the Affymetrix Mouse 430_2 genechip and had identified 794 (2hr), 1921 (8hr), 2019 (24hr) and 2076 (72hr) transcript clusters to be statistically significant. Cell proliferation was markedly increased at 72 hrs. Analyzing differentially expressed genes (DEGs) in the context of cell proliferation showed roughly 10% related to cell cycle at each time point with very little overlap across the time points. This suggests sequential non-overlapping regulatory events controlling cell cycle gene expression. Putative transcription factor binding sites were predicted in the 10 Kb 5' upstream region of DEGs at each time point by sequence analysis (using the TRANSFAC® 2008.2 database). Statistically significant transcriptional sub networks were inferred computationally from putative genetic-regulatory interactions. The putative transcription networks of regulated cell cycle genes were compared with published data. This work identified a number of genes that may underlie the increase in cell proliferation observed upon PPC exposure. This work is being conducted to model toxicity pathways in the USEPA Virtual Liver (v-Liver) project. This abstract does not necessarily reflect USEPA policy.

1046 MECHANISMS OF ZINC OXIDE-INDUCED IL-8 EXPRESSION IN HUMAN AIRWAY EPITHELIAL CELLS.

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Sponsor: I. Nacher.

Inhalation of zinc oxide (ZnO) in occupational settings has been associated with "metal fume fever," a flu-like illness characterized by increases in pulmonary tumor necrosis factor-alpha and interleukin-8 (IL-8). In this study, the effect of ZnO on IL-8 expression and the mechanisms underlying this event have been investigated in human airway epithelial cells. ZnO exposure induced increased expression of IL-8 in human primary airway epithelial cells and the airway epithelial cell line BEAS-2B. Pretreatment of BEAS-2B cells with the transcriptional inhibitor actinomycin D blocked ZnO-induced IL-8 expression, demonstrating the involvement of transcriptional regulation. This was corroborated by the observation of increased IL-8 promoter activity in ZnO-treated BEAS-2B cells transfected with a construct encoding IL-8 promoter-driven luciferase reporter. ZnO exposure was also shown to increase canonical NFκB-dependent promoter activity. Chromatin immunoprecipitation assay showed that ZnO stimulation increased binding of p65 to the IL-8 promoter in BEAS-2B cells. Relative to BEAS-2B cells that were co-transfected with wild type p65 constructs, cells overexpressing constructs encoding mutated p65 at S276, S529, and/or S536, showed significant reduction in ZnO-induced increase in IL-8 promoter activity. In addition, ZnO treatment also resulted in increased IL-8 mRNA stability. In summary, these data indicate that ZnO exposure can increase IL-8 expression through induction of p65 phosphorylation and stabilization of IL-8 mRNA in human airway epithelial cells. This work was supported by the US EPA COOP Agreement CR83546301.

1047 DISRUPTION OF THE LARGEMOUTH BASS (MICROTÉRUS SALMOIDES) STEROIDOGÉNE ACUTE REGULATORY PROTEIN BY ENDOCRINE DISRUPTING CHEMICALS: A ROLE FOR ESTROGEN RECEPTOR SIGNALING?

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The largemouth bass (LMB) is highly sensitive to endocrine disrupting chemicals (EDCs) and serves as a good model for EDCs studies. Steroidogenic acute regulatory protein (StAR), responsible for the rate-limiting step in steroidalogenesis, is a target for EDCs known to disrupt estrogen receptor (ER) signaling. To investigate the potential role of ERs in disruption of StAR by EDCs, testes from 4 recrested LMB were dissected, cultured, and treated for either 6hrs or 2hrs with 17βestradiol (E2), a potent inhibitor of ER signaling, alone and in combination with 1μM human chorionic gonadotropin (hCG), a potent activator of StAR transcription. Using quantitative real-time PCR, it was observed that hCG treatment induced StAR mRNA 2× after 6hrs. This response to hCG was not present at 2hrs, while when E2 was added in combination with hCG which showed a 2.5X induction of StAR mRNA from basal levels. To characterize transcriptional regulation of StAR, we used a 2.9kb portion of the LMB StAR promoter previously cloned by our group in transient transfections in MA-10 mouse Leydig cells. Initial transfections verified that the StAR promoter is hCG-responsive. In silico analysis of the promoter sequence revealed a putative estrogen response element (ERE). Prior to conducting further transfections, functionality of the ERE was verified using electro mobility shift assays (EMSA). EMSAs showed that specific proteins from MA-10 cells, including ERβ (verified by supershift), bind to the putative ERE. Transfections with the StAR promoter are being conducted to identify the role of ERs in regulating StAR, yielding insight into regulatory mechanisms and downstream effects of EDCs on StAR transcription. Research funded by NIEHS SBIR R01 ES015449.

1048 MICRORNA EXPRESSION AND PERMISSIVE TISSUE REGENERATION.

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Previously we identified a number of genes that are coordinately regulated during the course of zebrafish epimorphic tissue regeneration. To investigate whether microRNAs (miRNAs) play a role in the post-transcriptional regulation of regeneration specific genes, we conducted a series of miRNA expression studies using arrays that contained 219 miRNA targets. The caudal fins of adult zebrafish were surgically amputated and RNA was isolated at 1 day post amputation (dpa). miRNA microarray analysis was also conducted using RNA isolated from nonamputated (control) fin tissue. In total, 25 miRNAs were increased specifically in the regenerates. 19 miRNAs were repressed. miR-134 and 29 miRNAs were repressed. miRNAs that potentially regulate target genes previously reported as important for regeneration, such as wnt11a, dkk5, junb, mmp, and vmp, were predicted to be regulated by a number of the miRNAs significantly repressed during regeneration. Since optimal expression of regeneration-promoting genes is required to initiate tissue regeneration, these results suggest that repression of particular miRNAs must occur to permit expression of initiators of regeneration. We specifically hypothesize that repression of critical miRNAs is more important than induction of miRNAs for epimorphic tissue regeneration. Consistent with this hypothesis, the enzyme responsible for miRNA processing, Dicer, is dispensable for tissue regeneration. Collectively, these results indicate that the zebrafish regeneration model is a useful tool to identify the mechanisms by which miRNAs regulate gene expression during regeneration. This research was supported by NSF 0641409, NIH R01 ES01820, and NIH P50 ES00210.

1049 EVALUATION OF ESTROGENIC ACTIVITY OF ZEARALENONE BY TRANSCRIPTOME ANALYSIS OF ESTROGEN Responsive Genes.


Zearalenone (ZEA) is a Fusarium-derived mycotoxin commonly found in food commodities worldwide. Two major alcoholic metabolites of ZEA, α- and β-zearal- enol, have been associated with reproductive tract disorders in mammals due to
and C. D. Klaassen

Glycine-N methyltransferase (GNMT) is a hepatic cytosolic enzyme. It is responsible for the transfer of a methyl group from S-adenosylmethionine (SAM) to glycine forming S-adenosylhomocysteine (SAH) and sarcosine. Mutations of GNMT are associated with hepatocellular carcinoma and prostate cancer. In 1994, it was reported that the GNMT was also the 4S PAH-binding protein and could induce growth promoting activity in MCF-7 cells, as strong as E2. Gene expression profiles obtained from MCF-7 cells treated with E2 and ZEA compounds were analyzed by cluster analysis or using correlation coefficients or R-values. We found no significant differences in R-values between E2 and ZEA compounds. However, a wide range of R-values were found between pairs of ZEA compounds. This suggests that expressions of some genes are variables in response to various ZEA compounds. Furthermore, when we extended our analysis to six functional groups of genes (enzymes, signaling, proliferation, transcription, transport and others) we found that, except for the signaling-related genes, all showed common responses to ZEA compounds. Some genes in the signaling category revealed unique responses to different ZEA compounds and whose expression most likely contributed to the differences in R-values. In conclusion, DNA microarray-based gene profiling may help to explore the estrogenic activities of ZEA and similar compounds at the molecular level and to identify unique genes, which could be used as biomarkers for the investigation of environmental estrogenic compounds.

1050 EFFECTS OF SODIUM ARSENITE ON DNA METHYLATION, MTHFR, CMYC, MT 1/2, PROTEIN LEVELS AND CELL CYCLE ALTERATIONS IN MCF-7 CELLS.

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Arsenic (As) metabolism yields As-methylated species and contributes to depletes methyl donor pools, which may result in DNA hypomethylation and activation of genes, such as c-myc and MT, which regulate proliferation and apoptotic processes and are potentially important in oncogenesis. Our aim was to evaluate the effects of acute As exposure (0-10 μM) on global DNA methylation, methylene tetrahydrofolate reductase (MTHFR), DHFR, c-myc and MT1/2 protein levels and cell cycle profile in the human breast cancer cell line MCF-7. As-treated cells showed DNA hypomethylation and decreased specific PCR. Western blot unique showed a decrease in MTHFR protein levels without significant changes on DHFR. We also observed an increase in c-Myc and MT1/2 proteins along with an increased cell recruitment in S-phase of cell cycle as evaluated by FACS, as well as increases in cell proliferation evaluated by BrdU incorporation into cellular DNA. The reduction in MTHFR protein levels suggest that arsenite alters the one-carbon metabolic pathway which produces SAM, the major methyl group donor in the one-carbon cycle, DNA methylation events and cell cycle progression in mammalian epithelial cells.

1051 EFFECTS OF BENZO[A]PYRENE EXPOSURE ON FUNDULUS HETEROCLOTUS EMBRYONIC GNMT mRNA EXPRESSION AND ADULT HEPATIC ENZYME ACTIVITY.

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Glycine-N methyltransferase (GNMT) is a hepatic cytosolic enzyme. It is responsible for the transfer of a methyl group from S-adenosylmethionine (SAM) to glycine forming S-adenosylhomocysteine (SAH) and sarcosine. Mutations of GNMT are associated with hepatocellular carcinoma and prostate cancer. In 1994, it was reported that the GNMT was also the 4S PAH-binding protein and could induce CYP1A expression. To further understand the role of GNMT in benzo[a]pyrene (BaP)-related toxicity, full length Fundulus GNMT cDNA was cloned from adult liver. It was 1759 base pair (bp) long and contained a 5' non-coding region of 63 bp, an open reading frame of 888 bp, and a 3' non-coding region of 785 bp to the polyA tail. It encoded a deduced protein of 295 amino acids which is 74% similar to human GNMT. A 186 bp GNMT probe was designed for whole-mount in situ hybridization. Expression of GNMT mRNA was dose-dependently increased in the liver region of 7 and 14 dph Fundulus embryos exposed up to 100 μg/L BaP. In order to determine the effects of BaP-treatment on GNMT enzyme activity, a GNMT substrate assay using glycineS-adenosyl-L-[methyl-3H]methionine was optimized. Both control and Fundulus liver cytosolic preparations had GNMT activity, which averaged 12 ± 0.9 and 2.3 ± 0.12 pmol/mg/min, respectively. In addition, the linearity of GNMT activity with time or protein concentration was confirmed. In conclusion, altered expression of GNMT may represent a new target gene for consideration in BaP-mediated embryonic and liver toxicities (Supported by NIEHS R01ES012710).

1052 TISSUE DISTRIBUTION AND CHEMICAL INDUCTION OF THE CYSTINE TRANSPORTERS XCT AND RBAT IN MICE.

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Cationic amino acid transporter, y+ system (xCT) and related to b0, + amino acid transporter (rBAT) are cystine uptake transporters that aid in maintaining adequate intracellular concentrations of cystine, the rate-limiting amino acid in glutathione synthesis. The purpose of the present study was to determine the tissue distribution of these two cystine transporters in male and female mice, and whether hepatic xCT and rBAT mRNA expression are altered by chemical activators of xenobiotic sensors (AhR, CAR, PXR, PPARα and PPARγ). xCT mRNA was expressed highest in brain and gonads. xCT mRNA in brain was 135% higher in male than female mice, whereas xCT mRNA was 75% higher in ovaries than testes. rBAT mRNA was expressed highest in kidney, small intestine, and liver. rBAT mRNA in duodenum and liver was higher in female than male mice (1450 and 1300%, respectively). Activation of the xenobiotic sensors did not increase hepatic mRNA expression of xCT or rBAT. However, all three PPARα activators (clofibrate, ciprofibrate, di(2-ethylhexyl) phthalate) decreased hepatic rBAT mRNA 40-50%. In addition, clofibrate administered to PPARα-null mice did not lower hepatic rBAT mRNA expression, indicating that the decrease in rBAT is PPARα dependent. The current findings show that xCT is mainly expressed in brain and gonads, whereas rBAT is mainly expressed in kidney, small intestine, and liver. In addition, the hepatic expression of rBAT is decreased by PPARα activators, which is PPARα dependent. (Supported by NIH grants ES-09649, ES-013714, ES-09716, ES-07079, and RR-021940).

1053 PREGNANE X RECEPTOR REGULATES THE mRNA TURNOVER THROUGH INTERACTION WITH CCR4-NOT.

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Pregnane X receptor (PXR, NR1I2) is a nuclear receptor that regulates the metabolism of xenobiotics and endobiotics. PXR has been consid- ered the xenosensor because of its remarkable ability to bind a wide range of structurally diverse ligands and coordinately regulate the Phase I and Phase II drug metabolizing enzymes as well as the “Phase III” transporters. PXR plays an important role in drug–drug interactions, adverse drug responses, cholestatic liver diseases and colon cancer. In order to understand the mechanisms of transcriptional regulation of PXR, we performed yeast two-hybrid screenings to screen the interactive proteins in human liver cDNA library, using PXR ligand binding domain as the bait. More than one million independent clones were screened; the known PXR interactive partners were among the positive clones, including NcoR2 and RXXR. One positive clone was a partial cDNA of cNOT2 (amino acids 183-540). cNOT2 is a component of Crr4-NOT which is a multi-subunit protein complex highly conserved from yeast to humans. The Crr4-NOT complex plays critical roles in various aspects of transcriptional regulation, including initiation, repression, activation and mRNA degradation. Using a mammalian two-hybrid system in CV-1 cells and GST-pulldown assays, we confirmed the direct interaction between PXR and cNOT2. In HepG2 cells, over expression of cNOT2 suppresses the PXR-regulated luciferase gene activity, siRNA knockdown of cNOT2 potentiated PXR transcriptional activity. The immunoprecipitated cNOT2 complex contained deadenylase activity as determined by an in vitro RNA decay assay. The presence of transfected
Presented here illustrate that in the embryonic larval assay system TBBPA is more acutely toxic than BPA or TBBPA DME. Furthermore, these data suggest that one mechanism of TBBPA-induced lesions may be through alteration in MMP gene expression. Supported by the Hudson River Foundation.

**PS 1054**

**EXPRESSION/ACTIVITY OF GLUTATHIONE S-TRANSFERSASE ISOFORM (GST-0) IS ASSOCIATED WITH INCREASED SENSITIVITY TO 4-TERTIARY BUTYLPHENOL (4-TBP) TOXICITY: IMPLICATIONS FOR OCCUPATIONAL VITILIGO.

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Environmental exposure to 4-TBP causes patchy depigmented skin due to destruction of melanocytes in occupational vitiligo. Evidence suggests that oxidative stress induced by α-quinone of 4-TBP may be responsible for melanocyte cell death. Since glutathione S-transferase (GST) mediated conjugation with glutathione (GSH) protects against quinone induced oxidative stress, effect of inhibition of GST activity and cell GSH content on 4-TBP-induced toxicity was investigated. Melanoma cells (M19), MM.1 and SK.MEL-19 (M19) and SK.MEL-23 (C22) maintained in Dulbecco’s Modified Eagle Medium (10% bovine serum, 1% antibiotic/antimycotic) were seeded (1x10⁵ cells/well) in 96 well plates. Approximately 24 hours post-seeding, cells were exposed to 0, 100, 250 or 500 μM concentration of 4-TBP in the absence or presence of 100, 150, 200μg/ml GST inhibitors etharic acid (EA) or 1,2-epoxy-3,5-dinitrophenyl)propane (ENPP). Toxicity was determined at 72 hours using MTS cell proliferation assay and data expressed as percent control±SD. Expression of GST isoforms was identified by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) using specific primers. At the end of 72 hours, neither cell line was susceptible to cytotoxic potential of 4-TBP at all concentrations tested. Although total GSH content were comparable, M19 had significantly higher GST activities compared to C22 cells. Pretreatment of both cell lines (6 hours) with 1mM BSO significantly decreased cell GSH content but did not influence 4-TBP (≥250μM) toxicity. In presence of EA, a known inhibitor of GST isoforms (μ, π, α), both cell lines were equally sensitive to 4-TBP at 500μM concentration. In contrast, at 100μg/ml ENPP, which preferentially inhibits GST (α) activity, there was increased sensitivity of C22 cells to 4-TBP (250 ≤ 500μM) toxicity as compared to M19 cells. The above data and differential expression of GST isoforms between C22 & M19 cells suggest that GST (0) influences 4-TBP toxicity in melanoma cells.

**PS 1055**

**MICROBIAL TRANSFORMATION PRODUCTS OF BROMINATED FLAME RETARDANTS RESULTS IN CHEMICAL SPECIFIC LESIONS IN THE DEVELOPING ZEBRAFISH (DANIO RERIO) EMBRYO.

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Our goal is to determine the environmental fate and the effects of brominated flame retardants (BFRs) and their metabolites on aquatic species. This study focused on tetrabromobisphenol-A [TBBPA, 4,4’-isopropylidenebis (2,6-dibromophenol)], and its microbial transformation products, bisphenol A (BPA) and tetrabromobisphenol A –dimethyl ether (TBBPA DME). TBBPA is one of the most abundant BFRs in use today and is persistent in the environment. Because TBBPA exposure appears to be highest in young organisms, there is increasing concern about the potential developmental effects of these compounds. Our hypothesis is that embryonic exposure of developing fish to BFRs results in abnormal development, as a result of altered expression and activity of the matrix metalloproteinases (MMPs). MMPs are a family of Zn2+ and Ca2+ dependent proteases that are involved in the regulation of tissue morphogenesis through altering matrix metabolism, cell proliferation and angiogenesis. Developing zebrafish were exposed to TBBPA and its microbially-mediated transformation products, Bisphenol A (BPA) and TBBPA dimethyl ether (TBBPA DME). TBBPA (5, 1.5, 0.75 μM) exposed embryos exhibited the highest levels of mortality and lesion occurrence, including pathoneumonic lesions such as trunk and tail malformation. BPA (15, 10, 5 μM) and TBBPA DME (10, 5, 1μM) exhibited edema and hemorrhage in a dose-dependent manner. Additionally, both TBBPA and BPA delayed hatching of zebrafish embryos, unlike TBBPA DME, which did not appear to alter hatching. TBBPA exposure also increased expression of MMP-2, MMP-9 and MMP-13; whereas exposure to BPA or TBBPA DME exposure did not. The results presented here illustrate that in the embryonic larval assay system TBBPA is more acutely toxic than BPA or TBBPA DME. Furthermore, these data suggest that one mechanism of TBBPA-induced lesions may be through alteration in MMP gene expression. Supported by the Hudson River Foundation.

**PS 1056**

**DIFFERENTIAL REGULATION OF IL-8 EXPRESSION BY HUMAN AIRWAY EPITHELIAL CELLS EXPOSED TO DIESEL PARTICLES.


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Diesel exhaust particles (DEP) are a ubiquitous component of ambient particulate matter. Exposure to DEP induces inflammatory signaling characterized by activation of NFκB and AP-1 in cultured human airway epithelial cells (HAEc) and in bronchial biopsies from human subjects exposed to DEP. IL-8 is a major proinflammatory chemokine synthesized by HAEc whose expression is increased by inflammatory stimuli. Although DEP exposure is known to cause increases in IL-8 mRNA levels, the mechanism by which this occurs is not well understood. To identify signaling pathways through which DEP induces IL-8 expression, we examined the role of response elements in the IL-8 promoter in BEAS2B cells exposed to 10–9 to 10–7 mg/ml DEP containing low (L-DEP), intermediate (I-DEP), or high (H-DEP) organic content for 4 hr. Exposure to these DEP differentially induced IL-8 expression in both primary human lung epithelial cells and BEAS2B cells with similar ranked potencies: H-DEP > L-DEP > I-DEP. Through the use of IL-8 lentiviral promoter reporters, we show that treatment with H-DEP or L-DEP stimulates IL-8 promoter reporter activity while I-DEP fails to induce detectable IL-8 promoter reporter activity. Furthermore, mutation of the NFκB response element abolished H-DEP-induced IL-8 promoter reporter activity. In contrast, IL-8 promoter reporter activity induced by exposure to H-DEP was not affected by the ablation of the NFκB response element. Data obtained independently using BEAS2B cells transfected with an lentiviral promoter reporter carrying a tandem repeat of the consensus NFκB binding site confirmed that L-DEP, but not H-DEP activates NFκB -dependent transcriptional activity. These data show that DEP of varying organic content can induce IL-8 expression by multiple, yet specific, mechanisms in the human lung. This abstract of a proposed presentation does not necessarily reflect EPA policy.

**PS 1057**

**DEVELOPMENT AND VALIDATION OF AN INTEGRATED GENOME-WIDE APPROACH TO DETECT CHROMOSOMAL STRUCTURAL ABERRATIONS.

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Assessing the genotoxic properties of persistent and emerging contaminants is paramount in establishing proper safety standards and regulations. Current genotoxicity assays are optimized to detect single nucleotide mutations or large chromosomal aberrations and have limited capability of integrating alterations with a reference genome sequence. However, undetectable by most of these assays are structural chromosomal aberrations that range in size from a few thousand to a few million base pairs. These chromosomal aberrations can possibly lead to altered gene expression, adverse developmental effects, and cancer. It is thus important to develop a means by which to efficiently determine if specific compounds are capable of altering chromosome structure at this level and to directly integrate these structural alterations with a genome sequence of interest. In this study, a molecular cytogenetic technique called array comparative genomic hybridization (array CGH), which is used to detect and map chromosomal imbalances in an unbiased genome-wide fashion, was developed and applied in a genotoxicity assay to assess the potential for chemicals to induce chromosomal aberrations. The array CGH technology measures relative DNA copy number by competitive hybridization of a test and a reference sample to a microarray containing genomic DNA elements. Recently, a genome-wide array CGH platform for the zebrafish model system was constructed. The zebrafish array CGH platform contains 385,000 oligonucleotide probes spaced across the genome (with a median probe spacing of 3.2 kb). The goal of this study was to validate the use of the array CGH technology in a toxicological-based assay using known genotoxic compounds (e.g., cisplatin and ethyl methanesulfonate). Chemical-specific genomic imbalances are being detected, proving the utility of this approach to detect chromosomal structural alterations. Upon validation this assay will then be applied to start identifying biomarkers of genotoxicity following exposure to environmental stressors using the zebrafish model system.
DNA double-strand break (DSB) repair. To investigate whether VPA affects the activity of DNA DSB repair, CHO 33 cells containing the neo direct repeat recombinator substrate were transfected with either the Saccharomyces cerevisiae mitotic endonuclease I-SceI to induce a site specific DSB within the recombinator substrate or the empty plasmid, pGem. Cells were then exposed to 5 mM VPA for 24 hrs and two weeks later, the frequency of HR was determined by counting the number of functional neo expressing G418-resistant colonies per cell plated. A significant increase in the frequency of HR was observed in the presence (I-SceI) or in the absence (pGem) of a DSB after exposure to VPA; however there was no increase in the fold difference in HR between VPA and vehicle (media) exposed I-SceI transfected cells compared to cells transfected with pGem. To determine whether VPA induces DNA DSBs to elicit repair, CHO 33 cells were exposed to 5 mM VPA for 10, 16 or 24 hrs and γ-H2AX foci, a marker of DNA DSBs, was detected by immunofluorescence microscopy. A significant increase in the number of γ-H2AX foci per cell was observed for all time points with the greatest increase at 16 hrs after exposure to VPA. These results suggest that VPA does not affect the activity of HR but induces DNA DSBs to elicit HR repair. [Support: CIHR]

PS 1062 THE WERNER SYNDROME PROTEIN FUNCTIONS IN REPAIR OF CR(VI) INDUCED STALLED DNA REPLICA FORKS.

F. Liu, A. Batchowski and P. L. Opresko. Department of Environmental and Occupational Health, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA.

Werner syndrome, an autosomal recessive premature aging disease characterized by cancer predisposition and genetic instability, results from loss of the WRN helicase damage. Although a few reports on the cytotoxicity of CNTs have been published, very few report about their toxicity or genotoxicity in mammalian cells. We compared the clastogenic/genotoxic potential of pristine and purified oxidized multi-walled carbon nanotube (MWCNT) in bone marrow cells of Swiss-Webster mice; using mitotic index (MI), chromosome aberrations (CA), micronuclei (MN) formation and DNA damage in peripheral blood leukocytes as toxicological endpoints. Thirty five mice were randomly divided into seven groups; five for each group were used in this study. Pristine MWCNT was directly injected into the mice. Chromosome, micronuclei preparation was obtained from bone marrow and leukocyte DNA damage following standard protocols. The results demonstrated that MWCNT exposure significantly increased the number of structural chromosomal aberrations, the frequency of micro-nucleated cells, the level of DNA damage, and decreased the mitotic index in treated groups compared to control groups. In summary purified oxidized MWCNT has more clastogenic/genotoxic potential than the pristine form as measured by the bone marrow CA, MN and DNA damage in Swiss-Webster mice. Our studies do not imply that CNTs should be abandoned for biological or medical purposes. However, our proposed mechanism of toxicity of these nanomaterials based on their surface properties can assist material scientists to design/synthesize biocompatible materials. It also assist toxicologist to further characterize clastogenicity/genotoxicity of dispersed carbon nanomaterials in vitro or in vivo studies.

PS 1061 CYTOGENETIC EVALUATION OF MULTI-WALLED CARBON NANOTUBE TOXICITY IN SWISS-WEBSTER MICE.

A. Parilla1, S. Parilla1, S. Hussain1, J. Schalager2 and P. Tchounwou2, 1Jackson State University, Jackson, MS, 2Air Force Research Laboratory, Dayton, OH and Emory University, Atlanta, GA.

The development of nanotechnologies may lead to considerable release of nanomaterials in the environment, potentially harmful to human health. Among the nanomaterials, multiwalled carbon nanotubes (MWCNT) are commercially used in many products which can be in direct contact with populations. They not only are produced by high-technology laboratories but also are found in particular matters formed from ordinary combustion of fuel gases. Although a few reports on the cytoxicity of CNTs have been published, very few report about their toxicity or genotoxicity in mammalian cells. We compared the clastogenic/genotoxic potential of pristine and purified oxidized multi-walled carbon nanotube (MWCNT) in bone marrow cells of Swiss-Webster mice; using mitotic index (MI), chromosome aberrations (CA), micronuclei (MN) formation and DNA damage in peripheral blood leukocytes as toxicological endpoints. Thirty five mice were randomly divided into seven groups; five for each group were used in this study. Pristine MWCNT was directly injected into the mice. Chromosome, micronuclei preparation was obtained from bone marrow and leukocyte DNA damage following standard protocols. The results demonstrated that MWCNT exposure significantly increased the number of structural chromosomal aberrations, the frequency of micro-nucleated cells, the level of DNA damage, and decreased the mitotic index in treated groups compared to control groups. In summary purified oxidized MWCNT has more clastogenic/genotoxic potential than the pristine form as measured by the bone marrow CA, MN and DNA damage in Swiss-Webster mice. Our studies do not imply that CNTs should be abandoned for biological or medical purposes. However, our proposed mechanism of toxicity of these nanomaterials based on their surface properties can assist material scientists to design/synthesize biocompatible materials. It also assist toxicologist to further characterize clastogenicity/genotoxicity of dispersed carbon nanomaterials in vitro or in vivo studies.

PS 1059 CHARACTERIZATION OF VALPROIC ACID-INITIATED HOMOLOGOUS RECOMBINATION.

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Use of the first-line antiepileptic agent, valproic acid (VPA) during pregnancy is associated with an increased incidence of major congenital malformations; however the molecular mechanism of its teratogenicity has not been elucidated. Our previous study showed an increase in the frequency of homologous recombination (HR) repair in CHO 33 cells to Cr(VI) at noncytotoxic concentrations and stalled DNA replication forks induced by DNA damaging agents. Chromium acts as a human carcinogen through multiple mechanisms, including the induction of genomic instability by interfering with DNA replication progression through formation of bulky DNA adducts or interstrand cross-links. To test the hypothesis that WRN functions in the restoration of stalled and broken replication forks caused by Cr(VI), we exposed multiple cell lines to Cr(VI) at nontoxic concentrations and evaluated cellular response with various markers. We found that cells deficient in WRN were hypersensitive to Cr(VI), suggesting WRN functions in the repair of Cr(VI) induced damage.

PS 1058 TOPOISOMERASE II INHIBITION INVOLVED IN DNA DOUBLE-STRAND BREAKS BY EMODIN AND ITS GENOTOXICITY.

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The molecular mechanism of its teratogenicity has not been elucidated. We previously showed an increase in the frequency of homologous recombination (HR) repair in CHO 33 cells transfected with either the Saccharomyces cerevisiae mitotic endonuclease I-SceI to induce a site specific DSB within the recombinator substrate or the empty plasmid, pGem. Cells were then exposed to 5 mM VPA for 24 hrs and two weeks later, the frequency of HR was determined by counting the number of functional neo expressing G418-resistant colonies per cell plated. A significant increase in the frequency of HR was observed in the presence (I-SceI) or in the absence (pGem) of a DSB after exposure to VPA; however there was no increase in the fold difference in HR between VPA and vehicle (media) exposed I-SceI transfected cells compared to cells transfected with pGem. To determine whether VPA induces DNA DSBs to elicit repair, CHO 33 cells were exposed to 5 mM VPA for 10, 16 or 24 hrs and γ-H2AX foci, a marker of DNA DSBs, was detected by immunofluorescence microscopy. A significant increase in the number of γ-H2AX foci per cell was observed for all time points with the greatest increase at 16 hrs after exposure to VPA. These results suggest that VPA does not affect the activity of HR but induces DNA DSBs to elicit HR repair. [Support: CIHR]

PS 1057 ELEVATED ETHYL METHANESULFONATE (EMS) IN NELFINAVIR MESYLATE (VIRACEPT®, ROCHE): ANIMAL STUDIES CONFIRM TOXICITY THRESHOLD AND ABSENCE OF RISK TO PATIENTS.

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Background: Roche’s protease inhibitor nelfinavir mesylate (Viracept) produced between March 2007–June 2007 was found to contain elevated levels of ethyl methanesulfonate (EMS), a known mutagen (alkylator) – leading to a global recall of the drug. EMS levels in a daily dose (2500mg Viracept/day) were predicted not to exceed dose of ~2.7μg/day (~0.05 ng/kg/d) based on 50kg patient. As existing toxicity data on EMS did not permit an adequate patient risk assessment, a comprehensive animal toxicity evaluation of EMS was conducted. Methods: Two studies for DNA damage were performed in mice: chromosomal damage was assessed using a micronucleus assay and gene mutations were detected using the MutauMouse transgenic model. In addition, experiments designed to extrapolate animal exposure to humans were undertaken. Results: Studies for chromosomal damage and mutations in mice demonstrated a clear threshold effect with EMS at 25μg/kg/day, under chronic dosing conditions. Exposure analysis (Cmax) demonstrated that ~370-fold higher levels of EMS, than that ingested by patients, are needed to saturate known, highly-conserved, error-free, mammalian DNA repair mechanisms for alkylations. Conclusions: Animal studies confirmed that patients who took nelfinavir mesylate with elevated levels of EMS are at no increased risk for carcinogenicity or teratogenicity over their background risk, since mutations are prerequisites for such downstream events. These findings are applicable to >40 marketed drugs that contain mesylate.

PS 1056 THE WERNER SYNDROME PROTEIN FUNCTIONS IN REPAIR OF CR(VI) INDUCED STALLED DNA REPLICATION FORKS.

F. Liu, A. Batchowski and P. L. Opresko. Department of Environmental and Occupational Health, University of Pittsburgh Graduate School of Public Health, Pittsburgh, PA.

Werner syndrome, an autosomal recessive premature aging disease characterized by cancer predisposition and genetic instability, results from loss of the WRN helicase damage. Although a few reports on the cytotoxicity of CNTs have been published, very few report about their toxicity or genotoxicity in mammalian cells. We compared the clastogenic/genotoxic potential of pristine and purified oxidized multi-walled carbon nanotube (MWCNT) in bone marrow cells of Swiss-Webster mice; using mitotic index (MI), chromosome aberrations (CA), micronuclei (MN) formation and DNA damage in peripheral blood leukocytes as toxicological endpoints. Thirty five mice were randomly divided into seven groups; five for each group were used in this study. Pristine MWCNT was directly injected into the mice. Chromosome, micronuclei preparation was obtained from bone marrow and leukocyte DNA damage following standard protocols. The results demonstrated that MWCNT exposure significantly increased the number of structural chromosomal aberrations, the frequency of micro-nucleated cells, the level of DNA damage, and decreased the mitotic index in treated groups compared to control groups. In summary purified oxidized MWCNT has more clastogenic/genotoxic potential than the pristine form as measured by the bone marrow CA, MN and DNA damage in Swiss-Webster mice. Our studies do not imply that CNTs should be abandoned for biological or medical purposes. However, our proposed mechanism of toxicity of these nanomaterials based on their surface properties can assist material scientists to design/synthesize biocompatible materials. It also assist toxicologist to further characterize clastogenicity/genotoxicity of dispersed carbon nanomaterials in vitro or in vivo studies.
Consistent with this, WRN translocates into nucleoplasmic foci from the nucleoli in response to Cr(VI) in a time- and dose-dependent manner, and localizes to sites of Cr(VI) induced γH2AX foci, a marker of DNA damage. Replacement with Cr(VI)-free medium led to WRN relocation back to the nucleoli and a reduction in γH2AX foci within 48 h. However, γH2AX foci persisted long after WRN deficient cells following recovery. Furthermore, Cr(VI) stimulated accumulation of cells in S-phase, indicative of DNA replicative stress, and increased WRN nucleoplasmic and γH2AX foci in S-phase cells. Therefore, we conclude that WRN functions in repair of DNA damage resulting from replication fork stress induced by noncytotoxic levels of Cr (VI). This novel observation also implies that replicative stress inducing environmental genotoxins may enhance premature aging and age-related disease. Supported by NIEHS grant ES015051-01 (PLO).

PCB3-Cat significantly increased the SCE levels. After treatment with 7.5 – 10 μg/ml of PCB2- and PCB3-HQ ~40% and ~70%, respectively, of their metaphase chromosomes were dark-stained, indicating a strong delay in cell cycle progression. Most of the cells were tetraploids (4N). Such tetraploid cells, which are genetically unstable, can arise by a variety of mechanisms, including mitotic slippage, cytokinesis failure, viral-induced cell fusion or karyokinesis. Since nearly all cancer cells are hyperdiploid, polyploidization as an intermediate to aneuploidy due to uneven chromosome loss are hypothesized as underlying mechanisms of carcinogenesis. Understanding of the mechanism and structure-activity-relationship of this astonishing activity of some PCB hydroquinones is needed to fully understand the significance of this effect for human health. (Supported by NIEHS grant ES015661 and DOI DAMD17-02-1-6241)

Poly (ADP-ribose) polymerase-1 (PARP-1) is a key element in recognition and regulation of DNA repair or entry into apoptosis and is an important mechanism to keep homeostasis after exposure to noxious agents. The experiments were undertaken to determine the expression and function and inter-individual differences of PARP-1 in normal human lung cells. Another goal was to monitor PARP expression and function over a longer time period. Normal human bronchial epithelial cells (NHBE) and peripheral lung cells (PLC) from lung cancer patients were grown as explants. The expression of PARP-1 was examined by Western Blot analysis of the total protein fraction of lung cells. PARP-1 activity was determined immunohistochemically after induction of DNA damage with H2O2 (0.05 – 0.3 mM, 5 min). The fluorescence signal for ADP-ribose polymers attached to chromatin in nickel protein correlates well with the applied concentrations of H2O2. To investigate the influence of PARP-1 protein was expressed in all explant cultures from bronchial epithelium. The levels for PARP protein differed between individuals by a factor of 2.3 in the first explants. Three cases were followed for more than 100 days. The expression levels were measured or by a factor 1.3 to 1.4 over this time period. PARP-1 protein activity differed by a factor of 3.1 in NHBEs obtained from the first generation of explants from 11 cases. PARP-1 activity is present in NHBEs until the 8th and in PLCs until the 12th week and declined to about half of the start level. In summary, PARP-1 protein is well expressed over a long time by normal bronchial cells in culture. Its expression is variable in cells obtained from different individuals, which may be due to life style factors. PARP-1 protein expression and function is conserved in primary lung cells in vitro over a time period of 8 to 12 weeks. This indicates that primary lung cells cultures are a useful experimental model to study the control mechanisms for PARP-1 expression and function in human lung.

Carbon nanotubes are increasingly used in various consumer applications, but information on their possible carcinogenicity is still scarce. We examined the genotoxicity of single-wall (SWCNT; length 1-5 μm, outer diameter <2 nm; SES Research) and multi-wall (MWCNT; length 1-2 μm, outer diameter 10-30 nm; SES Research) carbon nanotubes in vitro. Genotoxicity was assessed by the analysis of DNA damage in human bronchial epithelial cells (BEAS 2B) and mesothelial cells (MeT SA) and micronuclei (MN) in BEAS 2B cells. The cells were cultured with various doses (5-80 μg/cm²) of SWCNT and MWCNT. The single cell gel electrophoresis (comet) assay was applied to study DNA damage, and the induction of MN was examined by the cytokinesis-block method. The treatment lasted for 24 or 48 h in the comet assay and 48 or 72 h in the MN assay. We also examined structural chromosomal aberrations (CAs) in 72-h cultures of isolated human lymphocytes after a 24-h or 48-h treatment with both materials at several doses (6.25-300 μg/ml). In BEAS 2B cells, only SWCNT induced DNA damage after the 48-h treatment, while both nanomaterials increased DNA damage in MeT SA cells. Only SWCNT induced MN in BEAS 2B cells. Both types of nanotubes produced an increase in the frequency of lymphocytes with CAs after the 48-h treatment, but not after the 24-h treatment. Both chomarid-type and chromosome-type aberrations were induced. In conclusion, MeT SA cells appeared to be more sensitive than BEAS 2B cells to the induction of DNA damage by carbon nanotubes. In BEAS 2B cells, SWCNT were more genotoxic than MWCNT. Further studies are required to clarify, whether the increase of CAs by carbon nanotubes is due to higher cumulative effects after the longer treatment time or to an increased ability of the nanomaterial to get access to the nucleus in cell division and to interfere with DNA and chromosomes.
1067 DRUG-INDUCED DNA DAMAGE IN CANINE PERIPHERAL BLOOD: A COMPARISON BETWEEN CYCLOPHOSPHAMIDE AND ETOPOSIDE

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A variety of studies have been conducted recently to clarify the risk of genetic damage following exposure to methylphenidate (MPH), a frequently prescribed drug for treatment of attention deficit/hyperactivity disorder. Although most reported no changes in genetic damage endpoints associated with MPH exposure, one recent study [Andreazza et al. 2007, Prog Neuropsychopharmacol Biol Psychiatry 31(6):1282-8] reported an increase in DNA damage detected by the comet assay in blood and brain of juvenile and adult male rats exposed to 1, 2, or 10 mg/kg MPH for up to 28 days; no increases in micronucleated lymphocyte frequencies were observed. To clarify this report of MPH-induced DNA damage, we treated adult male Wistar-Han rats with 0.2, 10 or 25 mg/kg MPH by gavage once daily for 28 consecutive days and assessed micronucleated reticulocytes (MN-RET) in blood, and DNA damage in blood, brain, and liver 4 hr after the last dosing. At the end of the 28-day exposure period, no clinical signs of toxicity were observed and weight gain in MPH-exposed rats was similar to controls. Flow cytometric evaluation of blood samples revealed no significant increases in MN-RET, and the %RET among total erythrocytes, a measure of bone marrow toxicity, was unaltered at any dose. Furthermore, evaluation of primary DNA damage in leukocytes, liver, striatum, hippocampus, and frontal cortex tissues using the alkaline (pH > 13) comet assay revealed no increases in MPH-treated rats in any of the three treatment groups. Finally, neurohistopathological examination of 30 sub-anatomic sites, including grey and white matter, in the 3 brain tissues assessed for DNA damage showed no treatment-related changes. Thus, the earlier reported observations of MPH-induced DNA damage in blood and brain of rats exposed for 28 days were not confirmed in this study.

1068 CYCLOPHOSPHAMIDE AND ETOPOSIDE CANINE STUDIES DEMONSTRATE THE CROSS-SPECIES POTENTIAL OF THE PERIPHERAL BLOOD MICRONUCLEATED RETICULOCYTE ENDPOINT

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Accumulating data suggests that peripheral blood micronucleated reticulocytes (MN-RETs) represent an endpoint of cytogenetic damage, even in species with efficient splenic filtration function. Studies performed evaluated both dose response and time-course of micronucleus (MN) induction in the bone marrow and blood of beagle dogs after dosing with cyclophosphamide (CP) or etoposide (Etopo). CP was administered daily via intravenous injection for 5 days at 0, 6.25, 12.5 or 25 mg/m2/day. Eto was administered via iv injection for 2 days at 0.1, 0.56, 6.25, and 12.5 mg/m2/day. Blood specimens were collected for analysis before dosing as well as at several intervals during treatment, and bone marrow was prepared at necropsy. Blood was prepared using the In Vivo MicroFlow® method and analyzed at Covance, while bone marrow was analyzed at Covance via microscopy (May-Grünwald and Giemsa stain) and acridine orange staining. Robust MN-RET induction was observed in the blood of all CP-treated dogs by Day 4, with dose-related increases evident by Day 3. Comparable dose-related increases were observed in the bone marrow with microscopy-based scoring. While significant MN induction was not observed in the bone marrow of dogs treated with Eto at 1.563 mg/m2/day, marked dose-related increases were noted in both compartments for the 6.25 and 12.5 mg/m2/day groups. Collectively, these results demonstrate the utility and sensitivity of blood-based automated MN-RET measurements in canines. These data have important implications in regard to the reduction and refinement of animal usage in genetic toxicology investigations.

1069 PERFORMANCE CHARACTERISTICS OF A MINIATURIZED AND AUTOMATED IN VITRO MICRONUCLEUS ASSAY

S. Bryce, S. Phonepheswath, S. Avlasevich, S. Raja, J. Benis and S. D. Dertinger. 1Covance Laboratories, Rochester, NY.

This laboratory has described a flow cytometric method for scoring in vitro micronuclei (MN) [Environ. Mol. Mutagen. (2006) 47:56-66]. This "In Vivo MicroFlow" method labels nontoxic and mid-late-stage apoptotic cells with ethidium monoazide. Cells are then washed, stripped of their cytoplasmic membranes, and incubated with RNase plus a pan-nuclear acid dye. Here, we report modifications to the method whereby all procedures are accomplished in the same 96 well plate. For these experiments, human TK6 cells were treated continuously for 24 - 30 hrs with 68 diverse chemicals over a range of concentrations up to 10 mM. With the assistance of liquid handlers, treatments and staining occurred automatically. Furthermore, a Becton Dickinson HTS device was used to deliver the contents of each well to a FACSCanto flow cytometer for walk-away data acquisition. Flow cytometry-based MN frequencies were calculated based on the analysis of 10,000 nuclei per well. Since a consistent number of latex particles were added to each specimen, nuclei to bead ratios were collected concurrently with MN frequencies, and these data were used to calculate relative survival values. Top concentrations were limited to 10 mM, solubility, or else 50% relative survival. Overall, the automated assay agreed well with expected in vitro cytogenetics results (correlation = 88.2%; positive predictivity = 87.5%, negative predictivity = 92.9%). These data suggest that in vitro MN screening can be accomplished in a miniaturized and highly automated format. Further work is needed to assess the transferability of the method through inter-laboratory trials.

1070 BATTERY OF GENOTOXICITY STUDIES CONDUCTED ON A GROUP OF STRUCTURALLY RELATED NITRILES


A battery of genotoxicity studies were conducted on a group of 8 structurally related nitriles (Benzonitrile; Cinnamyl nitrile; 3-Methyl-5-phenylpentanenitrile; 2,2,3-Trimethylcyclopent-3-enylacetonitrile; 3-Methyl-5-phenylpent-2-eno-nitrile; α-Cyclohexylidene benzeneacetonitrile; Citronellyl nitrile; Dodecanenitrile) that are used as fragrance materials. All the studies were conducted according to the OECD Testing Guidelines. In an in vitro chromosome aberration test using Chinese Hamster V79 cell line,citronellyl nitrile induced structural chromosome aberrations in presence of S9. In an in vitro micronucleus assay using V79 cell line, cinnamyl nitrile and 3-Methyl-5-phenylpent-2-eno-nitrile induced micronuclei in V79 cells in the presence and absence of S9, while 3-Methyl-5-phenylpentanenitrile induced micronuclei in the presence of S9 activation only. 2,2,3- Trimethylcyclopent-3-enylacetonitrile induced statistically significant increase in micronuclei in presence of S9, however there was a lack of dose dependency and the values were within the historical range of the control. No effects were observed in an in vitro chromosome aberration test using Chinese Hamster V79 cell line with Dodecanenitrile. No effects were observed in in vivo micronucleus assays in NMRI mice (high doses ranged from 200 to 2000 mg/kg) in all the five nitriles (cinnamyl nitrile; benzonitrile; 3-Methyl-5-phenylpentanenitrile; 3-Methyl-5-phenylpent-2-eno-nitrile; α-Cyclohexylidene benzeneacetonitrile) that were tested. It is concluded that these 8 nitriles are not genotoxic.

1071 HIGH CONTENT ANALYSIS OF PHOSPHO-H2AX AND PHOSPHO-ATM IN CONJUNCTION WITH THE IN VITRO MICRONUCLEUS TEST TO DETERMINE THE CLASTOGENIC POTENTIAL OF GENOTOXIC COMPOUNDS


The in vitro micronucleus assay is used to detect genotoxic chemicals that induce the formation of micronuclei within interphase cells. The test is capable of detecting both clastogens and aneugens, but is not capable of directly distinguishing between clastogenic and aneugenic compounds, three proteins involved in the cells response to DNA damage were studied: H2AX, ATM and p53. H2AX is a histone protein that is rapidly phosphorylated by ATM, a serine/threonine kinase, in response to DNA damage. ATM also autophosphorylates in response to genotoxic stress. TP53 is a tumor suppressor gene that is phosphorylated in response to DNA damage prohibiting its degradation resulting in the accumulation of p53 in the nucleus. For this study, BALB/3T3 A31 cells were treated with various concentrations of different aneugenic and clastogenic compounds for both 4h and 20h. After fixation, the cells were stained with either Phospho-ATM and p53 Activation HCS Reagent kit or Phospho-H2AX HCS Reagent kit. Plates were run on the Cellomics ArrayScan VT1 Reader to measure the percent micronucleus frequency, p-ATM and p-H2AX expression and p53 translocation to the nucleus. An increase in both p-ATM and p-H2AX after 4h treatment of the three clastogenic compounds was found. An increase in both p-ATM and p-H2AX was also seen after 20h treatment of mitomycin and cytarabine, but not with chlorambucil. Expression of p53 in the nucleus was unchanged for...
both treatment times. In contrast, aneugenic compounds showed no change or a
decline in p-ATM and p-H2AX staining at both 4h and 20h. These initial findings
indicate a correlation between clastogenic compound treatment and increases
in p-ATM and p-H2AX expression.

1072 USING HIGH CONTENT CONTENT TO EVALUATE THE USE OF RECOVERY TIME AND ITS EFFECT ON MICRONUCLEUS FREQUENCY IN MONONUCLEATE CELLS.

The micronucleus assay is used to detect potential aneugenic and clastogenic compounds in cells that undergo cell division. Not finding the correct doubling time of all cell lines could lead to false negatives or decreases in micronucleus frequency. In this study various cell lines (CHO-K1, CHL, and Balb 3T3/31) were treated with three aneugenic compounds (vinblastine, mitomycin C, and chlorambucil) for 20h, followed by either a 24h rest period (removing compound and replacing with media) or immediately fixing the plate. Cells were fluorescein stained so that the nuclei, micronuclei, and cytoplasm were visible. Plates were run on the Cellomics ArrayScan VTI Reader, evaluating a minimum of 1000 targeted cells per well. Values for Micronucleus Frequency were calculated and cells were evaluated based on set guidelines (intact cytoplasm, not mitotic, micronucleus 1/3 diameter of nucleus, etc.). Under normal circumstances, it is during cell division that micronuclei are created after compound treatment. For all cell types tested, both clastogens and aneugens exhibited a fold-difference over control for micronucleus frequency equal to or greater in cells that had a recovery period than those treated for only 20h. Therefore, letting the cells recover from treatment allowed the majority of cells to undergo cell division, giving rise to micronuclei at proper genotoxic concentrations.

1073 MECHANISM(S) OF DNA DAMAGE INDUCTION BY 4-MONOCHLOROBIPHENYL (PCB3) METABOLITES.
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PCB3 is readily metabolized to dihydroxy-metabolites and quinones. The PCB3 hydroquinone (PCB3-HQ) induces chromosome loss in Chinese Hamster V79 cells, whereas the para-quinone (PCB3-pQ) very efficiently induces gene mutations and chromosome breaks. We hypothesized that the genotoxicity of HQ requires enzymatic activation by peroxidases, whereas the p-Q reacts directly with DNA and/or proteins. We used myeloperoxidase (MPO)-rich HL-60 and MPO-deficient Jurkat cells to measure cytotoxicity, DNA damage (Comet assay), MPO activity, intracellular levels of reactive oxygen species (ROS), free –SH groups, and GSH after exposure to PCB3 metabolites and also examined the effects of normal/low temperature, pre-treatment with a MPO inhibitor (sucinylacetone, SA), or GSH depletion. PCB3-pQ increased intracellular ROS levels and induced DNA damage in both, HL-60 and Jurkat cells at 37°C and 6°C. It also strongly reduced intracellular free –SH groups and GSH in normal and GSH-depleted cells. Thus the ROS increase could be the result of GSH-depletion or non-enzymatic autoxidation of the resulting PCB3-HQ-GSH adduct. PCB3-HQ had no effect on GSH in HL-60 cells and reduced free –SH groups only at the highest concentration in GSH depleted cells. Moreover, PCB3-HQ induced DNA damage and ROS production only at 37°C in HL-60 cells, not at 6°C or in Jurkat cells at either temperature; no significant DNA damage and ROS production was observed in HL-60 cells at 37°C if MPO activity was inhibited by SA. These studies show that the effects of PCB3-HQ are enzyme dependent, i.e. PCB3-HQ is oxidized by MPO in HL-60 cells with the generation of ROS and induction of DNA damage. However, PCB3-pQ may produce DNA damage by direct interaction with DNA or nucleic acids, i.e. proteins like topoisomerase II, or possibly by indirectly increasing intracellular ROS levels by GSH depletion. These different modes of action explain not only the different types of genotoxicity observed previously, but also suggest different organ specificity of these genotoxicities. (Supported by NIEHS P42 ES013661 and DAMD17-02-1-0241)

1074 MECHANISM OF GENOTOXIC EFFECTS OF SILVER NANO Particles RELATED TO OXIDATIVE STRESS.
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In the single-cell gel electrophoresis (Comet assay) and micronucleus assay (MN assay) in human normal lung epithelial BEAS-2B cells, Ag-NPs stimulated DNA breakage and micronucleus formation in a dose-dependent manner (from 0.01
ug/ml to 10 ug/ml). The genotoxic effect of Ag-NPs (10 ug/ml) was partly blocked by scavenger agents such as mannitol (Mannitol, OH radical scavenger), catalase and sodium selenite (H2O2 radical scavenger), whereas SOD (superoxide dismutase, superoxide scavenger) completely blocked. Furthermore, in the modified comet assay using endonuclease HindIII, Ag-NPs induced a significant increase of DNA strand breaks compared with control levels, indicating that Ag-NPs stimulate oxidation of pyrimidine. Furthermore, in DCFH-DA assay, Ag-NPs showed a significant increase (p<0.01) of oxidative stress. These results indicate that oxidative stress of Ag-NPs, especially superoxide and oxidized pyrimidine play an important role in genotoxicity of silver nanoparticles.

1075 INVESTIGATIONS INTO THE GENOTOXIC POTENTIAL OF ORGANOPHOSPHATES.

Organophosphates (OP’s) are acutely toxic to man by inhibition of acetylcholinesterase (AChE) but chronic toxicity is less well explained. Several studies have suggested that OP’s can cause DNA damage in vitro. In this study the potential of dichlorvos (DCV), an oxon and chlorpyrifos (CPF) a thiophosphate to cause DNA damage was investigated using the Comet assay and micronucleus assay to measure the induction of cytotoxicity and DNA damaging signalling pathways. TK6 human lymphoblastoid cells in culture were exposed to either DCV or CPF (0.01, 0.1, 1.0 and 100μM) for 1 or 24 hours. DNA damage was measured using the Comet assay and cytotoxicity using the MTT assay. Western blotting was used to investigate DNA damage signalling and apoptosis by monitoring p33 induction and PARP cleavage in TK6 cells after 1, 2, 4 and 24 hours. The background DNA damage measured as olive tail moment (OTM) for 50 cells was <1 and increased with dose of OP. After 1 hour 100μM DCV gave an OTM of 4.04±0.04 (mean ± SE) whilst CPF gave a value of 2.35±0.72. 1μM DCV gave an OTM of 2.61±0.79 whilst 1μM CPF 1.45±0.26. After 24 hours 100μM DCV gave an OTM of 4.8±1.67 whilst CPF gave a value of 3.97±1.16. 1μM DCV gave an OTM of 2.15±0.69 whilst 1μM CPF 2.24±0.71. Both OP’s proved to be cytotoxic after 24 hours in TK6 cells at 100μM, a 40% or 80% loss occurred with DCV and CPF respectively. Western blotting analysis in TK6 cells showed that levels of cleaved PARP-1 slightly increased at 24 hours after 100μM DCV, indicative of apoptosis. The p33 level was increased, with a maximum induction at 4 hours after 100μM DCV. Lower doses of DCV showed no increase in p33 or cleaved PARP-1 after 24 hours. This study has confirmed that both DCV and CPF can induce DNA damage after exposure for an hour. This DNA damage leads to a subsequent induction of p33 and a loss in cell viability at the higher doses of OP. It will be important to investigate the nature of this DNA damage and the cellular repair mechanisms employed by the cell to re-store the integrity of the DNA (UK Home Office funded research)
**1077 COMET ASSAY ON RECONSTRUCTED HUMAN SKIN: AN ALTERNATIVE ASSAY IN GENOTOXICITY.**


Starting in March 2009, the testing ban in genotoxicity (and in other 3 endpoints) imposed by the 7th amendment to the European Cosmetic Directive will enter into force. To face this regulation, the EU Cosmetics Association’s (COLIPA) Genotoxicity Project Team has set up a project using reconstructed skin models. Generally, results obtained from monolayer cell-based in vitro genotoxicity assays are sensitive but lack specificity. The reconstructed tissues were chosen as biological models in the purpose to overcome this limitation and take into account the dermal route of exposure. The comet assay (together with the micronucleus assay) is one the endpoints extensively used in this project. Based on the scientific literature, the comet assay is a sensitive assay for screening genotoxic hazard. It is used in biomonitoring studies, early in drug/chemical development and for studying UV or other radiation related DNA damage. It can detect a large variety of DNA damage very early after the onset of the insult. Therefore, it can be used to screen for gene mutagens as well as chromosome damaging agents. Combined with antibodies and/or restriction enzymes, this assay can be used to get a mechanistic insight into the genotoxic insult. Several multi-laboratory studies have been conducted on the in vitro comet assay. Nevertheless no “validated” protocol is available. Here, the results obtained with the RealSkin (a reconstructed full-thickness skin model) are discussed. This model is a promising tool for detecting genotoxins as well as photo-genotoxins.

**1078 IN VITRO MICRONUCLEUS ASSAY USING A CO-CULTURE SYSTEM: TOWARDS NEW TOOLS FOR IN VITRO RISK ASSESSMENT OF DERMALLY APPLIED COMPOUNDS?**


In vitro reconstructed skin models such as Episkin® (reconstructed epidermis) and Realskin (reconstructed full-thickness: epidermis + living dermis) are biological models mimicking human skin. They are of growing interest in safety or efficacy pre-screening tests and for regulatory purposes as alternatives to animal testing (7th amendment to the European Cosmetic Directive, 2009). The aim of our work was to eventually the replacement of in vivo toxicity testing require the development of new complementary biological models and methods with improved ability to predict the genotoxic or other endpoint risk with in vitro data. This can be achieved if these new assays take into account the exposure conditions in a more relevant way than the current ones. To that end, new approaches using human reconstructed skin models for in vitro toxicology assessment are proposed. A co-culture system (made of Episkin® or RealSkin and target) cells was used to perform the micronucleus assay. The results obtained from six different compounds are shown. This way of using human reconstructed skin aims at improving the relevance of exposure conditions in in vitro genotoxicity assays for dermally applied compounds. The skin is indeed a biologically active barrier driving the exposure to compounds and their possible metabolites. The exposure of the target cells to a given substance can be assessed after topical application as was the case here. Episkin® and Realskin were used as a metabolically active tissue and a physiologic barrier. The test compound can be metabolized by the skin and/or by the target cells (± S9 if needed).

Metabolism is an important event to consider in genotoxicity and skin sensitization evaluation. Compared to cell models, a broad variety of chemicals with different pharmacokinetic properties can be evaluated in this system (after topical or systemic application). The data highlight the value-added of this co-culture system.

**1079 THE PHOTOCLASTOGENICITY OF FUCOCHRUMARINS: BERGAMOTTIN AND ISOPIMPINELLIN.**

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Phototoxic and photomutagenic properties have been reported for certain fucochrumarins, but cannot be attributed to all of the materials within the family since they constitute a highly diverse group of natural chemicals. Photoclastogenicity was tested for bergamottin and isopimpinellin, found in citrus oils, by chromosome aberration studies in cultured Chinese hamster ovary cells with and without the presence of ultra-violet light. Quadruplicate cultures were treated with the vehicle (DMSO) and duplicate cultures were treated with 6.597 - 120.0 μg/ml bergamottin, 5.0 - 225.0 μg/ml isopimpinellin, or the positive controls. After incubation in the dark for 15 minutes, flasks were exposed to 700 mJ/cm2 UV light or remained non-irradiated. An Atlas Suntest CPS+ solar simulator light source encompassing UVA and UVB wavelengths was used. Post irradiation, all flasks were incubated for at least 2 hours. Treatment media was removed and cultures were incubated for 17 hours before harvesting. Approximately 1.5 hours prior to harvest, colchicine was added to arrest dividing cells in metaphase. Slides were prepared with one hundred metaphases from each and analyzed for chromosome aberrations. The frequencies of cells with structural chromosome aberrations (including and excluding gaps) was determined and compared to negative (vehicle) controls and to equivalent concentrations treated in the absence of UV light. Treatment with bergamottin in the presence of UV light resulted in significant increases in structural chromosome aberrations at 8.246-20.13 μg/ml compared to vehicle controls. No such increases in structural chromosome aberrations were observed in the absence of UV light, indicating a photoclastogenic response. Isopimpinellin at 25-150 μg/ml induced structural chromosome aberrations that were similar to, and not statistically different from, vehicle controls in the presence of UV light. As such, isopimpinellin did not induce photoclastotoxicity.

**1080 COMPARISON OF BASELINE AND CHEMICAL-INDUCED DNA DAMAGE IN WISTAR HAN AND FISCHER 344/N RATS.**


The National Toxicology Program is considering the use of Wistar Han rats as a replacement for F344 rats in its toxicity and carcinogenicity bioassays. Therefore, studies were conducted to compare the baseline and chemical-induced DNA damage between these strains of rats. Three genotoxic chemicals evaluated previously in F344 rats were tested in Wistar rats using a combined 4-day protocol for in vivo evaluation of micronuclei induction in reticulocytes (MN-RET), as measured by flow cytometry, and DNA damage as assessed by the Comet assay. Two clastogens, ethyl methyisulfonate (EMS) and cyclophosphamide, and an aneugene, vincristine sulfate, were tested. Significant differences in MN-RET frequencies were measured between Wistar and F344 rats exposed to cyclophosphamide and vincristine sulfate, but not EMS. As assessed by the olive tail moment (OTM) endpoint in the Comet assay, there were significant strain differences in the extent of DNA damage in blood leukocytes following exposure to EMS and cyclophosphamide, but not vincristine sulfate. In liver, there were significant differences in OTM between the two strains when treated with EMS and vincristine sulfate. Statistical differences in DNA damage, as measured by OTM, was found in the duodenum, but not in blood leukocytes or liver. Our results indicate that, as compared to F344 rats, Wistar rats exhibit higher baseline levels of micronuclei and DNA damage in some tissues, and may be more susceptible to some genotoxic chemicals.
non-genotoxins. Taken together, these data indicate that flow cytometric scoring of MN can be accomplished in attachment cells using an efficient protocol that minimizes the number of manipulations required, and the high content assay appears capable of discriminating between aneugenic and clastogenic modes of action.

1082 PREGNANE X RECEPTOR (PXR) PROTECTS LIVER CELLS AGAINST DNA DAMAGES: EVIDENCE AND MECHANISMS.

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Pregnane X receptor (PXR) is a nuclear receptor that plays an important role in the pharmacokinetics of a broad spectrum of endogenous and xenobiotic compounds through coordinated-regulation of phase I, II enzymes and membrane bound transporters. Benzo[a]pyrene (BaP) is a known carcinogenic and ubiquitous environmental contaminant. In an earlier study, using HepG2 with stable-transfection of PXR, we found that PXR plays a role in reducing BaP-induced DNA damages as determined by P32-postlabeling experiments. There are three potential mechanisms to account for the overall reduction of BaP induced DNA damages: (1) PXR regulates metabolic detoxification of BaP; (2) PXR represses AhR thereby inhibiting AhR/CYP1A1-mediated metabolic activation of BaP; and (3) PXR regulates DNA repair machinery to reduce DNA damage. Evidence and mechanism (1) have been discussed in our earlier report (Toxi. Sci. 104, 67). In this study, we showed expres- sion of PXR in HepG2 cells suppressed AhR transcriptional activity. The CYP1A1 and CYP1B1 mRNA levels and EROD activity decreased significantly in PXR transfected HepG2 cells. Interestingly, the UV irradiation induced phospho-H2AX was significantly reduced in PXR-transfected HepG2 cells as determined by Immunofluorescence microscopy, suggesting PXR plays a role in DNA repair mech- anism. Taken together, these results suggest that in addition to metabolic detoxifi- cation, PXR protects cells against BaP induced damages through regulating DNA repair machinery as well as inhibiting AhR-mediated metabolic activation of the BaP. (This research is supported by ES09859 and ES09186).

1083 FLOW CYTOMETRIC EVALUATION OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES IN BONE MARROW AND MICRONUCLEATED RETICULOCYTES IN PERIPHERAL BLOOD FOLLOWING ACUTE AND REPEAT DOSE REGIMEN OF CHEMICALS.

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The flow cytometric (FCM) method for evaluating micronuclei (MN) in erythro- cytes has been developed and proven to be more sensitive, reliable and reproducible compared with traditional microscopic enumeration. To validate the FCM method at BioReliance using In Vivo MicroFlow®, we conducted a systematic evaluation of bone marrow and peripheral blood of mice treated with a number of well-charac- terized MN inducing drugs such as cyclophosphamide, methyl methanesulfonate, benzene and bleomycin, as well as nongenotoxic chemicals. Bone marrow and peripheral blood samples were harvested at 24 and 48 hours following a single dose, 24 hours follow- ing the 3rd repeat dose and on Days 1, 8, 15, 22 and 29 in a 4-week repeat dose stud- y. The frequencies of micronucleated polychromatic erythrocytes/reticulocytes (≤ MN-PCe/≤ MN-RETs) were determined by microscopy (BioReliance), and flow cytometry at both BioReliance and Litron. The MN frequencies deter- mined by FCM were essentially in agreement with the microscopic results and the FCM data obtained from BioReliance and Litron showed a high degree correlation. In addition, sampling once a week in a 4-week repeat dosing study provided valu- able insight into the generation, turnover and relationship between MN-PCe and MN-RETs in peripheral blood. The robustness of the assay was also examined where blood samples prepared from the same animals were analyzed by 4 independent FCM runs and the MN data were highly reproducible. In con- clusion, the presented data support the use of FCM to evaluate MN frequencies in bone marrow and peripheral blood of mice following chemical exposure. Future validation will be conducted in more species including rat, canine and human.

1084 CURCUMIN REGULATES CELL CYCLE PROGRESSION AND DNA REPAIR PROTEINS IN A P53-DEPENDENT MANNER.

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Benzo[a]pyrene-7,8-dihydridiol-9,10-epoxide (BPDE) is the ultimate carcinogen produced by benzo[a]pyrene bioactivation. BPDE is believed to play an important role in lung carcinogenesis by covalently binding to DNA. Although curcumin, a component in turmeric, shows promising effects against BPDE-induced carcino- genesis, its exact mode of action remains unclear. We propose that pretreatment with curcumin lowers the threshold of p53 activation in response to BPDE-in- duced damage. To test this hypothesis, lung epithelial cells were pretreated with or without curcumin for 24 h followed by 300 nM BPDE for 1 h. Here we show that p53(-) cells exhibited higher BPDE-DNA adduct levels than p53(+) cells after BPDE treatment. Curcumin pretreatment significantly reduced BPDE-DNA adducts in p53(-) cells yet had no effect on p53(+) cells. Flow cytometric analysis indicated S phase arrest between 6 and 16 h in p53(-) cells treated with BPDE alone. The p53(+) cells recovered from S phase arrest after 16 h. The time depend- ent S-phase arrest in p53(-) cells correlated with an induction of p53, p53 phos- phorylated at ser15 (p53S15), p21WAF1/CIP, and a down-regulation of phospho- rylated retinoblastoma (P-Rb). In contrast, pretreatment with curcumin further decreased the induction of P-pRb and increased S phase arrest and p53, p53S15, and p21WAF1/CIP expression. BPDE induced DNA damage recognition protein XPC in p53(-) cells while levels of DDR2 remained unchanged. Pretreatment with curcumin enhanced the expression of XPC. Moreover, human embryonic epithelial cells in p53(-), however pretreatment with curcumin had little affect. The expression of DDR2, but not XPC, was observed in p53(-) cells. DDR2 however was not in- duced in response to BPDE and curcumin did not affect its expression. In conclu- sion, the present study suggests that curcumin could play an important role in pre- venting lung carcinogenesis by modulating p53-regulated cell cycle progression and DNA damage recognition proteins in response to BPDE-induced damage. Supported by USPHS grants ES10617, CA119299 and ES014443.
bladders of mice after 6 h BMP exposure (300 mg/kg, PO) was determined using Affymetrix mouse ST arrays. In UROtsa cells BMP increased the expression of PARP-1, COX 2 and VEGF, and caused DNA strand breaks within 4 h. Following exposure in-vivo, the expression levels of genes associated with apoptosis (eg. Gadd 4, 2-fold), stress (eg. Atpase, 8 fold), DNA repair (eg. polymatase DNA direct), 2 fold), and cell cycle (eg. VEGF, 2 fold) were increased in the bladders of mice treated with BMP. These results demonstrate that BMP exposure can induce DNA damage and repair and affect genes associated with these processes. This research was supported in part by the NIH ES 005922 and the NIEHS-sponsored Southwest Environmental Science Center Grant Number P3 ES 06694.

**1087 DEVELOPMENT OF HUMAN CELL MODELS FOR ASSESSING THE CARCINOGENIC POTENTIAL OF CHEMICALS.**

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Sponsor: W. Chen

To develop human cell models for assessing the carcinogenic potential of chemicals, we established transgenic human cell lines and tested the sensitivity of known carcinogens using a cell transformation assay. A retroviral vector encoding an onco-protein (e.g., H-Ras (HEKR), implying that cell-type specificity is one of important factors determining the effectiveness of the assay. Using AFb1 (a) and BaP (Ba) as the representative pro-carcinogens, we also compared the efficiency of three different metabolic conditions in mediating cell transformation. Low dose chemical induction seems to be a prospective system used for metabolic activation of pro-carcinogens. Our findings provided direct evidence that a genetically modified human cell transformation model can be applied to the assessment of potent carcinogens.

**1088 CHEMINFORMATICS ANALYSIS OF EPA TOXCAST CHEMICAL LIBRARIES TO IDENTIFY DOMAINS OF APPLICABILITY FOR PREDICTIVE TOXICITY MODELS AND PRIORITIZE COMPOUNDS FOR TOXICITY TESTING.**

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An important goal of toxicology research is the development of robust methods that use in vitro and chemical structure information to predict in vivo toxicity endpoints. The US EPA ToxCast program is addressing this goal using ~600 in vitro assays to create bioactivity profiles on a set of 320 compounds, mostly pesticide actives that have well characterized in vivo toxicity. These 320 compounds (EPA-320 set evaluated in Phase I of ToxCast) are a subset of a much larger set of ~10,000 candidates that are of interest to the EPA (called here EPA-10K). Predictive models of in vivo toxicity are being constructed from the in vitro assay data on the EPA-320 chemical set. These models require validation on additional chemicals prior to wide acceptance, and this will be carried out by evaluating compounds from EPA-10K in Phase II of ToxCast. We have used cheminformatics approaches including clustering, data visualization, and QSAR to develop models for EPA-320 that could help prioritizing EPA-10K validation chemicals. Both chemical descriptors, as well as calculated physicochemical properties have been used. Compounds from EPA-10K are prioritized based on their similarity to EPA-320 using different similarity metrics, with similarity thresholds defining the domain of applicability for the predictive models built for EPA-320 set. In addition, prioritized lists of compounds of increasing distinctiveness from the EPA-320 have been produced, to test the ability of the EPA-320 models to extrapolate. These lists are further prioritized based on availability of toxicity data in the EPA ACToR database (actor.epa.gov/actor).

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

**1089 BIOLOGICAL PROFILING OF ENDOCRINE RELATED EFFECTS OF CHEMICALS USING TOXCAST.**


The Food Quality Protection Act of 1996 mandates that EPA implement a validated screening program for detecting estrogenic chemicals, as well as other endocrine targets deemed appropriate by the Administrator. EPA’s Endocrine Disruptor Screening Program (EDSP) has been developing and validating screening assays for disruption of estrogen (E), androgen (A) and thyroid (T) signaling pathways. The EDSP includes in vitro and in vivo assays for detecting E, A or T activity; and 73 chemicals have been proposed for initial screening. ToxCast is an EPA research program using a broad range of high-throughput screens to profile the bioactivity of chemicals and develop predictive signatures of toxicity, based on modeling in vitro assay data to in vivo toxicity phenotypes. ToxCast profiled 56 of the 73 EDSP chemicals using in vitro assays which characterized receptor binding, activation, inhibition and target gene regulation, providing biological fingerprints relevant to E, A, T and other endocrine related activities. Of the over 600 ToxCast assays, six detect E and 5 each are related to A and T receptor signaling. In addition to E, A and T endpoints, ToxCast also measured interactions with progesterone, glucocorticoid and PPAR receptors, aromatase activity, and other nuclear receptors including AhR, CAR, FXR, LXR and PXR that may modulate endocrine metabolism. Many assay targets were human proteins, but in some cases rodent or other species were targeted, affording cross-species comparisons. Results for the prototype EDSP chemicals were compared with the anti-androgenic or anti-estrogenic activity of ToxCast to identify potential endocrine disruptors, while screening other endpoints beyond E, A and T offers broader insights into the bioactivity of the EDSP chemicals. Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.

**1090 THE TOXCAST PATHWAY DATABASE FOR IDENTIFYING TOXICITY SIGNALS AND POTENTIAL MODES OF ACTION FROM CHEMICAL SCREENING DATA.**


The U.S. Environmental Protection Agency (EPA), through its ToxCast program, is developing predictive toxicity approaches that will use in vitro high-throughput screening (HTS), high-content screening (HCS) and toxicogenomic data to predict in vivo toxicity phenotypes. There are two major goals of this effort: 1) to produce toxicity signatures that can be applied to a large number of environmental chemicals for screening and prioritization; 2) to provide a more mechanistic understanding of chemical toxicity pathways. Towards these goals, we are developing a database and associated tools that organize the ToxCast data generated from over 600 different assays into pathways (e.g. toxicological, biochemical, metabolic, cellular), and then identifying pathway networks from which modes of action can be inferred. ToxCast in vitro assay data probe many biological domains and span multiple levels of biological organization. The ToxCast Pathway Database will be used to organize this extensive dataset, to perform network analysis, and to visualize putative modes of action that are activated by particular chemicals. Network analysis will facilitate the inference of predictive toxicological signatures by providing a visually intuitive representation of quantitative chemical effects across multiple levels of biological organization, including relationships to in vivo toxicity endpoints. This will define relationships between chemicals and disease states in whole animals, and potential disease states in humans. Initial analysis of ToxCast assays based on Entrez GeneID identified 113 human and 39 rat pathways within Ingenuity Pathway Analysis that contained at least one ToxCast assay target. This database will contribute to the larger goals of toxicogenomics by clarifying the role of gene-environment interactions in disease states. Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.

**1091 HIGH THROUGHPUT GENOTOXICITY PROFILING OF THE U.S. EPA TOXCAST CHEMICAL LIBRARY.**

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A key aim of the ToxCast project is to investigate modern molecular and genetic high content and high throughput screening (HTS) assays, along with various computational tools to supplement and perhaps replace traditional assays for evaluating
Novel Informatic Approaches to Analyze Gene Expression Data with the ToxCast 320 Chemical Library in Cultures of Primary Human Hepatocytes.


Prevaling methodologies in the analysis of gene expression data often neglect to incorporate full dose response and time response data but sensitivity with traditional microarray approaches. We have developed a high throughput assay suite using primary human hepatocyte cultures in vitro models that retain liver-like functionality (e.g. induction, metabolism and transport) to generate dose response microarray data across 40 chemicals. Using DEREK for Windows, results from these assays were compared to historical genotoxicity, genotoxicity, tumorigenicity and carcinogenic potential data collected from a variety of sources, including the ToxReID database. Historical data indicated some level of mutagenic concern for approximately 20% of the ToxCast chemcal libraries, over 90% of the chemicals caused some form of tumor in high-dose chronic tests, and approximately 20% of the chemicals were classified as possible or probable human carcinogens. Similar percentages of chemicals had either ToxCast HTS genotoxicity assay or DEREK in silico alerts for mutagenicity or carcinogenicity, and these data will be used for ToxCast predictive modeling.

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

Biochemical Activities of 320 Toxcast Chemicals Evaluated Across 231 Functional Targets.


EPA’s ToxCast research program is profiling chemical bioactivity in order to generate predictive signatures of toxicity. The present study evaluated 320 chemicals across 231 biochemical assays. Toxcast phase I chemicals include 309 unique structures, most of which are pesticide actives and metabolites. The battery of 231 assay services provided by Caliper Life Sciences (www.caliper.com) included 80 G-protein coupled receptors, 18 ion channels, 29 kinases, 19 phosphatases, 15 nuclear receptors, 21 transporters, 30 cytokines, and 45 other pharmacological assays. Compounds were initially screened at 25 μM in 0.4% DMSO, or 10 μM in 1% ACN for CYPS. Normalized data were scaled to +1.0 (100% inhibition) and Z-score provided a composite measure of dynamic range and variability. To assess screening ‘hits’ we used median absolute deviation (MAD2) or >30%-inhibition.

Analysis of the complexity of the responses. Rat primary hepatocytes were incubated with solutions of 0, 10, 30, and 100 μM of 4-aminopyrene, MEHP, myclobutanil, propiconazole, or triadimefon or 1, 3, and 1 μM of 21-PCB, 118-PCB, or 138-PCB. In all cases, the control consisted of the 1% DMSO dosing solution. RNA was extracted 72 hrs after dosing and analyzed using Affymetrix Rat Genome 230 2.0 arrays. Natural cubic splines were fit to the dose-response data for each gene. Statistical significance of each fit was assessed using bootstrap analysis. A set of curve shape and intensity features was extracted from the fit for each gene found to exhibit a significant dose-response feature. Features included first-derivatives, maximum/minimum expression, dose at maximum/minimum expression, and area under the curve. Using subsets of curve features, genes were first partitioned into groups that responded with a similar shape then into groups with similar expression intensities. Partitioning was performed on a chemical by chemical basis. Gene groups were subjected to functional and pathway analysis.

This work was reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy.

Screening for Chemical Effects on Neuronal Proliferation and Neurite Outgrowth Using High-Content Microscopy.

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The need to develop screening methods for developmental neurotoxicity to reduce the cost, time, and animals required for in vivo toxicity studies is well recognized. The EPA’s ToxCast program is developing rapid and high-content toxicity testing approaches. Data collected from various high-throughput assays to contribute to computational models designed to predict potential human toxicity. As part of this effort, we have screened a library of 320 chemicals (primarily pesticide compounds) using a variety of endpoints including cell proliferation and neurite outgrowth. Using high-content microscopy, chemical effects were assessed by BrdU incorporation and propidium iodide exclusion, respectively. NS-1, a cell line used in high-throughput assays, was evaluated using high-content microscopy, chemical effects (40 μM, 24 hr) on ReNcell CX cell proliferation and viability were assessed by BrdU incorporation and propidium iodide exclusion, respectively. NS-1 cells, a P12 subclone, were assessed for chemical effects (data across 40 μM, 96 hr) on neurite outgrowth (NOG) and viability by βIII-tubulin staining and ATP quantification assays, respectively. Effects on neurite outgrowth were defined as changes beyond 3x the standard deviation of control means. 112 chemicals inhibited ReNcell CX cell proliferation, while 62 decreased viability; of these, 49 chemicals decreased both measures. NOG in NS-1 cells was not necessarily reflected in the standard deviation of control means.
enhanced by 4 chemicals, but inhibited by 29 chemicals. NS-1 cell viability was decreased by 43 chemicals; all chemicals that inhibited neurite outgrowth also inhibited viability. Viability in both cell types was decreased by 28 chemicals, and 20 chemicals produced effects on all endpoints. These results demonstrate that these cell models can be used to screen large numbers of chemicals for effects on proliferation, neurite outgrowth, and viability using a high-content platform, and will contribute to ongoing data collection in ToxCast for the creation of predictive models for human chemical toxicity. (This abstract does not represent EPA policy)

**1096 IDENTIFICATION OF GENOMIC BIOMARKERS THAT PREDICT FUTURE ONSET OF DRUG-INDUCED RENAL TUBULAR INJURY**

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Drug-induced renal tubular injury is one of the major concerns in preclinical safety evaluation. Recently, toxicogenomics is becoming a promising tool for identifying chemicals with potential safety problems. In this research, we elucidated time- and dose-dependent global gene expression changes associated with drug-induced proximal tubular toxicity using 25 nephrotoxicants. Male Sprague-Dawley rats were dosed orally or intravenously once daily. The animals were exposed to 4 different doses (vehicle, low, middle, and high) of the compounds, and kidney tissues were collected on day 1, 4, 8, 15, and 29. Gene expression profiles were generated from kidney RNA using Affymetrix GeneChip. We previously reported the analysis of the gene expression profiles on day 4, 8, 15, and 29 in conjunction with histopathological findings, and identified 40 genomic biomarkers for concurrent diagnosis. This time, we identified genomic biomarkers for future onset prediction using the gene expression profiles on day 1, when histopathological changes of most of the nephrotoxicants (12/16) had not been observed yet. Filter-type gene selection and linear classifier were employed to discriminate future onset of positives (high dose of 16 compounds) from the negatives (low dose of 25 compounds). 5-fold cross-validation was executed by dividing 25 compounds into 5 subsets 100 times. In summary, we achieved the sensitivity of 82% and the selectivity of 90% with 72 genomic biomarkers. The gene list contains well-known biomarkers, such as Ki61, Timp1, Cpg, Gnp2, and also novel biomarker candidates, which are different from the previous genomic biomarkers for concurrent diagnosis. Toxicogenomics would be especially useful for future onset prediction of renal tubular injury.

**1097 INDEPENDENT VALIDATION OF GENE EXPRESSION-BASED HEPATOCARCINOGENIC PREDICTION MODELS**

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Identification of carcinogenic activity is the primary goal of the 2-year bioassay. The expense of these studies limits the number of chemicals that can be studied and therefore chemicals need to be prioritized based on a variety of parameters including suspected carcinogenic activity. Recent work has suggested gene expression signatures from target organs of subacutely exposed rodents can identify chemicals with carcinogenic potential. To determine whether such an approach can be used to prioritize a class of allyl/propyl benzene flavoring agents nominated to the NTP we developed a support vector machine-based, hepatocarcinogen classification model prioritizing a class of allyl/propyl benzene flavoring agents nominated to the NTP with carcinogenic potential. To determine whether such an approach can be used to identify hepatocarcinogens, estragole and safrole, and the most untested flavoring agents myristicin and isosafrole would be hepatocarcinogenic if untested flavoring agents myristicin and isosafrole would be hepatocarcinogenic if

1098 AN INTEGRATED SPECIES COMPARISON ANALYSIS BASED ON BIOLOGICAL PATHWAYS

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There are certain fundamentals remains irrevocable from bacteria to eucarya, therefore cross-species comparison provides insights into underlying principles behind complex biological phenomena. Evolutionary and organizational relationships among species have been investigated for through multiple sequence alignment of a single protein or genome. Yet, not only limited number of genome sequences is available but also comparing genome sequences may not represent the highest level organization of organisms such as metabolic pathways and protein interaction networks. Comparative analysis of metabolic and signaling pathways allows examining the biological processes rather than the individual elements. There have been bioinformatics approaches such as genomics and proteomics are used to determine biomarkers, for cross-species analysis. Phylogenetic analysis and bioinformatics approaches, taken together, raise the question whether an underlying similarity measure between the mode of action i.e. pathways, across species can be quantified; can we assess a pathway in human is more similar to its replacement in mice or rats? We used independent pathway similarity, enzyme sequence similarity and promoter region similarity of the genes, which codes for the enzymes. Pathway topology similarity and enzyme sequence similarity in a given pathway will indicate if the pathways function in the same manner and we explore how likely two pathways are regulated in a similar means by comparing promoter regions. We present the similarity trees based on the combined features for each pathway among a large collection of pathways that are constructed in KEGG. By doing so it may guide our selection as to the most appropriate model to use to study a given biological phenomenon.

3The views expressed are those of the authors and do not necessarily reflect the views or polices of the U.S.EPA.

1099 BENCHMARK DOSE DETERMINATION OF DIOXIN DOSE-DEPENDENT CHANGES IN BIOLOGICAL PATHWAYS IN PRIMARY HUMAN HEPATOCYTES

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Assessing health risks associated with mixtures of dioxin and dioxin-like compounds (DLCs) utilizes the toxic equivalency factor (TEF) approach that calculates a potency estimate for a DLC relative to that of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). This study used microarray-based gene expression changes in primary human hepatocytes treated with seven log-order concentrations of TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), or 2,3,4,7,8-pentachlorodibenzo-p-dioxin (PCDD) to calculate relative potencies for induced changes in biochemical pathways. The data were analyzed using the BMDexpress software application that combines traditional benchmark dose (BMD) methods with gene ontology classification in the analysis of dose-response data from microarray experiments. The BMD values for CYP1A1 expression were 0.4, 0.4, and 2.9 nM for TCDD, 4-PCDD, and TCDD, respectively. These BMD estimates are consistent with TEF values for these compounds and serve to validate the underlying gene expression data used in the analysis. However, when the gene expression changes were grouped based on biological processes, significant differences were noted between the three chemicals. Overall, 30% to 80% of the gene ontology categories had substantially different average BMD values when compared between TCDD, 4-PCDD, and TCDD. Changes in the expression of genes related to glucose transport and MAPK activity were among the most sensitive biological processes following treatment with TCDD and had average BMD values of 0.9 and 4.7 nM, respectively. By comparison, the total average BMD values for these categories ranged from 22 to 31 nM for 4-PCDD and TCDD. These results suggest that the inherent assumption in the TEF approach, that all DLCs produce equivalent responses based on relative potency, may not be appropriate when examined on a genome-wide transcriptional level, which may also extend to higher order biological endpoints.
Hepatotoxicity is a major cause of compound attrition in drug development. Primary hepatocytes treated in vitro hold potential as a surrogate system for detecting and understanding liver toxicity early in drug discovery. Gene expression profiles of cultured rat primary hepatocytes (RPH) differ from those derived from in vivo liver studies, however, raising concerns about the relevance of in vitro results. To characterize the similarities and differences in gene expression between in vivo and in vitro profiles, we compared gene expression data from rat liver and RPH compound treatments in the Entelo DrugMatrix® database. Seventy-four compounds were profiled both in vivo after 5 days treatment at the maximum tolerated dose, and in vitro for 24 hours at a concentration causing 20% cytolethality. For each compound, the correlation between liver and RPH was computed across all compounds and across specific classes of compounds with known in vivo outcomes such as DNA damage or hepatic necrosis. The set of probes most positively correlated across all compounds was significantly enriched with genes involved in metabolic pathways, such as beta-oxidation of fatty acids, regulation of glucose utilization, xenobiotic metabolism, and the P450 family, consistent with previous analyses. Additional pathways were significantly over-represented only for subsets of the compound treatments. The Wnt/b-Catenin signaling pathway was significantly associated with phospholipidosis in this analysis, but this association has not been reported in the literature. These findings demonstrate that many biological domains in RPH are predictive of liver gene expression in vivo, thereby increasing confidence that in vitro toxicogenomics assays can aid in drug development.

One of the aims of toxicogenomics is to classify compounds based on their toxicity. In order to compare the toxic response of compounds in different species, tissues and in vivo and in vitro, cross comparison of gene expression profiles is of great importance. The majority of the microarray analysis methods used within toxicogenomics are based on the single gene level. The use of gene groups can improve the sensitivity of analysis and has the advantage that interpretation of the data is more straightforward. Here, we created a web-application based on T-profiler [1]. T-profiler is a method that quantifies the expression of a gene group within a gene expression profile, by means of unpaired t-test calculation of an enrichment score (t-value). Our application is used for the analysis of gene expression profiles at gene group level, and the comparison of gene groups of interest to those derived from publicly toxicogenomics-related gene expression data. A possibility to upload custom gene sets is included together with a database of public available toxicogenomics experiments that can be used as reference material. We illustrate the utility of our application by showing gene specific response for compound classes and dose dependent responses for the HMG-CoA reductase inhibitor Atorvastatin. We also performed correlation analysis between t-values of gene groups and physiological parameters; this revealed mostly significant results for immunology related gene groups. Finally, we used cluster analysis to visualize the relation between the gene groups over all experiments. Altogether, we think that our web-application is a valuable contribution to the field of toxicogenomics.

this term. Query forms allow users to combine multiple criteria in structured queues to take advantage of the integration of data within MGI. Queries can simultaneously include parameters for phenotype, expression, biochemical function, subcellular location, sequence, and genome location. For example one can ask “What genes have products that are localized to the nucleus and have mutations resulting in alterations in oxidative stress baseline levels or induced responses?” Results from both the quick search tool and query forms may be viewed on the web or sent in tab delimited format to the MGI ftp site and downloaded. Integration and the use of structured vocabularies for annotations assure in robust and accurate data mining when posing such complex questions in both computational and individual formats at MGI.

The Mouse Genome Database (MGD) at MGI is supported by NIH/NHGRI grant HG0000350.

1105 THE COMPARATIVE TOXICOGENOMICS DATABASE: A TOOL FOR IDENTIFYING CHEMICAL–GENE–DISEASE NETWORKS.

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The Comparative Toxico-Anthropogenic Genomics Database (CTD; http://ctd.mbdlt.org) is a curated database that promotes understanding about the effects of environmental chemicals on human health. This is a critical area of research because the etiology of many chronic diseases involves interactions between environmental factors and genes and proteins that modulate important physiological processes. CTD combines manual curation and integration of diverse data sets to provide a centralized, freely available resource for exploring cross-species chemical–gene and protein interactions and chemical–gene–disease relationships. Over 122,000 interactions between 4,000 chemicals and 13,500 genes have been curated from 270 species, and 6,800 gene–disease and 2,200 chemical–disease relationships have been captured. By integrating these data, 390,000 gene-disease relationships and 93,000 chemical-disease relationships can be inferred. CTD also integrates external data sets like the Gene Ontology and KEGG pathways and maintains reciprocal links with many biological databases. Several new features were implemented to enhance data access and analyses including data download options, batch queries and the ability to compare associated data sets for chemicals, genes/proteins or diseases using a custom Venn Viewer tool. CTD’s integrative approach provides researchers with important connections between chemicals, genes/proteins and diseases that may not otherwise be apparent, but provide the basis for testable hypotheses about the mechanisms underlying the etiology of environmental diseases. This presentation will demonstrate the current scope and functionality of the database using a CTD-curated data set for arsenic.

1106 GENOME-LEVEL ANALYSIS OF GENETIC REGULATION OF SEX-SPECIFIC GENE EXPRESSION IN MOUSE LIVER.

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Sexual dimorphism in the expression of many genes is thought to play an important role in disease susceptibility, drug metabolism, and senescent response in both humans and other species. This study dissected the genetic regulation that controls sex-specific gene expression in mouse liver through a combination of mRNA expression analysis and genetic mapping in a reference population of BXD recombinant inbred mouse strains. While previous research has explored sex-specific gene expression patterns in liver following various chemical challenges, we explored gene expression differences in a population of naïve mice. We analyzed gene expression data in the livers of untreated male and female mice from C57BL/6J, DBA/2J, B6D2F1, and 37 BXD strains. As expected, thousands of transcripts exhibited considerable differences in expression between females and males. Functional analysis of the differentially expressed genes revealed several pathways related to xenobiotic metabolism that are heavily dependent on subject’s sex. Transcriptome factor binding site analysis of these same genes showed enrichment for the HNF1A, IRF2 and NR2F1 binding sites. Expression Quantitative Trait Loci (eQTL) mapping was also performed and revealed sex-dependent patterns of transcriptional regulation. Taking into account the influence of single nucleotide polymorphisms in microarray probes and the regions which are identical by descent between C57BL/6J and DBA/2J strains, we identified several eQTLs that are major sex-specific regulators of gene expression and proposed possible regulatory candidate genes that may define these loci.

1107 NOVEL CLASSIFICATION APPROACH FOR BIOMARKER IDENTIFICATION AND CARCINOGENICITY PREDICTION.

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We present a novel machine learning approach that utilizes gene expression profiles from livers of subacutely exposed rodents to predict the carcinogenicity of compounds. Our approach builds on the recursive feature elimination procedure using support vector machine that employs a leave one compound out training procedure along with novel scoring methodology to generate predictive models. We demonstrate the utility of our approach using liver gene expression profiles of rats exposed to structurally diverse hepatocarcinogens (aflatoxin B1, 1-amino-2,4-dibromoanisotroponine, N-nitrosodimethylamine, methyl/eguol) or non-carcinogens (acetaminophen, acetic acid, tryptophan). We begin the modeling procedure using 40K probes as individual features and optimize model using recursive feature elimination where 10% of features are eliminated at every step of cross validation. Models based on 2, 14 and 90 day exposures achieved 100% cross-validation accuracies using as few as 3, 6 and 13 array features respectively. We demonstrate the robustness of our approach by performing a validation using independent test dataset consisting of rat liver gene expression profiles where animals were treated with flavonoid agent compounds that are structurally different from those employed in the training set. Our models were able to differentiate between the hepatocarcinogens, estragole and safrole, and the non-hepatocarcinogens, euugenol and isoeugenol, all of which were previously studied by the NTP. The models also predicted that the untaken compounds myristostearic acid and methylsalicylic acid would be hepatocarcinogenic if studied at a dose level of 2 mmole/kg/day, whereas anethole (0.2 and 2 mmole/kg/day), isoanethole (0.2 mmole/kg/day) and myristostearic acid (0.2 mmole/kg/day) will not produce significant increases in hepatic cancer in rats. In summary, we have developed and validated a novel machine learning approach to produce classifiers that can accurately predict carcinogenic potential of compounds. Our approach is widely applicable for toxicogenomic biomarker identification.

1108 DEVELOPMENT OF LIVER TOXICITY KNOWLEDGE BASE (LTKB) TO EMPOWER THE FDA REVIEW PROCESS.

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A significant amount of drugs to be withdrawn from the market and to fail during clinical trial stage of development is due to liver toxicity. In order to understand liver toxicity at the mechanistic level and develop novel tools for identifying liver toxicity issues along the various stages of drug development, there is a need to develop content-rich resources to improve our basic understanding of liver toxicity and facilitate the efficient development of novel knowledge and tools for utilization by research, industry and regulatory groups. In the NCTR/FDA, we are developing Liver Toxicity Knowledge Base (LTKB) that will provide a wealth of focused knowledge and data mining tools for hepatotoxicity in the form of networks between chemicals (and drugs), molecular signatures, liver specific biomarkers, genes/proteins, functions, pathways and liver diseases (and pathology). Specifically, five sets of data/information are being generated and integrated through an in silico approach to develop LTKB. These are (1) Liver Ontology that characterizes liver pathology and toxicity and guide data collection/curation, classification and analysis; (2) Gene Expression Data was collected from public databases and through collaboration; (3) Conduct text mining on >18 millions abstracts in PubMed with an emphasis on liver-related data; (4) Collecting hepatotoxins and their associated mechanism Known Data through manual curation; and (5) Conducting in house omics experiment on >50 chemicals to augment and validate in silico results.

1109 NEW DEVELOPMENTS IN FDA/CDER’S COMPUTATIONAL TOXICOLOGY PROGRAM.

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The Informatics and Computational Safety Analysis Staff (ICSAS) at FDA’s Center for Drug Evaluation and Research (CDER) is an applied regulatory research group that creates databases of toxicological and clinical studies for the development of quantitative structure-activity relationships (QSARs). ICSAS employs leveraging mechanisms such as cooperative research and development agreements (CRADAs).
to obtain access to a variety of computational software programs that offer predictive (Q)SAR modeling capabilities (MCAPC, Leadscope Predictive Data Miner, Derek for Windows, MDL-QSAR). ICSAS is also involved in initiatives to harvest and publicly share toxicity data to support its modeling activities, and promotes the use of computational techniques that allow scientific knowledge to be extracted from proprietary chemical structures without revealing their identities. Recently, ICSAS has expanded its computational toxicology capabilities, including: (1) the creation of new comprehensive database and QSAR models suit describing adverse human health effects; (2) the assessment of pharmacological mechanisms of action and potential off-target effects; (3) the evaluation and application of several off-the-shelf metabolite prediction programs; and (4) the development of a weight-of-evidence approach for interpreting (Q)SAR predictions from multiple models and software platforms. In addition, ICSAS’ ongoing research program continues to upgrade its existing databases and models with new studies as they become available, and identify new areas of interest, such as QSAR modeling of phospholipidosis and maximum tolerated dose. This presentation provides case studies and describes ICSAS efforts to provide in silico tools for evaluating the toxicological profiles of chemicals for which little or no traditional test data are available or results are equivocal. This supports FDAs Critical Path Initiative which encourages the use of computational tools in a regulatory environment to enhance the approval process for FDA-regulated products.

1110 COMPUTATIONAL PREDICTION OF THE HUMAN METABOLISM OF HEPATOXIC DRUGS.

L. G. Valerio, E. J. Matthews, N. L. Krublak and R. Benet, CDER/OPS/SRS/ICASAS, Food and Drug Administration, Silver Spring, MD.

Predicting human drug metabolism is an essential part of drug discovery and the regulatory safety review of new drugs. Species-specific metabolism is a common problem encountered; drug metabolites identified in animal pharmacokinetic studies often differ from those formed in humans, leading to potential drug safety issues related to therapeutic efficacy, chronic toxicity, or drug-drug interactions. Computational prediction of human metabolic offers a rapid and inexpensive way of high-throughput screening for constructing metabolic profiles of drugs early in their development and for safety analyses. However, few computational prediction programs have been evaluated for their performance in predicting human drug metabolites. For the study reported here, 17 hepatotoxic drugs now withdrawn from the market were selected from the Drug Metabolic database program to assess the ability to predict human drug metabolites. Prediction scores for both phase I and II metabolic reactions were considered with a score of 0.8 being considered as a significant reaction. The results of this external validation study indicate that at least 25% of the predictions are above the 0.8 probability cut-off. Accuracy was judged by comparing the phase I and II in silico prediction with predicted metabolites of the hepatotoxic drugs determined in human clinical studies. This study found that the percentage of correct predictions for the drugs was 88% when considering all known human metabolites, while correct predictions based on major metabolites was significantly higher at 62%. In some cases the software was able to predict potential pathways for toxic produgs formation and metabolism. The results of this external validation study have been used in the MTD determination of new drugs.

1111 COMPUTATIONAL PREDICTION OF THE CARCINOGENICITY OF PESTICIDES.

E. J. Matthews and L. G. Valerio, CDER/OPS/SRS/ICASAS, Food and Drug Administration, Silver Spring, MD.

Estimating the carcinogenic and genotoxic potentials of a pesticide product, including its active ingredients(s), its metabolites and contaminants, is an essential part of the risk assessment and risk management safety review for pesticides. The presence of carcinogenic and/or genotoxic activities of this product can limit its commercial applications and its regulatory approval. Accurate knowledge of these toxicological activities could facilitate: (1) Tiered Testing: A sequential study of data from one tier of testing is used to determine the next step in testing; (2) Integrated Testing Strategies: A hypothesis-driven approach where data from one tier of testing is used to determine the next step in testing; (3) Lead Selection: A safer and more profitable lead selection and discovery process by the pesticide industry. Computational prediction of pesticide activities offers a rapid and inexpensive way of screening chemicals early in their development and performing safety analyses. However, few computational prediction programs have been evaluated for their performance in predicting pesticide activities with a comprehensive test set. In this validation study, 500 pesticide chemicals with known carcinogenic potential were evaluated using quantitative structure-activity relationship (QSAR) models generated with MCAPC, Leadscope FDA Model Applier (LFMA), and BioEpisteme software. Comparative predictive performance using coverage, specificity and sensitivity as indices were investigated using QSAR models enhanced incrementally with 100, 200, 300, 400, or 500 pesticides. Results of this study found there is an optimal number of rodent carcinogenicity bioassays required to accurately predict the carcinogenic potential of pesticide products using QSAR models.

1112 USING QSAR TO PREDICT ADVERSE EFFECTS OF ALKALOIDS IN BOTANICAL PRODUCTS.

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Purpose: Products containing herbs and other botanicals are becoming increasingly popular as a means of promoting health. The components of such products may contain hundreds of organic moieties, many of which are poorly described or understood. In particular, many botanicals contain alkaloids – a diverse group of nitrogen-containing compounds with high potential for biological activity. Our purpose is to use Quantitative Structure Activity Relationship (QSAR) models to identify potential adverse activities of alkaloids found in botanical products. Methods: The chemical compositions of 63 botanical species were found using mass spectral and electronic resources, primarily Dr. Duke’s Phytochemical and Ethnobotanical Databases (www.ars-grin.gov/duke). 179 distinct alkaloids (40 steroidal) were identified in these species. Two-dimensional molecular structures of these alkaloids were generated as machine-readable SMILES codes or mol files and submitted into approximately 100 validated QSAR tox models in each of three QSAR programs, including predictions for adverse reactions in human liver, kidney, bladder, or heart, rodent reproductive and development toxicity, genetic toxicity, and carcinogenicity testing. The QSAR modeling programs used were Model Applier (Leadscope), MCAPC (Multiusae), and BioEpisteme (Prous). Results: 80% of the alkaloids were in the chemical space of 60% of the current models, including most human organ models, rodent developmental models, and carcinogenicity models. Microbe, neurotox, and reprotox simulations were insufficient to make predictions for many alkaloids. 35 of the alkaloids showed activity in at least 20% of the models. 33 of the alkaloids showed activity for at least half of the human cardiac models. Conclusion: Upon QSAR analysis, alkaloids in botanicals were found to present signals for certain types of toxicities, indicating additional studies may be necessary to establish safe levels.

1113 PREDICTING MAXIMUM TOLERATED DOSE USING STRUCTURE-BASED SIMILARITY SEARCHING AND DOSE VS. EXPOSURE TIME DATA.

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Computational methods for predicting the toxicity of pharmaceutical substances are currently being developed and implemented by the Informatics and Computational Safety Analysis Staff at the FDA's Center for Drug Evaluation and Research. A new method for predicting the maximum tolerated dose (MTD) in rodents for pharmaceuticals and other organic chemicals is presented in this study. A database of approximately 5000 chemicals was compiled from National Toxicology Program studies, FDA archives, and various literature sources. Molecular structure data were then linked to the dosage and exposure information for each chemical. MTDs were obtained for study durations ranging from 2 days to 2 years, with some durations being more highly represented, reflecting common regulatory requirements. Modelable compounds were imported into Predictive Data Miner (Leadscope, Inc.), in which a similarity search using the Tanimoto coefficient was performed. Similarity searches using test compounds were then performed on the original dataset. Predictions of test compound MTD at different exposure times were made based on the time-dose equations of compounds sufficiently similar to each test chemical. Prediction performance was determined by adjusting the residuals of these predictions according to the degree of similarity between the test and training data set chemicals. The results of an external validation of this method are reported, indicating this method and the database are useful for predicting MTD in rodents.
1114 ADVERSE EFFECTS OF PHARMACEUTICALS: CONSTRUCTION OF A RELATIONAL DATABASE OF IMMUNOLOGICAL AND PULMONARY ADVERSE EFFECTS USING FDA ARCHIVES, PHARMAPENDIUM, AND PUBLIC SOURCES.

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The Informatics and Computational Safety Analysis Staff at the FDA's Center for Drug Evaluation and Research has created a database of drug adverse effects (AEs) and toxicology findings linked to drug chemical structure, class, clinical indication, and receptor target in humans. The database is being used to develop quantitative structure-activity relationship (QSAR) models that predict AEs of drugs. The preclinical, clinical, and post-marketing AE observations were obtained from: FDA's Spontaneous Reporting System, Pharmavendium (PP) software, and MicroMedex. PP includes information from FDA drug approval packages, Meyler's Side Effects of Drugs, drug monographs from Mosby’s Drug Consult, FDA’s Adverse Event Reporting System, and the published literature. PP's standard MedDRA vocabulary was employed for all AE endpoints and drug generic names in order to integrate the data from multiple sources. The total database contains ~4,000 drug substances, ~10,000 AE endpoints, and ~10,000,000 AE accounts. To account for variations in AE reports due to different patient populations for each drug, exposure was estimated based on the proportional reporting ratio (PRR) of reports for each drug expressed as a percentage of the total reports in the database. The PRR values were used to identify drugs with significant findings. This study reports an evaluation of two organ systems. The immune system included 1,030,961 reports corresponding to assorted hematological, dermatological, and immunological endpoints. The pulmon-ary system included 347,224 reports corresponding to breathing abnormalities, bronchospasms, laryngeal, respiratory tract, pulmonary disorders and additional endpoints. The results demonstrated that the AEs could be clustered into 52 endpoints that can be used for constructing models to predict immunological and pulmonary AEs of pharmaceuticals.

1117 STRUCTURE-ACTIVITY RELATIONSHIPS: SITE-SELECTIVE CARCINOGENESIS.

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Structure-activity relationship (SAR) models are used to predict the mechanisms of action of carcinogens and to predict the potential carcinogenicity of untested compounds. The cat-SAR model based on 1,049 carcinogens and noncarcinogens had a concordance of 73%. Considering the good predictivity of the models described here for organ-selective carcinogenesis, we speculate that the structural information that they contain can lead to insight for organ-selective carcinogenesis. In essence, just as traditional SAR models of carcinogens and non-carcinogens identified potential electrophilic moieties associated with DNA interaction, these organ-selective models may similarly indentify structural moieties associated with chemical interactions with molecular targets relating to cancer and uniquely expressed in the carcinogen-sensitive tissue.

1118 USING ALL THE DATA IN COMPREHENSIVE RISK ASSESSMENT OF THE MUTAGENIC POTENTIAL OF DRUGS.


The genotoxic potential in any candidate drug is carefully assessed during the discovery and development of new drugs. This is commonly done using in silico screens early on and continued with in vitro and in vivo experiments as the drug progresses closer to regulatory submission. In silico screens have several purposes, selection of compounds for experimental testing and/or to get early warning of any potential risk associated with the compound in the form of e.g. altering substructures. We have previously reported a comprehensive warning system (Genotoxic Warning System, GWS) for assessing the mutagenic potential of our compounds. This system is built on Ames assay data from public sources such as NTP and MultiCASE but also includes our internal data. Any molecule submitted to the system is passed through a series of steps and the output lists i) structural near neighbors with experimental results (Positive and Negative), ii) alerting substructures and iii) consensus Ames Q SAR results consisting of three different Q SAR models. A set of rules to aid the interpretation of the output and enhance the overall predictive performance was also derived and implemented. Using only Q SAR predictions, accuracy is ~80-85% with sensitivity being slightly lower than specificity. To improve our assessments, further development of the GWS was undertaken to include data from other experimental assays, such as carcinogenicity, mouse lymphoma, chron-
mosome aberration etc. These data are available as exact matched or structural near neighbours. The validity of structural alerts was also improved by comparing the statistical significance of each alert for the different assays. Finally, all the data were combined in a weight of evidence approach to give an overall assessment of the genotoxic risk associated with each compound. This approach proved to enhance both the predictive accuracy and the breadth of chemical coverage.

**1119 COMPARATIVE EVALUATION OF (Q)SAR MODELS FOR PREDICTING GENOTOXICITY ENDPOINTS.**

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New chemical regulations such as REACH are advocating the use of non-testing approaches to fill data gaps. The knowledge-based expert system DEREK, the rule-based statistical system TOPKAT and the hybrid expert system OASIS (TIMES) are among the QSAR models referred to in the European Chemical Agency's Guidance for REACH. However, the guidance does not provide an evaluation on the performance of these models. A comparative analysis of currently available QSAR models against experimental data is needed to gain experience surrounding the validation of these approaches as they relate to chemical-induced toxicity. The Dow Chemical Company has generated extensive data on the genotoxic potential of a wide variety of chemical substances using the Ames test (mutagenicity) and the in vitro chromosome aberration assay (clastogenicity). This has provided a unique opportunity to evaluate currently available QSAR models against experimental data for two endpoints included in the basic information requirements for REACH. In the scope of this project, 40 chemicals were analyzed with the models DEREK, TOPKAT and OASIS for mutagenicity and/or clastogenicity followed by an evaluation of the model predictions in comparison with experimental test data. For the mutagenicity endpoint, the overall concordance between experimental data and predicted values was 72%, 69% and 72% with a specificity of 76%, 72% and 79% and a sensitivity of 57%, 57% and 43% for the models OASIS, TOPKAT and DEREK, respectively. For the clastogenicity endpoint, the overall concordance between experimental data and predicted values was 67% and 75% with a specificity of 79% and 89% and a sensitivity of 20% and 20% for the models OASIS and DEREK, respectively. Overall the performance of the three models was comparable, showing a high specificity but rather low sensitivity. The data presented will provide valuable insight for developing approaches for the application of (Q)SAR data to chemical safety assessments.

**1120 EVALUATION OF (Q)SAR MODELS FOR THE PREDICTION OF MUTAGENICITY POTENTIAL.**


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The development of alternative methods to in vivo testing is critical to the cosmetic industry. The having of (Q)SAR models for predicting the mutagenic potential of a cosmetic is a key component of the safety assessment process for registration. Regulatory legislation such as REACH encourages the use of animal alternatives to address toxicology endpoints, including mutagenicity. There are currently no validated in vitro approaches for the assessment of mutagenicity, however, there are a number of in silico (Q)SAR models. (Q)SAR models with sensitivity and specificity values for the prediction of mutagenicity are currently not available. The development of alternative methods to in vivo testing is critical to the cosmetic industry and will provide valuable insight for the development of strategies for the incorporation of (Q)SAR models for the prediction of mutagenicity in (Q)SAR models.

**1121 COMPUTATIONAL APPROACHES FOR THE PREDICTION OF SKIN IRRITATION: EVALUATION AND IMPROVEMENT OF EXISTING TOOLS THROUGH DATA CATEGORIZATION AND ALERT EXTRACTION.**

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The 7th Amendment to the EU Cosmetics Directive has made in vitro and in silico approaches a key issue to warrant the safety of products. A ban on animal testing for chemicals to be used in cosmetics comes into effect in the EU in March 2009 for acute toxicity, genotoxicity, and skin or eye irritation. Over the past years there have been great efforts to develop alternative strategies, including in silico methods that would comply with regulatory constraints. Some commercial and free software tools integrate global models targeting the 2009 endpoints. For the cosmetic industry, the predictive performance of such models has been evaluated on chemical sets of interest: Chemical prioritization; Mechanistic understanding; and Elaboration of regulatory dossiers by providing additional information. In the present study, skin irritancy (SI) data of chemicals representative of a cosmetic industry portfolio were used to (i) challenge some of the global models available and (ii) extract knowledge to improve existing tools. Models for prediction of SI available in the Derek for Windows (V10.0) and Toxtree (v1.50) software were evaluated. The Toxtree module integrates both inclusion-rules based on structural alerts and exclusion-rules based on a selection of physico-chemical parameters (PCP). Since no PCPs were submitted in the present evaluation, only structural rules were applied. To improve the existing models, data were categorized on the basis of the structural features generated by the Leadscope Enterprise software. Multivariate analysis was performed to identify Leadscope chemical features most relevant to skin irritation. Additional molecules were compiled from the RTECS database for chemical domain analysis and validation of new or refined structural alerts.

**1122 COMPARATIVE EVALUATION OF THREE (Q)SAR MODELS FOR PREDICTING DERMAL SENSITIZATION POTENTIAL.**


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Determination of the dermal sensitization potential of a chemical is a key component of the safety assessment process for registration. Regulatory legislation such as REACH encourages the use of animal alternatives to address toxicology endpoints, including sensitization. There are currently no validated in vitro approaches for the assessment of dermal sensitization potential, however, there are a number of in silico (Q)SAR models. (Q)SAR models with sensitivity and specificity values for the prediction of dermal sensitization are currently not available. The development of alternative methods to in vivo testing is critical to the cosmetic industry and will provide valuable insight for the development of strategies for the incorporation of (Q)SAR models for the prediction of dermal sensitization in (Q)SAR models.

**1123 CHEMICAL REACTIVITY IN METABOLISM AND DEGRADATION REACTIONS IN RISK ASSESSMENT WORKFLOW.**

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Initiatives such as Canadian Domestic Substance List and REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) and 7th Amendment for Cosmetic Directive in European Union have put heavy emphasis on non-testing...
methods for hazard and risk assessment of chemicals. In particular, computational methods have become increasingly important for profiling environmental fate and evaluating toxicity of chemical compounds, which lead to risk assessment. Many of the currently available computational methods do not consider chemical reactivity and fate either in environment and biological compartments. In this paper, we will present a chemoinformatics approach for predicting chemical reactivity in metabolism and degradation reactions. Structural alerts on chemical reactivity can be used as indicators for safety issues due to reactivity. The application of reaction rules which combine reaction types with a physicochemical evaluation of the reactions allows a more sophisticated assessment of a chemical compound. The integration of the prediction of chemical reactivity into the workflow of the hazard and risk assessment process will be demonstrated. This paper will also report on two tools funded by ECB [1]: 1) START (Structural Alerts for Reactivity in Toxtree) [2]; 2) CRAFT (Chemical Reactivity and Fate Tool implemented in Ambit) [3]. [1] European Commission’s Joint Research Centre (JRC), Consumer Product Safety and Quality (CPSS&Q) Unit, formerly known as European Chemicals Bureau (ECB). [2] An open source tool for toxicology evaluation (http://ambit.acad.bg/toxT ree/) [3] An open source chemoinformatics data management system (http://ambit.acad.bg/)

1124 QUANTITATIVE STRUCTURE TOXICITY RELATIONSHIPS (QSTR) MODELS FOR PREDICTING ACUTE/SUB-ACUTE AND SUB-CHRONIC/CHRONIC ADVERSE EFFECT LEVELS.

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An important step in dose-response assessment is the identification of a critical effect level. The measure of critical effect often is presented as a no-observed-adverse-effect level (NOAEL), or a lowest-observed-adverse-effect level (LOAEL). Quantitative Structure Toxicity Relationships (QSTR) models are mathematical models that may be used to predict toxicity from physical characteristics of a chemical structure. In this research, QSTR models were developed to estimate critical effect levels from chemical structure. The modeling focus was on machine learning methods, Random Forest, k-Nearest Neighbor, and Support Vector Machine, as well as models using Partial Least Squares. The models were built using over 2700 toxicity endpoints for 439 unique chemicals. Models were built with three to nine descriptor sets (Dragon, MOE, Molconnz) and one descriptor set from the US EPA. The performance of each model was assessed using a validation set not used in model development. These models show that it is very reasonable to predict critical effect values from chemical structure based on predictions of the validation set. The mean absolute error for the log scaled LOAEL and NOAEL values was close to 0.5 across models, giving confidence in the order of magnitude of the predictions. In addition, the applicability domains for these models were also evaluated. Among the four modeling methods used, all methods, with the exception of PLS, performed equally well. The models can be used as an additional tool for dose-response assessment when experimental data pertaining to critical effect levels do not exist.

Disclaimer: This research does not necessarily represent the views of the U.S. Environmental Protection Agency.

1125 COMBINATORIAL QSAR MODELING OF RAT ACUTE TOXICITY BY ORAL EXPOSURE.

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Few Quantitative Structure-Activity Relationship (QSAR) studies have successfully modeled large, diverse mammalian toxicity endpoints. In this study, we have applied a combinatorial QSAR approach in the development of robust and predictive models of chemical acute toxicity of rat by oral exposure. To this end, we have compiled a comprehensive dataset of 7385 compounds with their median lethal dose (LD50) values in the oral exposure test of rats. To compare the predictive power of our models with an available commercial toxicity predictor, we used the same training set of 3472 compounds as the TOPKAT (Toxicity Prediction by Komputer Assisted Technology) software. The remaining 3913 compounds which were not present in the TOPKAT training set were used as the external validation set for our toxicity models. We have developed 7 different types of QSAR LD50 models for the modeling set. The internal prediction accuracy for the modeling set ranged from 0.52 to 0.96 as measured by the leave-one-out cross-validation correlation coefficient (Q2). The prediction accuracy for the external validation set ranged from 0.24 to 0.70 (linear regression coefficient R2). The use of applicability domain threshold implemented in most models generally improved the external prediction accuracy but led to the decrease in chemical space coverage. Finally, several consensus models were developed by averaging the predicted LD50 for every compound using all 7 models. We find that consensus models afford higher prediction accuracy for the external validation dataset with the highest coverage as compared to individual models with a specific best validated LD50 models developed by our collaboration can be used as reliable computational predictors of in vivo acute toxicity and will be made publicly available from the participating laboratories.

1126 RESOLVIN E2 PROTECTS AGAINST ACETAMINOPHEN (AA)-INDUCED HEPATOTOXICITY.

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Ingestion of toxic doses of AA causes centrilobular hepatic necrosis. Evidence suggests inflammatory mediators released by cytosolic macrophages contribute to the pathogenic process. Resolvins are naturally occurring anti-inflammatory mediators derived from eicosapentaenoic acid. They inhibit production of IL-1β, IL-12, TNF-α and nitric oxide, and suppress leukocyte chemotaxis. In the present studies, we analyzed the protective effects of resolvins against AA-induced hepatotoxicity. Treatment of B6129F2 mice with AA (300 mg/kg, i.p.) induced hepatotoxicity resulting in increases in serum transaminase levels within 3 hr peaking at 24 hr. This was correlated with histological evidence of centrilobular hepatic necrosis. Pretreatment of the mice with resolin E2 (RvE2) (50 µg/kg/day, i.p., 3 days) abrogated these effects. TNF-α exerts its biologic activity by binding to two distinct cell surface receptors, TNFR1 and TNFR2. AA-induced hepatotoxicity was associated with increased expression of TNFR2 in the liver. This response was inhibited by RvE2 pretreatment of the mice. RvE2 also reduced hepatic expression of cyclooxygenase-2, which mediates the generation of proinflammatory eicosanoids. Heme oxygenase (HOX-1) is a potent antioxidant known to protect against AA-induced liver injury. Western blotting and immunohistochemistry (IHC) demonstrated that HO-1 was up-regulated in mice treated with AA. This was predominantly observed in liver macrophages. RvE2 treatment, by itself, also upregulated HO-1 in the liver, but had no effect on expression of this antioxidant in AA treated mice. Similarly, RvE2 upregulated levels of glutathione in the liver. These data suggest that RvE2 may be useful in mitigating AA-induced hepatotoxicity by its ability to block the generation or activity of inflammatory mediators and upregulate antioxidants (Supported by NIH GM034310 and ES005022).

1127 S-ADENOSYMETHIONINE (SAME) REVERSAL OF ACETAMINOPHEN (APAP) EFFECTS ON HEPATIC GLUTATHIONE PEROXIDASE AND HEPATIC SAME LEVELS.

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Overdose of the over the counter analgesic acetaminophen (APAP) is associated with hepatic damage. Previous work form our laboratory and by others have shown that S-adenosyl-L-methionine (SAME) is protective for APAP induced hepatotoxicity. This study tested the hypotheses that: 1) SAME reduces APAP mediated oxidative stress by maintaining glutathione peroxidase activity and 2) SAME treatment prevents APAP mediated decline in total hepatic SAME levels. Male C57BL/6 mice (16-25 g) were randomly divided into 4 groups (n=5/group): Vehicle (VEH), APAP, APAP and SAME+APAP. Mice were fasted overnight, injected intraperitoneal (p) with water (VEH; 5ml/kg) or 250 mg/kg APAP (15 ml/kg) followed 1h later by ip injection of 1.25 mmol/kg SAME. Plasma and liver were collected 4 or 6h after APAP administration. SAME treatment did not alter hepatic function as assessed by ALT and liver weight (wt) were comparable to VEH values. APAP administration decreased plasma ALT levels and hepatic liver wt. when compared to VEH and SAME groups confirming hepatic toxicity. Hepatic glutathione peroxidase activity was diminished in (p<0.05) 4 and 6h after APAP injection compared to VEH. APAP mediated decline in hepatic glutathione peroxidase activity was partially corrected at 4 hr in the SAME+APAP when compared to the APAP treated mice. Total hepatic SAME levels were diminished 4h after APAP treatment when compared to the VEH and SAME groups. Depletion of total hepatic SAME levels was prevented from 0.24 to 0.70 (linear regression coefficient R2). The use of applicability domain threshold implemented in most models generally improved the external prediction accuracy but led to the decrease in chemical space coverage. Finally, several consensus models were developed by averaging the predicted LD50 for every compound using all 7 models. We find that consensus models afford higher prediction accuracy for the external validation dataset with the highest coverage as compared to individual models with a specific best validated LD50 models developed by our collaboration can be used as reliable computational predictors of in vivo acute toxicity and will be made publicly available from the participating laboratories.
in the SAmε+APAP group. These findings indicate that SAmε partially protects the liver from APAP induced oxidative stress and depletion of hepatic SAmε levels. (Supported by NIH Grant S2020R016477 to the West Virginia IDEA Network for Biomedical Research Excellence).

1128 TEMPORAL STUDY OF ACETAMINOPHEN (APAP) AND S-ADENOSYL-L-METHIONINE (SAME) EFFECTS ON SUBCELLULAR HEPATIC SAME LEVELS AND METHIONINE ADENOSYLTTRANSFERASE (MAT) EXPRESSION.

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Acetaminophen (APAP) is the leading cause of induced liver failure in the United States. The antidote, N-acetylcysteine, is most effective when given within 8 hours of APAP overdose. S-adenosyl-L-methionine (SAME) is the principle biological methyl donor participating in methylation of nucleic acids, proteins, and phospholipids. SAME also participates in the transulfuration pathway to provide cellular glutathione. SAME is critical for growth of liver cells which would be important in recovery of APAP toxicity. SAME is synthesized in cells through the action of methionine adenosyltransferase (MAT). MAT I/III is constitutively expressed in cells, while MAT II is expressed in regenerating cells. This study tested the hypothesis that hepatic mitochondrial SAME levels are decreased by APAP toxicity. A separate experiment examined alterations of MAT expression in response to APAP toxicity. Male C57BL/6 mice (16-22 grams) were treated with vehicle (VEH; water 15ml/kg ip injections). 250 mg/kg APAP (15 ml/kg, ip) or SAME (1.25 mmol/kg) which was administered 1 hour after APAP injection (SAME and SAME+APAP). Hepatic tissue was collected 4 and 6 hours after APAP administration. SAME levels were determined by HPLC. Adapted to western blot. APAP depressed mitochondrial SAME levels at 4 and 6 hours relative to the VEH group. SAME administration following APAP (SAME+APAP) prevented APAP associated decline in mitochondrial SAME levels. Hepatic MAT II expression was not present at 4 hr in any of the groups. In conclusion, the maintenance of SAME may provide benefit in preventing mitochondrial damage associated with APAP toxicity. (Supported by NIH Grant S2020R016477 to the West Virginia IDEA Network for Biomedical Research Excellence).

1129 ANALYSIS OF CYTOKINES AND RELATED SIGNALING MOLECULES IN A CO-CULTURE MODEL OF PRIMARY MOUSE HEPATOCYTES AND KUPFFER-CELL ACETAMINOPHEN EXPOSURE.

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Acetaminophen (APAP) is a popular pain killer that produces liver damage when ingested at high doses. Kupffer cells are liver macrophages that become activated with toxic APAP treatment. Depletion of Kupffer cells prior to APAP treatment protects mice against hepatotoxicity. In the present study, a mouse Kupffer cell and a hepatocyte co-culture system was used to investigate the interaction of these cell types during APAP toxicity and the gene expression profile of cytokines. Kupffer cells and hepatocytes were isolated from naive C57Bl/6 mice and cultured. Cocultures and mononucleus cultures were exposed to APAP (50μM) and cytotoxicity was determined by lactate dehydrogenase (LDH) leakage into the culture media at 24 hr. Cytokine gene expression was analyzed by real-time PCR at 6 and 24 hr after APAP. Treatment of hepatocytes with APAP resulted in a significant release of LDH into the media as compared to control hepatocytes. In contrast, LDH released into the media was significantly less when hepatocytes were co-cultured with Kupffer cells, indicating that Kupffer cells protect hepatocytes from APAP toxicity. Tumor necrosis-alpha (TNFα) gene expression was increased only when hepatocytes were co-cultured with Kupffer cells either in the presence or absence of APAP. Gene expression for all interleukins examined (IL-1α, IL-1β, IL-6 and IL-8) were elevated only in mono-cultures of hepatocytes exposed to APAP and not in control hepatocytes or any of the co-cultured groups of cells. In addition, nitric oxide synthsate 2 and prostaglandin synthsate 2 gene expressions were also elevated only in APAP-treated hepatocytes. By contrast, cyclooxgenase-1 gene expression was higher in co-cultures of hepatocytes and Kupffer cells exposed to APAP. Collectively, these results indicate that Kupffer cells protect hepatocytes from APAP toxicity and the presence of Kupffer cells along with hepatocytes in culture is needed for the expression of certain cytokines in response to APAP.
dimethylsulfoxide (DMSO) and 5 mM epidermal growth factor (EGF). Furthermore, PMHs appear to dedifferentiate during the first week in culture, and then recover some liver-specific functions, peaking at 3 weeks. Comparing PMHs isolated from male C57BL/6 mice and cultured for less than 24 hours to those cultured for 3 weeks, some advantages are apparent in the long-term cultures. PMHs remain fairly isolated shortly after attachment whereas, by three weeks, they orient towards cords. Western immunoblotting indicates that albumin, GST, and Cyp1A1 are all upregulated at 3 weeks, while GSTT1 is slightly downregulated. ATP levels are depleted by pre-isolation, but dramatically recover (70-fold) after 3 weeks in culture. RT-PCR results indicate that the week 3 cultures treated with 10 mM acetaminophen (APAP) mimic better in vivo APAP treatments (300 mg/kg). The in vivo 6 h treatment was better represented by a 6 h treatment in vitro than a 2 h treatment in vitro, suggesting the toxicokinetics in vivo and in vitro may be similar. Further support for similar toxicokinetics is found in the peak appearance of APAP-protein adducts in APAP-treated cultures after 2 hours of treatment, which is similar in vitro, suggesting the toxicokinetics in vitro and in vivo may be similar. This work was supported by NIEHS grants 1U19ES11387, R01ES016189 and P30ES07035.

1133 GENE EXPRESSION PROFILES IN LIVERS FROM DICLOFENAC-TREATED RATS REVEAL INTESTINAL BACTERIA-DEPENDENT AND -INDEPENDENT PATHWAYS ASSOCIATED WITH LIVER INJURY.

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Diclofenac (DCF) is a nonsteroidal anti-inflammatory drug (NSAID) that is associated with idiosyncratic adverse drug reactions (IADRs) in humans. Previous studies revealed a crucial role for intestine-derived bacteria and/or lipopolysaccharide (LPS) in DCF-induced hepatotoxicity. We explored further this mechanism by conducting gene expression analysis of livers from rats treated with LPS or a hepatoxic dose of DCF (100 mg/kg) with or without oral antibiotic pretreatment. K-Means clustering revealed similarity between the gene expression profiles of the toxic DCF dose group and the LPS group. Genes for which expression was altered by DCF were divided into two groups: genes with expression altered by antibiotic treatment and those unaffected by antibiotics. The former group of genes represented the ones for which DCF-induced alterations in expression depended on intestinal bacterial endotoxin of the latter group of genes was likely changed by direct effect of DCF rather than by intestinal bacteria. Functional analysis of genes in the former group revealed fatty acid metabolism, protein transport and trafficking as well as LPS-related signaling, which further suggested a role for bacterial endotoxin in the liver injury. Functional analysis of genes in the latter group revealed changes in signaling pathways related to inflammation, hypoxia, oxidative stress, the aryl hydrocarbon receptor and peroxisome proliferator-activated receptor alpha (PPARα). Hypoxia occurred in the livers of rats treated with DCF, and in vitro hypoxia rendered hepatocytes sensitive to DCF-induced cytotoxicity. These results suggest that both intestinal bacteria-dependent and -independent mechanisms contribute to DCF-induced hepatotoxicity and that hypoxia plays an important role in the pathogenesis. (Supported in part by NIH grant GM 57865.)

1134 HEPATOITIC INTERACTION OF SULINDAC WITH LIPopolysaccharide: ROLE OF THE HEMOSTATIC SYSTEM.

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Sulindac (SLD) is a nonsteroidal anti-inflammatory drug (NSAID) that has been associated with a greater incidence of idiopathic hepatic necrosis in humans than other NSAIDs. In previous studies, lipopolysaccharide (LPS)-induced inflammation potentiated SLD-mediated liver injury in rats. Neither SLD nor LPS alone caused liver injury. The goal of this study was to evaluate the role of the coagulation system in SLD/LSD hepatotoxicity. Cotreatment with SLD/LSD led to increased activity of serum biomarkers of both hepaticcellular injury and cholestasis. Histological evidence of liver damage was found only after SLD/LSD cotreatment. Thrombin-antithrombin dimer, a biomarker of coagulation system activation, as well as plasminogen activator inhibitor-1, a down-regulator of the fibrinolytic system, was elevated in the plasma of SLD/LSD-coated rats. As a result of activation of hemostatic mechanisms by SLD/LSD, coagulation, fibrin and hypoxia were prominent in the livers of cotreated rats before the onset of the hepatotoxicity. Therefore, we tested the hypothesis that SLD and LPS interact to cause liver injury by a mechanism that involves the hemostatic system. Pretreatment of rats with heparin reduced hepatic fibrin deposition and hypoxia and protected against liver injury induced by SLD/LSD cotreatment. These results indicate that the hemostatic system is activated by SLD/LSD cotreatment and plays an important role in the development of SLD/LSD-induced liver injury. (Supported by NIH GM075865 and ES004193.)

1135 ACTIVATION OF CASPASE-8, BUT NOT CALPAIN, IS CRITICAL IN DICLOFENAC-INDUCED BID TRUNCATION AND MITOCHONDRIAL PERMEABILIZATION IN HUMAN HEPATOCYTES.

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Diclofenac (DCF) -induced lethal injury to cultured human hepatocytes is a cellular model for reactive metabolite-mediated mitochondrial permeabilization and cell death. We have previously demonstrated that an oxidative metabolite of DCF causes increases in [Ca2+], which is critical for cleavage of the cytosolic protein Bid and which results in mitochondria-mediated apoptosis. However, the signaling pathways that link [Ca2+] and Bid truncation are unknown. The aim of this study was to explore the mechanistic role of various death pro tease s that activate Bid and thus initiate mitochondrial permeabilization. Immortalized human hepatocytes (HC-O4) exposed to cytotoxic concentrations of DCF (>500 μM) for up to 48 h underwent apoptotic cell death as demonstrated by LDH release and staining of apoptotic nuclei with Hoechst 33342. As early as 4 h, DCF increased the mitochondria-specific production of superoxide as demonstrated with the mitochondrially-targeted fluorescent probe, mito-HE (1 μM) with superoxide-specific new assay conditions (596 nm exc/580 nm ems). Diclofenac (1 mM) caused activation of caspase-8 that became apparent at 12 h and which decreased to control levels at 24 h. The caspase-8 inhibitor, Z-IEET-DFMK (10 μM) significantly decreased the extent of DCF-induced cell injury. In contrast, calpain was activated and remained high at 6, 12, 18, and 24 h. The calpain inhibitor-III, Z-Val-Phc-CHO (500 μM) blocked calpain activity but failed to rescue HC-O4 cells from DCF-induced apoptosis. Combined treatment of cells with DCF and cyclosporin A (2 μM), an inhibitor of mitochondrial permeabilization, completely abolished caspase-8 activation, suggesting a likely mitochondrial origin of procaspase-8. Collectively, this results indicate that calcium- and/or superoxide-mediated activation of caspase-8, but not calpain, is critically involved in mitochondrial permeabilization and cell death induced by DCF in hepatocytes.

1136 LIVER EFFECTS IN MICE GIVEN TRICHLOROACETIC ACID (TCA) IN A 14-DAY DRINKING WATER STUDY ARE PPAR-DEPENDENT.

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Exposure of mice to perchloroethylene (perc) is known to increase peroxisomes and cell proliferation in the liver, hepatocellular hypertrophy, and liver tumors. TCA is the major metabolite of perc and is known to induce the same responses, including tumors, in the livers of mice when given in drinking water. This study was conducted to determine whether the liver effects seen in mice exposed to TCA in drinking water are PPAR-dependent. Male and female B6C3F1 mice, male 129/sv wild-type (WT) and male PPARα-null mice were given 0, 1 or 2.5 g/L TCA in drinking water for 5 (male B6C3F1 mice only) or 14 days. Liver tissue was examined by light and electron microscopy, cell proliferation was quantified by BrdU labelling, and apoptosis was measured by TUNEL staining. Effects in B6C3F1 mice were: increased relative liver weight (1.2 and 1.5 g/L); increased palmitoyl CoA oxidase activity, with males > females (1 and 2.5 g/L); increased number and volume density of peroxisomes (1 and 2.5 g/L); non-statistically significant increase in cell proliferation in males at 5 days (316% and 267%, respectively); dose-dependent decrease in apoptosis in males at 5 days (12% and 31%, respectively); and eosinophilia and/or hepatocyte hypertrophy (1 and 2.5 g/L). Similar effects were seen in the WT male mice at 14 days, except that there was no change in cell proliferation. Palmitoyl CoA oxidase activity was increased to a greater extent in male WT mice compared to B6C3F1 mice. No liver effects were seen in the PPARα-null mice except for evidence of fatty vacuolation in all PPARα-null mice, which appeared more widespread in the treated versus non-treated mice. Thus, peroxisome proliferation occurring in the livers of TCA-treated mice is dependent on PPARα. The results also suggest that perc-induced liver effects in mice are mediated by PPARα.

1137 EFFECT OF PPARα AGONISM DURING PRE-NATAL DEVELOPMENT.


Recent work indicates that PPARα is required to mediate perfluorooctanoic acid (PFOA)-induced post-natal lethality but the mechanisms identifying this effect are uncertain. The present study examined the effect of administering two PPARα-
ionox (clofibrate or Wy-14,643) on pre- and post-natal development using wild-type, PPARα-null or PPARα humanized mice. Mice were mated overnight, and the presence of a copulatory plug was indicative of pregnancy and considered gestation day (GD) 0. Plugged female mice were fed either a control diet or one containing clofibrate (0.5%) or Wy-14,643 (0.005%) diet until GD18. These concentrations of PPARα agonists were chosen to potentially model exposure to PFOA which is a considerably less potent PPARα activator as compared to Wy-14,643. Mice were euthanized on GD18 to isolate maternal and fetal livers and assess developmental parameters in the dam and fetuses. No significant changes in the maternal weight gain and average uterine weight between treatment groups were observed. An increase in relative maternal liver weight was found in response to clofibrate and Wy-14,643 in wild-type mice and this effect was not observed in the similarly treated PPARα-null mice. An increase in relative liver weight in fetuses was not found in response to either PPARα agonist. Additionally, no significant changes in average fetal weight, crown:rump ratio or placental weight were found. Interestingly, the mRNA levels of Cyp4a10 and acyl-CoA oxidase (Aox), markers of PPARα activation, were significantly increased by clofibrate and Wy-14,643 in wild-type maternal and fetal livers but not in PPARα-null mice. These results demonstrate clear PPARα-dependent changes in both the maternal and fetal compartments. In terms of changes in the different species, post-natal studies are currently being examined in ongoing studies. Additionally, whether there is a species difference in the results will be determined from the studies with PPARα-humanized mice. (Supported by unrestricted gifts from 3M Company and Dupont Haskell Global Centers of Health and Environmental Sciences).

1139 ACUTE ETHANOL-INDUCED LIVER MITOCHONDRIAL DEPOLARIZATION IN VIVO IS DEPENDENT ON ALCOHOL DEHYDROGENASE (ADH) AND CYTOCHROME-P450 2E1 (CYP2E1).

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Our recent study showed that ethanol ingestion causes widespread hepatic mitochondrial depolarization (on <15% of hepatocytes after 24 h). Loss of cell viability, as indicated by nuclear PI fluorescence, was minimal after acute ethanol treatment. Ethanol-induced mitochondrial depolarization was dose-dependent in the range of 1-6 g/kg. This mitochondrial depolarization caused by ethanol was associated with hepatic steatosis but little overt cell injury. Ethanol-induced mitochondrial depolarization was independent of the mitochondrial permeability transition, opening of mitochondrial K<sub>ATP</sub> channels and uncoupling protein-2. Without ethanol treatment, hepatocytes of ADH-positive and ADH-negative mouse livers remained polarized mitochondria. After ethanol treatment, mitochondria depolarized in most hepatocytes of ADH-positive mouse livers. By contrast, mitochondrial depolarization did not occur in most hepatocytes of ADH-negative mouse livers. The cytochrome P450 inhibitor, aminobenzotriazole (100 mg/kg), blunted mitochondrial depolarization in 25% of hepatocytes. Ethanol-induced mitochondrial depolarization was similarly decreased in CYP2E1-deficient mice. Taken together, the data show that acute ethanol causes widespread, reversible hepatic mitochondrial depolarization. Ethanol metabolism by ADH plays a major role in ethanol-induced mitochondrial depolarization whereas CYP2E1 plays a more minor role (NIDDK).

1140 THE ROLE OF ETHANOL METABOLISM IN ALCOHOL-INDUCED HEPATOPOTOXICITY.

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The role of ethanol (EtOH) metabolism in liver injury remains a topic of controversy. In the current study, groups of male Sprague-Dawley rats (n = 4-8) were infused with 12.5 g/kg/d EtOH as part of isocaloric liquid diets fed via gastric tube at a rate of 2.5 g/kg/d for 45 d. Some groups were given 200 mg/kg/d dialediosulfate (DAS) in the diet which prevented induction of CYP2E1 apoprotein or activity after EtOH treatment. Other groups were infused diets supplemented with 164 mg/kg/d of the acetylation inhibitor aminobenzotriazole (4MP) or dosed at 2.5 g/kg/d of DAS to maintain blood glucose concentrations in the 200-400 mg/dl range. Resulting liver pathology scores and levels of apoptosis did not differ significantly between EtOH, EtOH + DAS or EtOH + 4MP groups, although serum ALT values were lower after 4MP treatment (p < 0.05). Hepatic oxidative stress measures (lipid peroxidation, (TBARS) and reduction in GSH concentrations) were increased after EtOH (p < 0.05), but unaltered by DAS or 4MP DAS and 4MP blocked EtOH increases in TNF alpha mRNA (p < 0.05), and 4MP prevented EtOH increases in expression of the chemokine CXCL1 (p < 0.05). However, neither inhibitor prevented EtOH suppression of IL-4 or IL-12 mRNA or increases in TGF beta mRNA (p < 0.05). The ER stress marker TRB3 was elevated by EtOH (p < 0.05) and while this was partly attenuated by 4MP, DAS had no effect. DAS in combination with EtOH increased the number of hepatocytes in S-phase (p < 0.05). These data suggest that oxidative stress and many of the cytotoxic changes associated with hepatic injury following EtOH exposure are not mediated by CYP2E1 or acetaldehyde, but that EtOH metabolism by CYP2E1 may be linked to induction of TNF alpha; whereas, acetaldehyde production may be linked in part to necrosis, chemokine production and ER stress. (Supported in part by R01 AA08645 T.M.B.)

1141 NFR2 DELETION IMPAIRS GLUCOSE TOLERANCE AND EXACERBATES HYPERGLYCEMIA IN TYPE 1 DIABETIC MICE.

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The transcription factor Nrf2 induces numerous protective detoxification and antioxidant genes following electrophilic or oxidative stress. Nrf2 aids in liver regeneration by altering insulin signaling, however, whether Nrf2 plays a role in hepatic glucose homeostasis is unknown. In the present study, blood glucose, serum insulin, urine output, and hepatic expression of glucose-related genes were examined in male diabetic wild-type (C57BL/6) and Nrf2-null mice. Type 1 diabetes was induced with a single dose of streptozotocin (STZ 200 mg/kg ip). Blood glucose levels were elevated in both genotypes by 3 days after STZ. Five days after STZ, Nrf2-null mice had higher blood glucose levels (714 mg/dl) than wild-type mice (442 mg/dl) (control mice:160-180 mg/dl). Ten days after STZ, polyuria occurred in both genotypes with higher urine output from Nrf2-null mice (11-fold) than from wild-type mice (7-fold). Histopathology and decreased serum insulin levels confirmed reduced pancreatic islet β-cells in both genotypes. Higher urine output and blood glucose levels in STZ-treated Nrf2-null mice corresponded with enhanced glucokinase expression. No differences in the expression of glucokinase and glucose-6-phosphate dehydrogenase, and reduced glycolysis (pyruvate kinase) related mRNA expression in liver. STZ reduced hepatic glycogen content in both genotypes, with lower levels observed in Nrf2-null mice. To examine glucose sensitivity in naïve mice, a glucose tolerance study was conducted. Blood glucose increased within 15 min after an ip dose of
Gadolinium chloride has been used extensively to deplete and/or inactivate Kupffer cell function and to manipulate immune responses. In this study, mature female mice were ovariectomized (OVX) or sham-operated (SHAM) and treated with 15 mmol/kg halothane (ip), and hepatotoxicity was monitored. To study the influence of ovarian hormones on the susceptibility of female mice to halothane-induced toxicity, females in estrous phase developed more severe hepatic injury at 12 hrs than mice which the mice were administered halothane. When treated with 5 mmol/kg (ip), less injury (ALT ~ 2000 U/L) was observed in OVX mice than in controls. However, life-threatening agranulocytosis or hepatotoxicity occurs in about 1 in 2000 patients. AQ forms a reactive iminoquinone metabolite and these idiosyncratic reactions appear to be immune-mediated but the details of how this reactive metabolite might induce an immune response is unknown. The purpose of this study is to establish an animal model of idiosyncratic hepatotoxicity. Methods and Results: Male Wistar or Brown Norway (BN) rats were treated with AQ at 62.5 mg/kg/day, 6 days/week for 5 weeks via gavage. A significant increase in serum ALT was detected in both Wistar (65.8 U/L ± 3.2 in treated vs 26.7 U/L ± 4.2 in controls) and BN (65.5 U/L ± 6.7 in treated vs 36.8 U/L ± 3.0 in controls) rats after 4 weeks of treatment, which then returned to almost normal despite continued treatment. 24 serum cytokines and chemokines in Wistar rats were determined by Lumines; RANTES and MCP-1 were significantly increased. The number of E. coli stained Kupffer cells and lung macrophages were significantly increased in both rat strains but the increase was greater in BN rats. Using the TUNEL stain, an increase in apoptotic cells in lung and liver were found in BN rats. Covalent binding of AQ was found in lung, spleen, and liver and it was much greater in Kupffer cells than in hepatocytes. Co-treatment with poly I:C (TLR 3) or imiquimod (TLR 7) appeared to delay the onset; however, none of the co-treatments had a significant effect on the level of ALT increase. Conclusion: AQ binding was greatest to Kupffer cells/macrophages and presumably was responsible for their activation. It also caused a delayed onset mild liver injury but the response was limited and not significantly increased by agents that stimulate the immune system via toll-like receptors. It appears that AQ damage is limited by tolerance and the key to developing an animal model of drug-induced idiosyncratic liver failure is overcoming tolerance. This research was supported by grants from CIHR.

### 1144 DEPLETION OF MOUSE KUPFFER CELLS BY CLODRANATE LIPOSOMES VERSUS GADOLINIUM CHLORIDE TREATMENT: DIFFERENTIAL GENE EXPRESSION ANALYSIS.

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Gadolinium chloride has been used extensively to deplete and/or inactivate Kupffer cells from rodent livers. Animals with compromised Kupffer cell function are less susceptible to hepatotoxins such as acetaminophen (APAP) and carbon tetrachloride. Recently, administration of clodronate encapsulated in liposomes has been used as an alternative approach to deplete Kupffer cells. Interestingly, mice treated with liposomal clodronate are actually more susceptible to APAP hepatotoxicity. The reason for this differential effect is not known. In this study, C57BL/6j mice were treated with either clodronate liposome treatment significantly decreased expression of genes involved in several immune processes, including migration (CCL2), binding and adhesion (MARCO), signaling and activation (C1QA) as well as inflammatory response. Similarly, the expression of genes involved in arachidonic acid metabolism (PTGS1) and leukocyte extravasation signaling (VCAM1) was also suppressed in clodronate liposome treated mice. By contrast, treatment with gadolinium chloride did not result in the down regulation of many of the immune-specific genes altered by clodronate liposomes. This indicates that suppression of immune response and Kupffer cell function differs between these two modes of Kupffer cell depletion, which could explain the differential susceptibility to the action of hepatotoxins in rodents treated with these agents.
cumulative oxidant stress and mitochondrial dysfunction) were significantly increased. FLU treatment also resulted in drastically higher numbers of apoptotic hepatocytes and produced small areas of hepatic necrosis in Sod2−/−, but not wild-type, mice. In addition, microarray analysis of 542 mitochondria-related genes (MicroChip) revealed striking alterations in hepatic transcript expression, including lower levels of mtDNA-encoded genes of complexes I and III and mitochondrial tRNAs, as well as downregulated pathways of fatty acid oxidation. These results demonstrate that FLU can cause oxidative damage and overt liver injury in mice with underlying compromised mitochondrial function. The data are compatible with our hypothesis that individuals with mitochondrial abnormalities may be predisposed to DILI-2 caused by mitochondria-targeting drugs.

1147  EFFECTS OF PERINATAL EXPOSURE TO A COMMERCIAL POLYBROMODIPHENYLETHER (PBDE) MIXTURE ON LIVER GENE EXPRESSION AND PROTEIN EXPRESSION IN SD RATS.


PBDEs are a mixture of chemicals used as flame retardants in a variety of textiles, plastics, and furniture. A study using the PBDE mixture DE-71 orally administered to Sprague-Dawley (SD) rats was undertaken to assess the impacts of PBDEs on developing rats. Adult (F0) rats were administered PBDEs in the form of DE-71 technical mixture by gavage at dosages of 0.5, 5, or 25 mg/kg body weight (bw/kg)/day for 10 weeks, after which the rats were bred. Weanling (F1) rats received the DE-71 mixture by gavage from postnatal day (PND) 21 to 42. Liver has been previously identified as a target organ for toxicity by PBDEs. Liver samples from F1 PND 42 male and female rats were examined for changes in gene expression between control and high dose rats via Agilent 22k rat microarray and by monitoring specific gene expression using real-time PCR. Western blots were used to examine changes in protein expression for selected targets. Analysis of microarray data from rat liver found gender specificity with greater effects in females due to PBDE treatment (24 significantly differentially expressed genes) than males (6 significantly differentially expressed genes). Further gene expression analysis using real-time PCR found multi-fold changes in gene expression for CYP1A1, CYP1A2, CYP2B1, CYP2A5, 2D6, 2C9, 2C19, 2C8, 2A6, 1A2, 1B1, 1A1, and 1B10. Further gene expression analysis by real-time PCR found multi-fold changes in gene expression for CYP1A1, CYP1A2, CYP2B1, CYP3A5, UDPGT2, ABCG5, G6PBP1 and CEACAM10. Protein expression monitored via western blots observed significant increases in CYP protein levels for medium and high dose groups. Phase I and Phase II genes and proteins were generally up-regulated in male rat livers compared to treated females. Pathways analysis of female liver data indicate changes in PPAR/RXR pathways which may relate to previously reported changes in serum cholesterol levels. Analyses also indicates pathways for thyroid homeostasis and lipid metabolism are affected. Changes in gene expression of CEACAM also indicate DE-71 exposure may increase the potential for cellular changes related to carcinogenesis.

1148  ROLE OF METALLOTHIONEIN INDUCTION IN HEPATOPROTECTION AGAINST CARMUSTINE TOXICITY IN NORMAL AND GLIOMA-BEARING RATS.

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PB has pleiotropic effects on the liver of rodents, both in the short and long term. Phenobarbital (PB) is a classic, thoroughly studied rodent hepatocarcinogen acting via non-genotoxic mechanisms, involving the CAR nuclear receptor. Exposure to PB has pleiotropic effects on the liver of rodents, both in the short and long term. Short-term administration of PB causes liver enlargement via hypertrophy, and transient cell proliferation. MicroRNAs (miRNA) are non-coding RNA molecules that play important roles in metabolic activation in 1-bromopropane-induced hepatotoxicity.

1149  HEPATOPROTECTIVE EFFECTS OF SELECT WATER-SOLUBLE PARP-1 INHIBITORS.


Induction of Poly(ADP-ribose) polymerase-1 (PARP-1) is one possible mechanism of carbon tetrachloride (CT) hepatotoxicity. Therefore, inhibition of PARP-1 may be an important pathway for reducing hepatic injury. We hypothesize that PARP-1 inhibition may ameliorate CT toxicity by preserving hepatic ATP levels required for apoptosis or cell repair. The current study assesses the ability of water-soluble PARP-1 inhibitors, including 3-aminoazobenzamide (ABA), 3-aminoisothiouronium (AIQ) and PJ-34, to attenuate CT-induced hepatotoxicity in male ICR mice. Dose dependent PARP-1 induction followed CT treatment at doses of 0.3, 0.6 and 1.2 mL/kg. Each dose significantly elevated serum ALT levels, up to 13.6 times controls. Lipid peroxidation was also increased in liver tissue, up to 5.3-fold. Hepatic tissue glutathione (GSH) and superoxide dismutase (SOD) levels were decreased 2.2 and 2.3-fold, respectively. A dose dependent reduction of mitochondrial SOD was also observed. In a previous investigation, results with water-soluble PARP-1 inhibitors. In contrast, the current study indicates that select water-soluble PARP-1 inhibitors can alter CT-induced hepatotoxicity. Treatment with 3 mg/kg AIQ 1 hour before CT reduced elevation of serum ALT 2.1-fold. The most profound reduction followed PJ-34, where ABA pretreatment also reduced ALT elevation. Pretreatment of animals with PJ-34, ABA or AIQ significantly decreased PARP-1 activity in liver tissue (3.4, 2.0 and 1.9 times, respectively) following hepatotoxic doses of CT. Biochemical data indicate that inhibition of PARP-1 can alter hepatotoxicity and protect the liver against CT-induced cytotoxicity. All of the tested PARP-1 inhibitors reduced CT-induced lipid peroxidation and partially restored GSH levels. There were no visible tissue defects in liver from the animals treated with a PARP-1 inhibitor and CT. Thus, select water-soluble PARP-1 inhibitors can attenuate chemical-induced hepatotoxicity.

1150  POSSIBLE ROLES OF METABOLIC ACTIVATION IN 1-BROMOPROPAINE-INDUCED HEPATOTOXICITY.

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1-Bromopropane (1-BP) would be metabolized by two different pathways: the formation of both glutathione (GSH) conjugates by glutathione S-transferase and metabolism by cytochrome P450 (CYP). In the present study, the possible role of CYP in 1-BP-induced hepatotoxicity was investigated in mice. The depletion of GSH by the formation of GSH-conjugate was involved in the increased hepatotoxicity in 1-BP. The formation of reactive metabolites of 1-BP, such as CYP metabolites and by cytochrome P450 (CYP), would be involved in the increased hepatotoxicity. The decreased content of hepatic GSH by 1-BP was parallel not only with the increased activities of ALT and AST but also with the increased content of hepatic malondialdehyde in the group that metabolic enzymes were induced by pretreatment with phenobarbital. The hepatotoxicity induced by 1-BP was significantly protected in mice by the co-treatment with N-acetyl cysteine used to supply the biological content of GSH and silmarin as an antioxidant. Taken together, the formation of metabolites by CYP as well as the depletion of GSH in the CYP-induced animals would play important roles in hepatotoxicity induced by 1-BP. (Supported in part by the U.S. Department of Defense Contract #DAAD15-01-C-0055)

1151  THE NON-GENOTOXIC HEPATOCARCINOGEN PHENOBARBITAL ALTERS MICRONA EXPRESSION IN THE MALE FISCHER RAT.

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Phenobarbital (PB) is a classic, thoroughly studied rodent hepatocarcinogen acting via non-genotoxic mechanisms, involving the CAR nuclear receptor. Exposure to PB has pleiotropic effects on the liver of rodents, both in the short and long term. Short-term administration of PB causes liver enlargement via hypertrophy, and transient cell proliferation. MicroRNAs (miRNA) are non-coding RNA molecules that play important roles in metabolic activation in 1-bromopropane-induced hepatotoxicity.
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1152 TROFLOXACIN AND TUMOR NECROSIS FACTOR α SYNERGIZE TO CAUSE LIVER INJURY IN A MURINE MODEL OF IDIOSYNCRATIC TOXICITY.

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Therapy employing the fluoroquinolone antibiotic, troflloxacin (TXV) was curtailed due to idiosyncratic hepatotoxicity. The incidence of TXV-induced hepatotoxicity was estimated at 1 in 18,000 patients, and in 10% of these patients the pathogenesis progressed to severe liver injury, including liver failure. Liver biopsies from affected patients revealed centrolobular necrosis and the presence of inflammatory cells. Previous studies in rodents showed that an inflammatory stress induced by lipopolysaccharide (LPS) rendered nontoxic doses of TXV hepatotoxic and that TXV/LPS-induced liver injury was TNF receptor-dependent. The interaction of TXV with tumor necrosis factor α (TNFα) was explored further in this study. TXV given 3 h before LPS administration caused significant hepatocellular damage. TXV/LPS-induced liver injury was significantly attenuated in both p55−/− (TNF-R1 knockout) and p75−/− (TNF-R2 knockout) mice. Since TNFα is a critical mediator of TXV-induced toxicity, we explored whether TXV could interact with TNFα. A nonphaptoxic dose of TXV synergized with a nonphaptoxic dose of TNFα to cause liver injury. The hepatic lesions were primarily localized to perivenous regions and were characterized by biliary necrosis and apoptosis. TXV pretreatment enhanced early TNFα-induced inflammatory stress, as evidenced by an increase in hepatic neutrophil accumulation. In addition, a nonphaptoxic dose of TXV sensitized a murine hepatocyte cell line to cytotoxicity induced by TNFα. In summary, TXV/LPS-induced liver injury was dependent on both TNFα receptors. Furthermore, TXV interacted directly with TNFα to cause liver injury by both enhancing TNFα-induced inflammation and sensitizing hepatocytes to TNFα-induced cell death. (Supported by NIH grant DK61315.)

1153 HYPEROXIA-INDUCED OXIDANT STRESS IN THE LIVERS OF NEWBORN MOUSE PUPS.

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Hyperoxic lung injury is a serious medical complication in vulnerable populations such as premature infants. Systemic responses to inhalation or injury have been described and often involve reciprocal responses in the lung and liver. These studies tested the hypothesis that oxidant stress would be evident in the liver of the newborn mouse exposed to hyperoxia. Newborn mice were placed in either room air (RA) or >95% O2 for 1, 3, 7, or 14 days. Data were analyzed by two-way ANOVA, and a modified t-test was used for post hoc testing. There were no differences in liver-to-body weight ratios between the two exposure groups. Analysis of hepatic GSH content indicated an effect of day, an effect of treatment, and an interaction between day and treatment with lower GSH contents in the pups exposed to >95% O2 than in the RA controls at 5, 7, and 14 days (0.9 vs 2.9; 4.8 vs 6.1; and 4.2 vs 6.2 mg/g tissue, respectively). An effect of day was observed on GSSG levels but not on hyperoxia exposure was detected. Thioredoxin-1 and thioredoxin-2 protein levels were greater in the livers of pups exposed to >95% O2 than the RA controls at day 1 but were not different at days 3, 7, and 14. As markers of mitochondrial redox status, CoASH and CoAOSG levels were measured. An effect of day and an interaction be-

tween day and exposure were observed on CoASH levels with higher levels in the >95% O2-exposed animals than the RA controls at day 1 but lower levels at day 14. An effect of exposure was observed on CoAOSG levels with greater levels in the >95% O2 group than the RA group by day 7 (4.1 vs 5.8 nmol/g tissue). These data indicate that oxidant stress is present in the livers of newborn mouse pups exposed to hyperoxia. We speculate that hepatic oxidant stress contributes to the pathogenesis of hyperoxic lung injury.

1154 ACUTE EFFECTS OF ORAL EXPOSURE TO MIDDLE EAST PM10 DUST ON SYSTEMIC PARAMETERS IN LABORATORY MOUSE (MUS MUSCULUS).

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Inhalation of particulate matter (PM)-associated silica, metal and microorganisms can result in adverse pulmonary and systemic health effects. In addition, U.S. military personnel deployed in Iraq and other parts of the Middle East have experienced health effects, particularly Desert Storm pneumonia, as a result of exposure to desert sand or desert dust, a major component of ambient particulate matter (PM). We hypothesize that Iraq desert dust oral exposure will result in circulation and increased bioavailability of PM-associated components resulting in a significant systemic response. Here, we examine in mice the acute effects of oral exposure to desert sand. In this study, mice received a single dose oral gavage of either PM10 Middle East dust in saline or saline alone. At 4 hr and 24 hr following gavage administration, animals were euthanized to analyze the tissue distribution of PM components and the generation of reactive oxygen species (ROS) in tissue. Bioavailability of metals found in PM was assayed in major organs including heart, liver, skin and brain by Inducively Coupled Plasma Mass Spectrometry (ICP-MS). In addition, ROS scavenger phenyl-N-tet-butylamine (PBN) was administered 30 min prior to sacrifice, to trap radical species for electron paramagnetic resonance (EPR) spectroscopy. As a positive control for ROS formation, mice were orally gavaged with LPS, which generates elevated ROS in liver within 4 hr of exposure. This work may provide a broader understanding of the mechanistic basis for PM-associated health effects such as severe acute pneumonia.

1155 COMPROMISED KUPFFER CELLS COULD LEAD TO TCE-MEDIATED SLE-LIKE DISEASE.

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We have shown that trichlorehroethene (TCE) causes SLE-like disease in female MRL+/- mice. We hypothesize that TCE-mediated SLE-like disease is due to increased apoptosis and/or decreased clearance of apoptotic bodies. Therefore, female MRL+/- mice exposed to TCE through drinking water (0.5 mg/ml) were sacrificed after 24, 36, and 48 weeks. The livers of exposed and age-matched control mice were analyzed for histopathological changes, apoptosis, and functional status of Kupffer cells. Hematoxylin and Eosin (H&E) staining demonstrated absence of Kupffer cells. Hematoxylin and Eosin (H&E) staining demonstrated absence of lymphocytic infiltration at 24 weeks, but showed diffuse periprofiral and central vein infiltration at 36 weeks, and increased infiltration and areas of mild hepatocellular necrosis at 48 weeks. TUNEL staining showed increased apoptosis at 24 weeks, but no significant changes at 36 and 48 weeks in TCE- treated mice compared to controls. When apoptotic bodies are not cleared by Kupffer cells, secondary necrosis and inflammation can result, which was observed at 48 weeks. The delayed clearance could be due to compromised endocytosis by Kupffer cells. To evaluate the functional status of Kupffer cells, we used the monoclonal antibody RM-4, which selectively binds to Kupffer cells. The intensity of antibody binding reflects endocytic function. In mice treated with TCE for 36 and 48 weeks, Kupffer cell function was decreased. These studies suggest that increased apoptosis and compromised Kupffer cell function may be a potential mechanism leading to TCE-mediated SLE-like disease.

1156 LIPIDOMICS OF ALCOHOL-INDUCED FATTY LIVER.

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Hepatic steatosis (fatty liver) is an early and reversible stage of alcoholic liver disease which is a major cause of liver-associated illnesses and deaths. To study the mechanism, and identify early biomarkers of alcoholic disease, Fisher 344 rats were fed...
Carbaryl is a widely used insecticide with significant human exposure. Detoxication metabolism of carbaryl occurs by hydrolysis and oxidation. While these pathways have been investigated in vitro, the metabolism kinetics of carbaryl in isolated liver cells has not been reported. The goal of the current study was to determine the kinetic constants of carbaryl biotransformation in isolated hepatocytes and use this information in a human PBPK model to assess risks from carbaryl in humans using biomonitoring data (supported by EPA G-2006-STAR-N1).

1158 KINETICS OF CARBARYL BIOTRANSFORMATION BY FRESHLY ISOLATED SPRAGUE-DAWLEY RAT HEPATOCYTES.

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Carbaryl is a widely used insecticide with significant human exposure. Detoxication metabolism of carbaryl occurs by hydrolysis and oxidation. While these pathways have been investigated in vitro, the metabolism kinetics of carbaryl in isolated liver cells has not been reported. The goal of the current study was to determine the kinetic constants for carbaryl biotransformation in isolated hepatocytes and use this information in a physiologically based pharmacokinetic (PBPK) model for carbaryl. Hepatocytes (2.0-2.5×10^6 cells/well) were cultured for 4-5 hours, then washed and exposed to carbaryl (1-7.5μM) and rates of carbaryl disappearance and metabolite formation were determined. Carbaryl and its metabolites were quantified using HPLC with known standards. The identity of the metabolites was confirmed by LC-MS. Chemical hydrolysis of carbaryl to 1-naphthol (0.800 μM, 77.7% yield) was followed by enzyme-mediated hydrolysis in the cells. Most of the 1-naphthol was rapidly converted to the sulfate conjugate, which was the major metabolite observed. Two hydroxylated metabolites were identified, 3-hydroxy- and trans-5,6-dihydroxy-carbaryl. The glucuronide conjugate of carbaryl was not observed. Formation of the identified metabolites followed Michaelis-Menten kinetics, with apparent KM values of 60-150 μM and apparent Vmax values 0.5-1.5 mmol/min. Metabolic rates for formation of each identified metabolite were calculated and the sum of the individual rates was used to simulate total carbaryl disappearance kinetics in hepatocytes. The observed carbaryl kinetics were in close agreement with the determined carbaryl metabolism rates in hepatocytes. This information on carbaryl metabolism in hepatocytes will be extrapolated to whole animals to refine the previously developed PBPK model for carbaryl in rats. Predictions from the refined model together with data from in vitro kinetic studies of carbaryl in human hepatocytes will be used to develop a human PBPK model to assess risks from carbaryl in humans using biomonitoring data (supported by EPA G-2006-STAR-N1).

1159 DEVELOPMENTAL EXPOSURE TO AMITRAZ ALTERS THE DOPAMINE SYSTEM.

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Amitraz is a formamidine pesticide. The reported biological activity of the formamidines includes MAO inhibition, inhibition of prostaglandin synthesis, local anaesthetic and alpha-2 adrenoceptor activity. The continue presence of amitraz residues may be of particular concern to children and pregnant women. The objective of the present study was to investigate if perinatal amitraz exposure of rats during gestation and lactation alters dopaminergic neurochemistry in their offspring. Pregnant Wistar rats were orally gavaged with amitraz (20 mg/kg) or with corn oil (1.0 ml/kg). The dosing period covered the period from day 6 of gestation through day 10 postnatally. Within each group, a total of 24 offsprings of different litter sizes (6 males and 6 females) were randomly culled on the lactation day 10. At 60 days of age, male and female offspring of control and amitraz-treated animals were killed and their brains removed. The hippocampus, frontal cortex and striatum were dissected and analyzed for content of dopamine (DA) and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) using a HPLC method with electrochemical detection. Developmental amitraz exposure decreased DA levels of male and female offspring at 60 days of age in the hippocampus (32%, P<0.001 and 8%, P<0.05), frontal cortex (17%, P<0.001 and 29%, P<0.001) and striatum (12%, P<0.01 and 13%, P<0.001). In contrast, amitraz caused a statistically significant increase in the turnover ([DOPAC+HVA]/DA) of male and female offspring at 60 days of age respectively, in the hippocampus (60%, P<0.001 and 58%, P<0.001) and striatum (41%, P<0.001 and 42%, P<0.001) respect to corn oil controls. These results suggest there are long-term alterations in the dopamine system after developmental amitraz exposure that might lead to enhanced vulnerability of the dopamine system. Work supported by projects No. CGC07-UCM/AGR-2618 & Consolider-Ingenio 2010 No.CSD2007-363 (MEC), Spain.
**1161 A DIETARY DOSE RANGE-FINDING AND TOXICOKinETIC (TK) STUDY OF 2,4-DICHLOORPHENOXACETIC ACID (2,4-D) IN ADULT CRL:CD(DR) RATS AND THEIR OFFSPRING: I. TOXICOKinETICS**

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TK and toxicity data from a range-finding one-generation study were used to determine appropriate doses for an extended one-generation dietary toxicity study of 2,4-D in rats. Groups of 10 male (M) and female (F) CD rats were treated with 0, 100, 200, 400, 600, 1200 or 1600 ppm 2,4-D in diet from pre-breeding through weaning. F1 offspring were exposed through postnatal day (PND) 35. 2,4-D AUC24 was calculated from 3 plasma samples collected at 6, 9 AM and 5 PM on designated sample days (based on Saghir et al., TAAP, 211:245, 2006). 2,4-D in milk was measured on the lactation day (LD) 20/34 and LD14 and in plasma of nursing pups by collection of a single 9 AM sample on PND4, 14, 21 and 28. Due to saturation of renal clearance, nonlinear TK was observed in parental M rats at 1600 ppm (test day T2D18) and 1200 ppm (TD17). Onset of nonlinear TK in parental females was observed at ~900 ppm (T2D21 and 95) AUC100 ppm ~ 25, AUC1400 ppm ~ 201 µg/ml h-1). During gestation and lactation F2 2,4-D levels were higher due to higher diet consumption; nonlinear TK occurred at ~400 ppm (GD17 and LD14) and ~4000 and ~8000 ppm (LD14). The shift in nonlinear TK on LD14 may have been due to excessive milk (levels approach 50% of F plasma). Plasma 2,4-D in pups was 30-70% lower than the dam on LD4 (M/F TK nonlinear <800 ppm) but similar to or higher than dam on LD14 (M/F TK nonlinear <400 ppm). Maximal 2,4-D in pups was reached on PND21 when pups were consuming 2,4-D diet in addition to nursing, but dropped to levels equal to dams on T2D29 and T2D29 levels on PND35. Thus, the kinetically-derived maximum dose (KMD) was 400 ppm (17.25 mg/kg/day) for F and 1200 ppm (65 mg/kg/day) for M. Maximal AUC during lactation supported dietary TK dose-adjustment during lactation. TK data support a definitive study high dose for females and lower than that derived by traditional toxicity. (Sponsored by the Industry).
centration (mg/cm²), A = surface area exposed (cm²), and t = exposure time (h/ev). In line with this acceptance, over the years many investigators have proposed various equations to estimate the Kᵢ of chemical vapors. These equations all used one or more of a vapor's physiochemical properties as input parameters, such as octanol-water partition coefficient (Kᵢ), Henry's Law constant (KH) and vapor pressure (VP). In this study, a revisit of these equations indicated that overall the one expressing \( \log Kᵢ = a - b \log Kᵢ \text{c} - c \log VP \) appeared to be most effective. Four datasets of experimental Kᵢ available in the literature were used to support the equation selection, as well as to determine the values of the constants (a, b, c, etc.) in each equation attempted. For the above selected equation, the correlation coefficients r (for predicted vs. experimental Kᵢ) ranged from 0.73 to 0.97. The selected equation was deemed highly practical because both the input parameters VP and Kᵢ of an organic vapor are readily obtainable. The equation was also selected to have great biological plausibility in that the DI of a vapor is supposed to be a function of the chemical's volatility (e.g., as measurable by Kᵢ or VP) and lipophilicity (e.g., as measurable by Kᵢ). The four datasets on human or rodent skins were all for volatile organic nontoxic pesticides. Insofar as these dataset elicits the extent to which derived can be extended to (highly) volatile pesticides (e.g., those with KH > 10⁻³ atm-m³/mol), the Kᵢ of regulatory concern are most likely < 2 cm/h. This in turn suggests that regardless of the pesticide vapor concentration involved, the DI time is negligible when compared to the inhalation component, unless the person is required to wear a self-contained breathing apparatus during the exposure period.

1166 A PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODEL FOR CYPERMETHRIN IN THE RAT AND HUMAN.

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Cypermethrin, known as (RS)-α-cyano-s-phenoxbenzyl (1RS)-cis-trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate, is a type II pyrethroid that plays a critical role in intracellular pH regulation and nutrient uptake among yeast and other fungi. The antifungal activity of ebselen (2,3,5,7,8-pentamethylinderselenophene), which has been widely used in clinical practice for its antitumoral activity, was found to decrease in order of magnitude as follows: S. cerevisiae > S. eubayanus > S. cerevisiae. Based on mean IC₅₀ values, the antifungal potency was found to decrease in order as follows: S. cerevisiae > S. eubayanus > S. cerevisiae. The study has also examined the inhibitory potential of these three compounds toward ebselen and its analogs in each case, a chromatographic spot having a migration behavior different to that of the organoselenium compound. Future studies will be required to determine the number and location of the cysteine residues in Pma1p that are susceptible to modification by ebselen and its analogs.

1168 COMPARATIVE CHLORPYRIFOS PHARMACOKINETICS VIA MULTIPLE ROUTES OF EXPOSURE AND VEHICLES OF ADMINISTRATION.

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There have been numerous studies evaluating toxic and pharmacokinetic effects of the organophosphate insecticide chlorpyrifos (CPF) in which, CPF has been administered using different routes and vehicles. The objective of this study was to compare CPF pharmacokinetics using oral, intravenous (IV), and subcutaneous (SC) routes as well as corn oil, saline, and dimethyl sulfoxide (DMSO) vehicles in the rat. In the first study, CPF and isotopically labeled CPF (L-CPF) were co-administered, at doses of 5 mg/kg each, orally and IV in a saline with 5% Tween 20 vehicle for the second study; the same doses of CPF and L-CPF were co-administered SC using corn oil and DMSO. Blood and urine samples were collected and analyzed for CPF and trichloropyridinol (TCPy). Oral CPF administration resulted in the highest peak concentration of TCPy (7-11 times higher), which also occurred most rapidly (1.5 hr prior to the next closest). SC administration with a corn oil vehicle led to faster absorption/metabolism of CPF than did SC administration using DMSO as a vehicle (ka: 0.6 and 0.2 μmol/hr, respectively). IV administration of CPF caused bimodal pharmacokinetics, probably due to CPF partitioning into to determine the body. For all exposure routes and vehicles, ~85% of the molar dose of CPF was excreted as some conjugated form of TCPy. Overall, orally administered CPF underwent much more extensive and rapid metabolism than did other routes investigated, probably due to first-pass metabolism. This suggests that CPF administered systemically could result in a greater body burden of CPF than would an equivalent dose administered orally, potentially leading to a higher CPF concentration at target tissues. These results will be useful for elucidating differences regarding potential uncertainty in CPF pharmacokinetics due to varying exposure routes and vehicles of administration. (Funded by CDC/NIOSH Grant R01 OH08173 and AGR05FED40077.02)

1169 THE EFFECTS OF ZIRAM ON RAT HIPPOCAMPAL ASTROCYTES.

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Ziram (zinc-bis(dimethyldithiocarbamate)) is an agricultural dithiocarbamate fungicide that is used on a variety of plant diseases. In recent years, epidemiological studies have linked exposure to environmental agents such as pesticides with an increased risk of developing neurodegenerative diseases. In spite of their generally acknowledged low toxicity, dithiocarbamates are known to cause a wide range of neurobehavioral effects as well as neuropathological changes in the brain. Furthermore, the dithiocarbamate, ziram, has been reported to cause hepatotoxicity, thyroid toxicity and have adverse effects on the reproductive system and the developmental process. This investigation studied the effects of ziram on rat hippocampal astrocytes. Cells were maintained in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum. Astrocytes were exposed to 1μM of ziram and 300 μM of zinc chloride for one hour at 37 degrees C and then re-fed with complete medium for 24 hours. Morphological observations, by phase contrast and scanning electron microscopy, in treated astrocytes showed blebbing, increased numbers of retraction fibers and distortion of cellular processes. Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) demonstrated, in all cases of treatment with Ziram, a significant increase of copper when compared to controls. However, zinc levels in the ziram treated astrocytes and the control astrocytes were not statistically different. The ICP-OES analysis of the zinc chloride treated astrocytes revealed a significant increase of zinc levels and no statistical difference of copper levels when compared to control astrocytes. The total antioxidant assay revealed a significant decrease in the antioxidant capabilities of ziram treated astrocytes. This preliminary data suggests that the cytotoxic effects observed in the ziram treatments may be related to the increased copper levels resulting in oxidative stress. Further studies will be needed to elucidate the mechanism of action induced by ziram on rat hippocampal astrocytes.
In pursuit of the Agency's goal for the protection of human health and the environment, the Office of Pesticide Programs (OPP) is examining alternative methods to enhance the comprehensiveness of pesticide risk assessments. Toxicity testing in the 21st century will feature alternative methods such as in silico technologies that will potentially reduce or refine animal testing requirements while increasing the efficiency and confidence in risk assessments. Although submissions for pesticide registration generally include comprehensive toxicity data sets, OPP is investigating the use of predictions from Quantitative Structure Activity Relationship (QSAR) models to support the assessment of pesticides and/or to demonstrate the need for additional testing. Commercial (QSAR) programs TOPKAT®, MultiCASE®, and DEREK for Windows® were assessed for their ability to predict carcinogenicity, genotoxicity, and developmental toxicity individually and in a consensus modeling approach. Model results were compared to empirical data for approximately 50 pesticides spanning 10 classes. Individually, the models were equivalent in their ability to correctly predict cancer, genotoxicity, and developmental toxicity endpoints compared to the empirical data. Concordance values were highest and lowest for the genotoxicity and developmental endpoints, respectively. Furthermore, confidence in the developmental endpoint predictions was low because the pesticides routinely fell outside the models' applicability domain. Consensus modeling generally provided greater predictive accuracy than the individual models alone. This study indicates it is critical to populate training sets with pesticide data to increase the applicability of these (Q)SAR models for pesticide structures. The approaches investigated in this study will contribute to EPA ongoing efforts to work toward the vision of toxicity testing in the 21st century.

The views expressed in this abstract are those of the authors and do not necessarily reflect those of the U.S. EPA.

**1170 PREDICTIVE CAPACITY OF SELECTED QSAR MODELS FOR PESTICIDES IN A REGULATORY SETTING.**


**1171 ASSESSMENT OF PESTICIDE CYTOTOXICITY USING CEREBROMICROVASCULAR ENDOTHELIAL CELLS.**

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The blood-brain barrier (BBB) acts as a protective interface between peripheral and central environments and limits brain exposure to environmental toxicants. P-glycoprotein (P-gp) is expressed in BBB cerebromicrovascular endothelial cells (CEC) and plays an important role in protecting the brain from toxic insults.

The aim of this study was to (i) identify a series of pesticides reported to interact with P-gp, (ii) assess pesticide toxicity towards CEC (iii) establish a physiologically representative in vitro BBB model.

Methods: Identification of pesticides: Thorough literature searches using Pubmed and Medline were carried out to identify structurally diverse compounds. Isolation of CEC: Freshly obtained porcine brain tissue was homogenised and the microvesicles treated with collagenase, trypsin and DNase I. Cytotoxicity assay: CEC were cultured on Transwell inserts over seven days in the presence and measurement of product formation at 550nm. Culture of CEC on Transwell inserts was carried out in 96-well plates. Pesticide toxicity was assessed using the MTT assay and DEREK for Windows® were assessed for their ability to predict carcinogenicity, genotoxicity, and developmental toxicity endpoints.

**1172 AVERMECTIN TRANSPORT BY MDR1 IN SH-SY5Y NEUROBLASTOMA CELLS.**

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Avermectins are used as agricultural insecticides and as anti-helmintic treatments in man. Avermectins have a good safety profile in a variety of species; however toxicities observed in the CF-1 mouse model initiated investigations into the relevance of this model for human risk assessment. The CF-1 mouse lacks a functional xenobiotic efflux transporter, multidrug resistance protein or P-glycoprotein (mdr1a or P-gp). In humans a non-functional MDR1 has yet to be identified, although polymorphisms do exist and their significance in efflux transport needs to be better understood.

The aim of this study was to assess the MDR1 efflux transport of avermectins in human neuroblastoma cells (SH-SY5Y), previously characterised for uptake and efflux transporter expression. SH-SY5Y cells were loaded with the MDR1-substrate Hoechst 33342 (H33342) in the absence or presence of avermectin (50ng/ml) and the intracellular retention of H33342 was measured by fluoroescence spectroscopy. The impact of avermectins (5μM) upon the intracellular retention of H33342 was compared with that of cyclosporin A (MDR1 substrate). Cytotoxicity was measured using the MTT assay to confirm integrity of the model system.

Inhibition of cells with abamectin or emamectin resulted in a significant (p<0.05) increase in H33342 dye retention by the cells which was of a similar order of magnitude as that achieved in the presence of cyclosporin A. Dose response curves for the ability of abamectin and emamectin to inhibit MDR1-mediated H33342 dye efflux gave apparent Ki values of 2.51±0.30 and 0.20±0.09μM respectively. These values compared with a Ki of 2.52±0.31μM for interaction of cyclosporin A with MDR1. SH-SY5Y cells are a good model in which to investigate the efflux of avermectins and confirm the involvement of human MDR1 in avermectin disposition. Future work will involve establishing in vitro overexpression systems to investigate the role of specific polymorphisms in avermectin efflux.

**1173 ISOFENPHOS-INDUCED DNA DAMAGE IN HUMAN LYMPHOCYTES IS MEDIATED VIA THE PRODUCTION OF OXIDATIVE STRESS.**

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Recent studies suggest that individuals who are chronically exposed to organophosphate pesticides may suffer higher incidences of chromosomal damage and leukemia. An organophosphate which may pose significant carcinogenic risks is Isofenphos (IFP) and its metabolite isofofosfate-oxygen (IFP-oxygen). IFP is utilized to control soil-based insects and is applied to fruits and vegetables including corn, carrots and bananas. It is also used to control termite population in edifices. It should be noted that IFP persists in soil and water for several months and weeks and its metabolite IFP-oxygen may be just as persistent as the parent compound. The objective of this study was to determine if Isofenphos exposure promotes genomic instability in human lymphocytes. Isofenphos (IFP) produced dose-dependent toxicity in Jurkat lymphocytes. Exposure to IFP for 24 hours induced dose dependent toxicity and dose dependent reductions in cellular GSH levels. Pretreatment with Atropine (10μM), Mecamylamine (100μM), L-NAM (100μM), Caspase inhibitor QVDOPh (50μM) failed to alter IFP-induced toxicity. However, the addition of antioxidants N-acetylcysteine (5mM) and Vitamin E (50μM) significantly reversed the effects of IFP (p<0.05). In the comet assay, IFP caused significant DNA damage in a dose-dependent mechanism. Moreover, IFP-induced DNA damage was partially attenuated by pretreatment by 100μM Vitamin E (p<0.05). Our data indicates that IFP is indeed genotoxic in the environment and that IFP induced DNA damaging properties is mediated by the production of oxidative stress rather than by cholinesterase inhibition.

**1174 TISSUE DISTRIBUTION AND ELIMINATION KINETICS OF FLUOROSULFATE (FSO3) AND FLUORIDE (F) IN ADULT RATS EXPOSED TO SULFURYL FLUORIDE (SO₂F₂).**


Sulfuryl fluoride (SO₂F₂) is a structural and post-harvest fumigant and a key alternative to methyl bromide, a stratospheric ozone-depleting fumigant scheduled for phase-out by the Montreal Protocol. Inhaled SO₂F₂ is rapidly hydrolyzed to fluorosulfate (FSO₃⁻) and one equivalent of fluoride (F⁻). FSO₃⁻ is subsequently hydrolyzed to sulfate and a second F⁻ equivalent. Fluoride appears to play a role in the mechanism of action of SO₂F₂ in both insects and mammals. Groups of adult male F344/DuCr rats were exposed to 0.3, 30, or 300 ppm SO₂F₂ for 4 h in order to examine the absorption, distribution, metabolism and elimination of inhaled SO₂F₂ and its metabolites FSO₃⁻ and F⁻. Plasma, kidney, cerebrum, olfactory bulb, gut, natal and pulmonary tissues were analyzed for FSO₃ and F⁻ levels at 0, 2, 4, and 8 h after exposure. Rats were fed a low F diet and deionized water for two weeks prior to exposure to enhance the ability to detect elevated F levels in treated vs control groups. Plasma SO₂F₂ levels were measured using ion chromatography with negative ion electron spray ionization mass spectrometry (IC/NEES/MS), while F was measured by micro-diffusion with ion-selective electrode detection (MD/ISE). Fluoride concentrations above the lower limit of quantitation (0.027 μg F/g tissue) were measured in all tissue samples from 0 ppm (control) rats. Immediately after exposure,
FSO, and F were measured in approximately equimolar amounts in plasma, kidney and lung. Tissue F levels were roughly dose proportionate in rats exposed to 30 or 300 ppm SO₂F₂. Both FSO, and F were rapidly cleared from tissues with elimination half-lives of less than 3 h. These data will aid in the development of a multi-compartment PBPK model which can be utilized for human risk assessments.

1175 OXIDATIVE STRESS AND GENOTOXIC EFFECTS INDUCED BY IN UTERO AND LACTATIONAL EXPOSURE TO ENDOSULFAN ON RAT PITUITARY. A. Lefuerne, T. Cabaleiro, A. Caride, B. Fernandez-Perez and A. Anadon. 1Toxicology Laboratory, Faculty of Sciences, Vigo University, Orense Campus, Orense, Spain and 2Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Universidad Complutense, Madrid, Spain.

Endosulfan is an organochlorine insecticide that is considered an endocrine disruptor and induces oxidative stress in a number of species. We hypothesized that endosulfan treatment during gestation and lactation may alter the pituitary gland, which controls the endocrine system. To test this hypothesis, we investigated the effects of endosulfan on pituitary gland expression of prolactin, luteinizing hormone (LH), growth hormone (GH) and thyroid stimulating hormone (TSH) and on the expression of antioxidant enzymes (SOD, GST, GPX, NOS) in rats treated with endosulfan. Male Sprague-Dawley rats were divided into four groups: control (C), low-dose (LD), mid-dose (MD) and high-dose (HD). On the day of weaning, all groups were sacrificed and the pituitary glands were collected. The expression of prolactin, luteinizing hormone (LH), growth hormone (GH) and thyroid stimulating hormone (TSH) and on the expression of antioxidant enzymes (SOD, GST, GPX, NOS) were measured. Compared to the control group, rats in the endosulfan-treated groups showed significant increases in the expression of prolactin, LH, GH and TSH. The expression of SOD, GST and GPX was also increased in the endosulfan-treated groups. In addition, the expression of NOS was decreased in the endosulfan-treated groups. These findings suggest that endosulfan exposure during gestation and lactation may alter the pituitary gland and affect the expression of pituitary hormones and antioxidant enzymes.
1180 PHARMACOKINETIC EVALUATION OF PERMETHRIN ENANTIOMERS IN RAT TISSUES.


Pyrethroids are neurotoxic insecticides that are used in agricultural, residential, and public health activities. The ubiquitous exposures to pyrethroids require understanding of the risk posed to human and ecological health. Since identification of the active toxophore is essential for improved risk analysis, understanding the active stereoisomer is not only important but remains largely unresolved. While evaluation of the exposure, toxicity and disposition of pyrethroid isomers has been examined at the enantiomer level, information on the pharmacokinetics is limited. Thus, this study aimed to develop a LC/MS method to identify and quantitate all four permethrin stereoisomers in rat tissues for absorption, distribution, metabolism, and excretion before analysis by LC/MS. Stereoseparation was achieved on a Chiralcel-OJ stationary phase with 98:2 hexane:ethanol mobile phase at 0.8 mL/min. Detection of selected ions was by atmospheric pressure chemical ionization mass spectrometry in the positive mode. For both blood and brain, the limit of quantification was between 50-150 μg/kg per permethrin enantiomer. Matrix based calibration curves were linear for each enantiomer over a range of 50-5000 μg/mL with regression coefficient exceeding 0.99. Initial findings indicate that the IV and oral area under the plasma concentration time curve (AUC) for the cis-isomers is greater than the trans-, indicating slower degradation/elimination of cis-permethrin. However, there was no significant difference in the AUC among the cis- and trans-enantiomers. These studies suggest that the pharmacokinetics of the permethrin diastereomers are different but at the enantiomer level, the pharmacokinetics are similar. (This abstract does not reflect EPA policy).

1181 PHEROMONES - TOXICOLOGY AND PUBLIC HEALTH.


This study evaluates the toxicologic properties and components of a public health program in addressing the use of pheromones (Straight chain lepidopteran pheromones, SCLP) as an integrated pest management tool in California for combating the light brown apple moth which is native to Australia. SCLPs are a class of chemicals with low acute toxicity (high LD50s, irritation). Subchronic tests showed no or minimal effect. SCLPs have been granted federal exemptions from food tolerances and long-term testing. They are used in various formulations and in tools such as dispensers, sprays and traps. These synthetic chemicals are identical to natural pheromones that attract (but do not kill) the moths and are used in a manner to prevent mating and propagation of future generations. Aerial applications of a synthetic pheromone formulation in 2007 at very low volumes were followed by reports of symptoms, mostly self-reported irritation in nature. We analyzed these reports including those from physicians under the current Pesticide Illness Reporting system and were unable to identify an associated cause. We conducted additional analyses of the physician reports and surveys to identify possible ways of improving this reporting system. We are setting up a toll-free telephone system to provide a resource for health related information and a centralized means for future symptom reporting. We conducted education and outreach activities to address health issues of concern relating to the active and inert chemical ingredients involved and routes and forms of exposure. We evaluated prior experience in the U.S., Australia, New Zealand and Canada regarding invasive pests and pesticide use, toxicology and health issues. Results indicated that notification, knowledge of toxicology, recognition of concerns, education, and with risk assessments are important components in addressing the use of pesticides to combat invasive species.

1182 ROLE OF HUMAN SERUM ALBUMIN IN THE DETOXIFICATION OF ORGANOPHOSPHORUS INSECTICIDES.


The capability of human serum albumin (HSA) to detoxify the organophosphorus compounds (OPs) paraoxon and chlorpyrifos-oxon was studied at high and low OP concentrations. OP hydrolysis was studied using classical colorimetric methods, while the assay at low concentrations were performed by a method based on the comparison of the capability of OP solutions to inhibit acetylcholinesterase just before and immediately after incubation with HSA. Chlorpyrifos-oxon was hydrolyzed by HSA with a Km of 2.6 mM and Kcat of 6.4 x 10-4 s-1, while the corresponding figure for paraoxon hydrolysis were 0.38 mM and 2.4 x 10-5 s-1. Thus, HSA hydrolyzed chlorpyrifos-oxon with a catalytic efficacy around 3.7 times higher than paraoxon. Paraaxon and chlorpyrifos-oxon also were hydrolyzed by HSA at significant rates at OPs concentrations in the same order of magnitude than their respective acetylcholinesterase inhibition constants for 5 minutes of exposure. Chlorpyrifos-oxon was again better substrate than paraaxon for HSA. The incubation (8 h at 37 °C) of a 2 μM chlorpyrifos-oxon solution in presence of 13 mg HSA/mL caused 93% of hydrolysis (estimated by recording a reduction of acetylcholinesterase inhibition power of 67%). However, the hydrolysis was only 20% (90% of acetylcholinesterase inhibition reduction) after incubation in the same conditions of a 14 μM paraaxon solution. It is concluded that HSA exhibits capability to detoxify paraaxon and chlorpyrifos-oxon even at concentrations 27 and 1300 times lower than the respective Km and despite the low Kcat. Furthermore, taking into consideration the high HSA concentration circulating in blood (around 40 mg/mL) these data suggest that HSA might play a relevant role in the detoxication of OPs.

1183 PARAOXONASE 1 STATUS AND ACTIVITY LEVELS AS RELATED TO RACE AND SEX IN HUMAN POPULATIONS IN THE DEEP SOUTH.

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Paraaxonase1 (PON1; a calcium-dependent A-esterase) can hydrolyze and thereby detoxify a variety of organophosphates (OPs), including the active metabolites (oxons) of a number of insecticides, such as chlorpyrifos and diazinon. Comparisons of paraaxonase to diazoxonase activities in the serum can characterize an individual into one of three genotypes (i.e., PON1 status: QQ, QR or RR) for the most important PON1 polymorphism, the Q27H polymorphism. PON1 is associated with the HDL particle and is involved in hydrolyzing oxidized lipids and protecting cardiovascular health. The Q form of PON1 is more efficient at hydrolyzing diazoxon and is more protective of cardiovascular health, while the R form is more efficient at hydrolyzing paraaxon and chlorpyrifos-oxon and is less protective of cardiovascular health. With 200 purchased serum samples, African American populations in the South displayed a higher proportion of the RR genotype than Caucasians, which corresponds to their higher prevalence of cardiovascular disease. Cardiovascular health status of these individuals was not known. Males and females displayed similar activity levels of both paraaxonase and diazoxonase. Arylesterase activity, monitored with phenyl acetate hydrolysis, did not characterize genotypes as effectively as diazoxonase activity. None of these samples were from smokers, as indicated by low cotinine levels. A pilot study is in progress that obtains blood samples from individuals in Mississippi with known cardiovascular health status. PON1 status of these Mississippi individuals corresponds to the same relationships observed with blood samples, i.e., a higher prevalence of the QQ genotype in the Caucasian individuals. Both the genotype and the activity levels with respect to OP substrates of interest will determine the susceptibility of an individual to potential toxicity following OP insecticide exposure. Supported by NIH R21 ES015107.

1184 EXPOSURE TO PYRETHRIDS AND CONTRIBUTING FACTORS IN RESIDENTS OF URBAN AND RURAL AREAS OF THE PROVINCE OF QUEBEC, CANADA.


Residents of rural (agricultural) areas are often suspected of being exposed to higher levels of pesticides than residents of urban areas. However, only a limited number of studies have specifically evaluated the impact of the geographical area of residence on pesticide exposure in the general population. This study aimed at characterizing pyrethroid exposure between an urban and rural population of the Province of Quebec, Canada. A total of 154 urban (Montreal) and 154 rural (Monteregie) participants provided a complete overnight timed-urine collection and filled a self-administered questionnaire. Urine samples were analyzed for pyrethroid metabolites, chrysanthemum dicarboxylic acid (CDCA), dichloro- and dibromo-vinyl-dimethylcyclopropene carboxylic acids (tDCCA, cDCCA and DBCA), and phenoxybenzoic acids (PBA and FPBA). Levels of pyrethroid metabolites (pmol/12 h/kg bw) and their frequency of detection in the two populations were compared.
Cross-comparisons with questionnaire data were also conducted. tDCCA and PBA treatment was higher in the rural population than in the urban population, and the differences were statistically significant. There was a higher frequency of detections of PBA in a population from the rural area than in the urban area, and the differences were statistically significant. In addition, the results of tDCCA and PBA treatment were higher in the rural population than in the urban population, and the differences were statistically significant. However, there was no statistically significant difference between the two populations in terms of tDCCA and PBA treatment. Therefore, it can be concluded that tDCCA and PBA treatment are not associated with the rural-urban residence of individuals.

1187 ACTION PLAN OF JAPAN NATIONAL CHILDREN’S STUDY (JNCS).

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In 2007, the Ministry of Environment, Japan decided to work on a nation-wide birth cohort study (Japan National Children’s Study; JNCS). According to the Advisory Commission’s recommendation in 2008, the working group of JNCS (WG) has set up and been discussing the action plan. The WG proposed 10 working hypotheses to clarify the effects of the exposure to chemical and physical environmental factors on the health and development of children in Japan. Pilot studies have started at three areas by the WG in advance. The full-scale survey will start in 2010. In WG will select 10 centers of communities which participate in JNCS. Each community registers maternity hospitals in it as many as possible (more than 70%) and participants (pregnant women) are recruited at their first visit at the hospitals. Another 2,000 pregnant women will be recruited from 2010 to 2012 for three years. Their children are observed from fetus to age 12. In order to evaluate the exposure to environmental factors, we are going to measure heavy metals, dioxins, POPs and so on in biospecimens from mother and their children besides questionnaire surveys and interviews. The targeted outcomes are physical development (low birth weight, developmental retardation, etc.), congenital anomalies (hypospadias, cryptorchidism, cleft palate, GI obstruction, venous septal defect, Down’s syndrome, etc.), psycho-neurodevelopmental impairments: autism (learning disorder, ADHD, hyperactivity disorder, etc.), immunologic impairments (allergy, atopic dermatitis, asthma, etc.), metabolic/endocrinoimpairments (genital hypoplasia, brain sexual differentiation, thyroid dysfunction, impaired glucose tolerance, obesity, etc.). The WG will publish findings obtained from JNCS at appropriate time.

1188 EXPOSURE TO DIOXINS AND FURANS IN THE POPULATION LIVING NEAR A HAZARDOUS WASTE INCINERATOR: A FOLLOW-UP STUDY.

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Although incineration is an effective way of treating municipal and hazardous waste (HW), in many countries the potential health risks associated with stack emissions, especially polychlorinated dibenzo-p-dioxins and furans (PCDD/F), have become a cause of great controversy and concern. In 1999, the only HW incinerator (HWI) in Spain initiated regular operations in Tarragona County (Catalonia). Since then, a wide environmental and biological monitoring program is being carried out. In 2007, human samples of plasma (n = 20), milk (n = 15) and adipose tissue (n = 15) of individuals living near the facility were collected. The concentrations of PCDD/F were determined by HRGC/HRMS and compared with those found in 2002. The current mean PCDD/F levels (pg WHO-TEQ/g lipid or fat) were 9.5, 7.6 and 14.6, vs. 17.8, 8.3 and 9.9, obtained for plasma, milk and adipose tissue, respectively. It means decreases of 40% and 8% for plasma and milk, respectively. In contrast, the mean PCDD/F concentration in adipose tissue increased (P < 0.05) a 47%, although it was still notably lower than the mean level found in the baseline (1998) survey, 40.1 pg WHO-TEQ/g lipid. On the other hand, the dietary PCDD/F intake of the assessed population was also determined, being currently 27.8 pg WHO-TEQ/day vs. 63.8 pg WHO-TEQ/day in 2002. These results indicate that emission of PCDD/F from the stack of the HWI do not mean an additional significant exposure for the population living near the facility. Moreover, it seems obvious that the levels of PCDD/F in plasma are a better biological indicator of exposure to these pollutants than those in adipose tissue.
Despite smoking fewer cigarettes daily, metabolic and pharmacogenetic explanations for these observations have been offered, as has speculation that differences in cigarette preferences or cigarette smoking behavior may play a role. Further clarification of black/white differences in biomarkers of cigarette smoke constituent exposure is indicated. We conducted the current analysis to determine whether racial differences in smoke constituent exposure biomarkers may be evident among smokers of cigarettes having a machine-measured smoke yield of about 10 mg "tar". A total of 28 black & 84 white smokers (mean 27.6 & 27.8 cigarettes/day, respectively) participated in a study to determine whether ad librum smoking resulted in differences in smoke constituent exposure biomarkers. Carboxyhemoglobin (COHb) levels were measured in blood samples drawn at mid afternoon following two 24-hour urine collection periods. Six urinary nicotine metabolites and respective glucuronides were determined as measures of tyrosine intake, and urinary 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone (NNK), COHb values did not differ significantly by race (6.2% for blacks vs. 6.6% for whites). Neither creatinine adjusted NNAL (C-NNAL) nor creatinine adjusted nicotine equivalents (C-NEq) significantly by race (6.2% for blacks vs. 6.6% for whites). Neither creatinine adjusted NNAL nor creatinine adjusted nicotine equivalents were determined significantly by race. There was also no significant difference in the trans-3'-hydroxycotinine to cotinine ratio, a measure of CYP2A6 activity (2.4 for blacks vs. 2.4 for whites). These findings indicate that in this sample, black and white smokers exhibit essentially the same levels of biomarkers of smoke constituent exposure.

A WEIGHT-OF-EVIDENCE APPROACH TO EVALUATING EPIDEMIOLOGICAL DATA ON STYRENE CANCER HAZARDS.

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The May 22, 2008 National Toxicology Program (NTP) Report on Carcinogens: Draft Background Document for Styrene describes the state of the literature on the carcinogenicity of styrene. With regard to the epidemiological literature, NTP describes several key studies, primarily of 12 cohorts, and their strengths and weaknesses. We conducted a weight-of-evidence analysis of the association between styrene exposure and the risks of each of the cancer types on which NTP focused in these cohorts, which includes cancers of the esophagus, pancreas, larynx, and lung, all lymphohematopoietic cancers combined, as well as lymphoma, Hodgkin’s disease, multiple myeloma, and leukemia. We assessed the consistency of risk estimates within and among each cancer type, and across studies, with an emphasis on comparing estimates that were based on the same exposure metric. We also assessed associations across industries. We found that, although some positive associations were reported in certain exposure groups of some cohorts in some studies, so, too, were null and negative effects. There were no consistent findings of effect for any cancer type in the reinforced plastics and composite (RPC), styrene-butadiene rubber (SBR), or the polystyrene/styrene (PS) industries. If styrene were a causal factor for any of these cancers, one would expect to see associations in the RPC industry, which has the highest exposures to styrene and the lowest likely confounding exposures, and particularly in the highest-exposed RPC workers. This was not the case. Taken together, the epidemiology data do not support a causal association between styrene and cancer risk in humans. This work was supported by the Styrene Information and Research Center (SIRC).

NO EVIDENCE OF INFECTION WITH AVIAN INFLUENZA AMONG U.S. POULTRY WORKERS, DELMARVA PENINSULA.

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Poultry workers are recognized as the front line for transmission of avian influenza (AI) to humans. Studies of AI transmission between poultry workers and workers in industrial facilities are limited, due in part to the perception that these facilities are biosecure and biocontained. To assess frequency of industrial poultry worker exposure to AI, we analyzed serum of poultry workers and community residents from the Delmarva Peninsula, one of the top five poultry production areas in the United States, for antibodies against five subtypes of AI. We interviewed a convenience sample of workers and community residents and collected serum samples in 2003 and 2005. Microneutralization assays were used to detect antibodies to five AI subtypes (H4N6, H5N2, H6N2, H7N2 and H9N2) representative of those circulating in poultry the US. Sera were also analyzed for antibodies that recognize currently circulating H1N1 and H3N2 human influenza A viruses using the hemagglutination inhibition assay protocol. We observed no evidence of seroconversion among poultry workers or community residents to the AI subtypes in our study, implying a lack of exposure to these viruses. Low levels of reported use of personal protective equipment suggest that worker protection does not explain this negative finding. The most likely explanations are that the workers in our study were either not exposed to AI viruses because none were present in their occupational environments, or that the viruses present were incapable of infecting humans. We observed high rates of seroconversion to human influenza subtypes in both the poultry worker and community residents. Since co-infection with avian and human influenza viruses is a risk factor for the emergence of a novel influenza strain, the prudent vaccination of poultry workers against human influenza is critical.

DEVELOPMENT OF ENANTIOSELECTIVE POLYCLONAL ANTIBODIES TO DETECT STYRENE OXIDE PROTEIN ADDUCTS.

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Styrene has been reported to be hepatotoxic and pneumotoxic in humans and animals. Styrene oxide, a major reactive metabolite of styrene, has been found to form covalent binding with proteins, such as albumin and hemoglobin. Styrene oxide has two optical isomers and it was reported that the (R)-enantiomer was more toxic than the (S)-enantiomer. The objective of this study was to develop polyclonal antibodies that can stereoselectively recognize proteins modified by styrene oxide enantiomers at cysteine residues. Immunogens were prepared by alkylation of thiolated keyhole limpet hemocyanin (KLH) with styrene oxide enantiomers. Polyclonal antibodies were raised by immunization of rabbits with the chiral immunogens. Titration tests showed all six rabbits generated high titer of antiserum that recognize (R) or (S) coating antigens according. No cross-reactivity was observed toward the carrier protein of the coating antigen (BSA). All three rabbits immunized with (R)-immunogen produced antibodies which show enantioselectivity to the corresponding antigen, while only one among the three rabbits immunized with (S)-immunogen generated antibodies with enantioselectivity of the recognition. The enantioselectivity was also observed in competitive ELISA and immunoblot analysis. Additionally, competitive ELISA tests showed that the immuno-recognition required the hydroxyl group of the hapten. Immunoblot analysis demonstrated that the immuno-recognition depended on the amount of protein adducts blotted and hapten loading in protein adducts. In summary, we successfully developed polyclonal antibodies to stereoselectively detect protein adducts modified by styrene oxide enantiomers.

SERUM COTININE LEVELS OF SMOKELESS TOBACCO USERS AND CIGARETTE SMOKERS: AN ANALYSIS OF NHANES 1999-2006 DATA.


Cotinine is widely used as a biomarker of nicotine uptake, and of tobacco consumption in general. Reports indicate that smokeless tobacco (ST) users tend to have higher levels of serum cotinine compared to cigarette smokers, with moist snuff users possibly having higher levels than users of chewing tobacco. The relationship between serum cotinine levels and the use of snuff (any form), chewing tobacco (chew), and cigarettes was examined using data collected from the National Health and Nutrition Examination Survey (NHANES) Mobile Examination Centers for 1999-2006. Multiple linear regression models for the natural-log transformed serum cotinine values (accounting for the design of the surveys) were used to compare the cotinine levels for the three tobacco categories, adjusting (when necessary) for age, gender, ethnicity, poverty status, region of residence (regional differences), smoking status (never, former, current), age of smoking onset (ages of 18 years or last five), and time since last used. The data suggest that snuff and chew users have higher levels of serum cotinine compared to users of cigarettes (p < 0.05) when the models include frequency of use. There is some evidence of higher levels for snuff users compared to chew users, and of an interaction between tobacco category and frequency of use, but both failed to achieve statistical significance (0.05 < p < 0.10). When the analyses are done separately for each frequency of use category (used 5 days, 4 days, or <3 days out of last 5 days), snuff users had the highest levels, followed by users of chew and cigarettes, with the statistical significance varying for
the frequency categories. These results are consistent with others in that cotinine levels are higher for ST users, with a possible difference between snuff and chew.

Interpretation of these findings as suggesting a difference in nicotine exposure should be made with caution since systemic cotinine levels are affected more than systemic nicotine levels by the pre-systemic first pass metabolism of the nicotine in swallowed tobacco juice.

1194 PRENATAL EXPOSURE TO MERCURY, PARTITIONING OF TOTAL AND ANA IMMUNOGLOBULINS IN MATERNAL AND CORD BLOOD, AND ASSESSMENT OF A BIOMARKER OF MERCURY IMMUNOTOXICITY.

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Mercury (Hg) is a ubiquitous environmental contaminant with known neurodevel-

opmental effects at high exposures. Recent studies on the toxic properties of Hg have highlighted the implications at lower exposures particularly on the immune system. Low level prenatal exposure through maternal contaminated fish consumption has the potential to impact the immune system of the fetus. Many studies have been undertaken to identify a reliable biomarker of maternal and cord blood Ig. We have previously reported that antigen-specific autoantibodies (anti-nuclear, ANA) may be informative biomarkers of Hg induced immunotoxicity. In this study, we assessed the effects of prenatal exposure to Hg on total and ANA immu-

noglobulins (Ig) in a cross sectional sample from a prospective mother-infant cohort study in Amazonian Brazil. We compared maternal and cord blood Hg and antibody levels with maternal covariates obtained by questionnaire. The geometric mean of Hg in mothers was 6.9 and in cord blood it was 9.6 μg/L. 36 percent of maternal and 52 percent of cord blood samples were above the US EPA estimated safe level of 5.8 μg/L and fetal-maternal Hg levels were correlated but not equiva-

lent. We found that total and ANA IgG, but not IgM, levels were correlated with both maternal and cord blood Ig. These findings are consistent with research on the ability of IgG, but not IgM, to cross the placenta in the absence of infection.

In this study, we've identified five known human neurotoxicants: ethyl acetate, methyl ketone, toluene, xylenes, and n-hexane, from air samples collected during bridge painting from 2005 to 2007 in New York, New Jersey, and Pennsylvania. Based on enzymes kinetics, ten metabolic enzymes of these neurotoxicants were se-

lected for assessment. Data were collected on an epidemiological data set examining the relationship between ex-

posure to women of PCB mixtures 62 congeners analyzed) and risk of en-

dometriosis (Buck-Louis et al., Human Reprod 20:279-85, 2005). In that study, the association between endometriosis and the sum of four antiestrogenic PCBs (PCB 105, 114, 126, 169) was significant (P=0.001), whereas there was no associa-

tion between endometriosis and the sum of 12 estrogenic PCBs. This finding was inconsistent with the widely held notion that endometriosis is an estrogen-dependent disease, prompting further statistical analyses to explore these associations in more detail. An optimization algorithm was developed that determines weights in the linear combination of scaled PCB levels which maximized the standardized slope esti-

mate. Application of this method to the antiestrogenic PCB subgroup revealed that only one, PCB 114, was responsible for nearly 100% of the association. The fact that PCB 114 is neither the most potent antioestrin nor the most abundant anti-

estrogen in the mixture suggests that the association might be driven by a different mechanism. Use of this statistical weighting method to further analyze the 12 es-

terogenic PCBs showed a stronger, but not statistically significant, association with endometriosis (P=0.060) driven mainly by PCBs 99 and 188. These results demon-

strate how the integration of refined statistical methods coupled with toxicological interpretation can generate testable hypotheses that might not otherwise have been generated. Funded by American Chemistry Council, Arlington, VA.

1198 EXPOSURE TO POLYCHLORINATED BIPHENYLS (PCBs) AMONG AFRICAN AMERICAN NEONATES.

B. L. Williams and M. S. Magumbura. NCEH, Centers for Disease Control, Atlanta, GA. Sponsor: D. Barr.

There are relatively limited data on exposures to transplacental toxicants such as polychlorinated biphenyls (PCBs) in neonates. The purpose of this study was to quantify exposure to PCBs among a sample of high risk neonates. We measured concentrations of PCBs congeners in umbilical cord serum and examined sources of environmental exposures to PCBs in a predominantly African American population. We collected reproductive histories, questionnaire data, and blood samples
from 102 women, aged 16-45 years, who delivered at an inner city hospital in Memphis, TN. The distribution and determinants of exposures to PCB's in this population will be discussed.

1199 EXPOSURE SCIENCE FOR CHEMICAL PRIORITYIZATION AND TOXICITY TESTING.


Currently, a significant research effort is underway to apply new technologies to screen and prioritize chemicals for toxicity testing (Dix et al. 2007, Toxicol Sci; NRC, 2007, Toxicity Testing in the 21st Century; Collins et al. 2008, Science). US EPA is completing the Phase I pilot for a chemical prioritization research program, called ToxCast™. Here EPA is developing methods for using computational chemistry, high-throughput screening (HTS), and toxigenomics to predict potential toxicity and prioritize testing. These high visibility efforts raise important research questions and opportunities for exposure scientists. As an expansion of HTS data for in vitro toxicity assays becomes available, exposure science is required to provide critical real-world information for interpreting these toxicity data, and predicting risks. The EPA National Center for Computational Toxicology has identified the immediate need to include exposure information for chemical prioritization. The EPA Exposure Science Community of Practice (ExpoCoP) formed in May 2008 to advance application of exposure science to inform and support EPA research in computational toxicology. The goal of ExpoCoP is to provide input on the research program in exposure science to address EPA needs for chemical prioritization, screening, and toxicity testing. The ExpoCoP, with a membership representing over 15 public and private sector organizations, is focusing initially on issues associated with classifying chemicals based on potential for human exposure and linking results with ToxCast™. An important component is to consider how best to consolidate and link human exposure data for this purpose. These sorts of linkages have the potential to bring toxicity and exposure science research communities into closer alignment and to improve risk assessment. In this presentation, potential linkages between research in computational toxicology and human exposure science were identified and activities of the ExpoCoP are highlighted. This work has been reviewed and approved by the US EPA for publication but does not necessarily reflect Agency policies.

1200 WATER ADHERENCE FACTORS FOR HUMAN SKIN.

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Dermal exposure to chemicals in water occurs in occupational and environmental settings in situations that involve incidental contact such as a splash. In which case, one key exposure factor is the amount of water that adheres to the skin. Although soil adherence factors have been developed by researchers and adopted by EPA and other regulatory agencies for risk assessment, measurements of water adherence on human skin have not been described to date. Currently, dermal dose is based upon other regulatory agencies for risk assessment, measurements of water adherence on human skin have not been described to date. Currently, dermal dose is based upon the amount of body linkages that have the potential to bring toxicity and exposure science research communities into closer alignment and to improve risk assessment. In this presentation, potential linkages between research in computational toxicology and human exposure science were identified and activities of the ExpoCoP are highlighted. This work has been reviewed and approved by the US EPA for publication but does not necessarily reflect Agency policies.

1201 CRYPTOSPORIDIUM EXPOSURE AND RECREATIONAL WATER CONTACT IN PERSONS WITH HIV/AIDS IN BALTIMORE, MARYLAND.

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Exposure to urban surface waters poses a risk for recreational water illnesses (RWIs) to persons who engage in recreational water activities. Cryptosporidium, a common infectious pathogen associated with RWIs, is prevalent in surface waters across the US and is not detected by standard microbiological methods of recreational water testing. Persons with HIV/AIDS are at increased risk of serious morbidity and even mortality from cryptosporidiosis of waterborne etiology. Few studies have examined risks of exposure among this population associated with water recreation. Baltimore City has one of the highest prevalence rates of HIV/AIDS in the US and is situated within a watershed that is extensively used for recreation. We undertook a study to determine if persons with HIV/AIDS are at risk of exposure to pathogens in the Baltimore watershed. Methods: We enrolled patients attending the Johns Hopkins Moore Orphan Patient AIDS Clinic in Baltimore, Maryland. Oral interviews were conducted in 2007 on a convenience sample of 157 HIV/AIDS patients to ascertain the sites utilized for recreational water contact within Baltimore watersheds. A 5-month environmental sampling was undertaken at 5 of the most frequently utilized recreational sites identified by patients. A combined fluorescent in situ hybridization and direct Immunofluorescence antibody (FISH/IFA) technique was used to detect the presence of viable Cryptosporidium oocysts in water samples. Results: 48% of patients reported taking part in recreational water activities; female patients were as likely to engage in recreational water activities as were males; 67% of patients reported consumption of their own seafood catch and/or wild-catch from friends and family members; and 61% of patients who reported recreational water contact are their own catch. Cryptosporidium oocysts were detected in water samples. Conclusion: This is the first study to show that persons with HIV/AIDS are in contact with Cryptosporidium-contaminated waters in Baltimore.

1202 BISPHENOL A EFFECTS ON GENE EXPRESSION OF ADIPOKINES.

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Bisphenol A (BPA) is generally used for polymerization of plastic materials to produce food containers, baby bottles and dental sealants, and human exposure BPA mainly through oral uptake. BPA has been considered as an endocrine-disrupting chemical to interfere with endocrine system and cause adverse health effects of reproduction and development in biological organisms and their progeny. Adipokines (e.g. leptin and adiponectin) secrete from adipose cells and regulate energy metabolism, endocrine system and immune homeostasis relevant to metabolic and degenerative diseases. Because of persistent nature, this organic pollutant BPA would accumulate in adipose cells and have the potency to affect adipokine secretion. This study was to determine BPA level in maternal and fetal core blood, and investigated its effect on gene expression of leptin and adiponectin in placenta tissue. With informed consent and physician interviews, maternal and umbilical cord blood, and placenta tissue (n = 100) were obtained during childbirth. Preliminary results of high performance liquid chromatography showed the average BPA level in maternal blood was 27.55 ± 4.59 ng/ml (mean ± SEM, median 15.26 ng/ml), and the BPA level of fetal cord blood was consistent with that of maternal blood. Additionally, the result of quantitative polymerase chain reaction displayed that maternal subjects with higher BPA level had repression of leptin (r = -0.306, p = 0.136) but augment of adiponectin (r = 0.124, p = 0.470). It suggested that fetuses exposed to BPA from their parents, and the increased BPA level affected gene expression of adipokines. Regarding median BPA value as cutoff and interference factors, analysis of binary logistic regression appeared that subjects with higher BPA level had potentially risk to decrease the ratio of leptin over adiponectin (OR = 2.06, 95% CI= 1.52-8.34). These findings are consistent with our cellular study. It is necessary to notice BPA manipulation.

1203 TURNING A BLIND EYE ON SMOKING DURING PREGNANCY – NICOTINE METABOLITES IN URINE OF PREGNANT WOMEN.

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Maternal smoking results in low birth weight. In our study, 50 pregnant women (including 17 smokers) underwent multiple consulting sessions during pregnancy. All women were informed about the general risks of drug abuse and in particular were advised to quit smoking and remove themselves from areas with environmental tobacco smoke. At the hospital prior to delivery, smoking habits, socio-economic status and use of medication were assessed by questionnaire, and the status of fetal health was assessed by ultrasound. Spot urine samples were collected to analyze nicotine (NIC), cotinine (COT) and 3-hydroxycotinine (3-OHC) by high performance liquid chromatography and tandem mass spectrometry (2D-HPLC-
MS/MS). Limits of quantification were ≤0.1 ng/L depending on the analyte examined. A high concordance (98%) on smoking habits between self-reported questionnaire information and analytical results were observed. Despite prior counseling, only 1 out of 17 maternal smokers quit smoking during pregnancy. Based on actual analytical results, NIC, COT and 3-OHC in urine of smokers were 464.2, 1979.0 and 2724.7 μg/L, respectively, whereas 1.3, 3.7 and 4.6 μg/L was found in nonsmoking mothers. NIC and COT as well as COT and 3-OHC were highly associated with each other (P≤0.0001, r²=0.79), whereas a moderate association between NIC and 3-OHC was observed (P=0.001, r²=0.50). Smoking habits were associated to socio-economic status as COT levels in women with low, middle, and high school degrees were 80.0, 12.6 and 2.6 μg/L respectively. Similar decreases were observed for both NIC and 3-OHC. The prevalence of low birth weight in the offspring was 25% for maternal smokers and 15% for nonsmokers, and two newborns (both of smoking mothers) showed either polycystic kidneys or intraterrine growth retardation. Our results show that the advice to avoid smoking during pregnancy is generally not heeded by the majority of women. Therefore, smoking must be considered a serious confounder in developmental studies after exposure to environmental toxicants.

1204 ORGANOPHOSPHATE PESTICIDE RESIDUES IN HOME ENVIRONMENT DUST OF ORCHARD WORKERS.

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Farmworkers and their families are at risk for chronic organophosphate pesticide (OP) exposure and it is necessary to characterize these exposures to predict health risks from OP toxicity. Data is from 203 orchard workers in Eastern Washington enrolled in a community randomized intervention trial to reduce the take-home pathway of OP exposure. Dust samples were collected in the room most commonly used by a referent child (aged 2–6) and family vehicles. A total of 172 dust samples were collected from carpeted surfaces and 27 from smooth surfaces. Dust was collected from 183 living rooms, and 20 other types of rooms and analyzed for azinphos-methyl (AZM) and five other OPs. Markov Chain Monte Carlo Methods were used to analyze the OP data as a multivariate log-normal distribution with measurements below the limit of detection treated as left-censored. AZM was the OP in the highest concentrations. Thick or thin carpeted surfaces had statistically significant greater mass of AZM per square meter than smooth surfaces—geometric means (GM): thick carpets 1430 ng/m², thin carpets 1550 ng/m², smooth surfaces 110 ng/m². The mass of AZM in the living room was not significantly different from other rooms—GM: living room 3450 ng/m², other rooms 1000 ng/m². There were no significant differences for the mass of AZM from vehicles when classified by type (marine auto 5630 ng, truck 6260 ng, other 2570 ng) or floor surface coverings (GM: plush mat 3220 ng, hard mat 4190 ng, or no mat 5100 ng). The results provide support for the theory that pesticide residues accumulate on carpeted surfaces protruding exposure of people in the household. Intervention efforts to reduce in-home exposure should target carpeted surfaces and frequent cleaning of all areas where young children play in the home. (Supported by grant 2-P01-ES09061 from National Institutes of Environmental Health Sciences and RD-8370901-0. STAR RD-83273501-0 from Environmental Protection Agency.)

1205 A META-ANALYSIS OF THE RELATIONSHIP BETWEEN OCCUPATIONAL EXPOSURE TO HEXAVALENT CHROMIUM AND CANCERS OF THE GASTROINTESTINAL TRACT.

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Recent rodent cancer bioassays have demonstrated that high concentrations of hexavalent chromium (Cr(VI)) in drinking water cause oral cancers in rats and small intestine tumors in mice at levels ≥20 μg/L. To evaluate the risk of human gastrointestinal (GI) tract cancers from Cr(VI) exposure, we conducted a systematic literature review and meta-analysis of epidemiologic studies of GI tract cancers, including oral cancer, esophageal, stomach, colon, and rectal, among workers occupationally exposed to Cr(VI). Meta standardized mortality ratios (meta-SMRs) were calculated using random-effects models to report risk estimates for GI cancers, and for subgroups within studies based on level of Cr(VI) exposure, industry, and geographic region. Meta-SMRs for any Cr(VI) exposure were, for oral cancer: 1.02 (95% CI=0.77–1.34); esophageal cancer: 1.14 (95% CI=0.87–1.49); stomach cancer: 1.08 (95% CI=0.93–1.25); colon cancer: 0.91 (95% CI=0.71–1.16); and rectal cancer: 1.16 (95% CI=0.97–1.39). Confounding by smoking could not be ruled out. None of the three studies that evaluated small-intestine cancer reported a statistically significant increased risk. Elevated SMRs were found for esophageal cancer in the high Cr(VI) exposure subgroup and the Cr(VI) group, but these analyses were limited by the small number of studies in each subgroup. Occupational exposures are estimated to be generally consistent with the current US chromium drinking water standard (0.1 mg/L) but lower than those that caused cancer in rodents. These meta-analysis results of epidemiologic studies conducted for Cr(VI) do not experience a greater risk of GI cancers than the general population, and that GI tract cancers in rodents may be of limited relevance to Cr(VI)-exposed humans due to the high doses of Cr(VI) administered in these studies or to species-specific differences in the cancers detected.

1206 URINARY AFLATOXIN M1, AND 1-HYDROXYPYRENE LEVELS IN A U.S. POPULATION COMPARED TO A HIGH RISK POPULATION IN GHANA.

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Aflatoxin (AF) B1, a metabolite produced by Aspergillus fungi, is a potent hepatocarcinogen in humans. An association between increased incidence of hepatocellular carcinoma (HCC) and co-exposure to AFs and polycyclic aromatic hydrocarbons (PAHs) has been evidenced in a recent study in Taiwan. In our current study levels of aflatoxin M1 (AFM1) and 1-hydroxypyrene (1-OPH) were measured in urine collected from participants in a predominantly Hispanic population in a dense maize consuming region of Texas known to have an increased incidence of HCC. A cross-sectional study was conducted within three zip code regions in San Antonio, Texas, A sociodemographic survey was administered to 185 participants and urine samples were analyzed for biomarkers via HPLC analysis with fluorescence detection. Of the total samples analyzed, 11.6% had detectable levels of AFM1. Detectable levels of 1-OPH in the urine totaled 62.3%; however, of these participants with measurable 1-OPH values, greater than 50% were identified as tobacco smokers by the questionnaire. Results from this study were compared to our previous findings, determining urinary biomarkers in a West African population known to be at high risk for aflatoxicosis. Of the total samples collected from participants in the Ashanti Region of Ghana, 88.3% and 98.3% had positive peaks for AFM1 and 1-OPH, respectively. Furthermore, tobacco smokers accounted for only 5.5% of the study population implying a predominant environmental exposure. Our findings suggest that the study population in San Antonio is not at high risk for aflatoxicosis. Further work is warranted to standardize data with creatinine clearance to ensure that screening levels in individual urines in order to compare exposure levels with those found in Ghana. (Supported by NIEHS ES 09106)

1207 ETIOLOGICAL CHARACTERIZATION OF EMERGENCY DEPARTMENT ACUTE POISONING.

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An investigation of emergency department (ED) poisonings was conducted to characterize poisoning demographics and evaluate associations with co-morbidities. Risk factors for suicidal poisoning and the relationship with mental illness was also evaluated. The study population consisted of 649 poisoning cases admitted between 2004 and 2007 to the ED of Mount Sinai hospital in Chicago. Ethnicity, age, and gender were socio-demographic factors with substantial impact on the population distribution as poisoning cases were predominantly African Americans (79.9%) between 36-45 years old with a 1.3 male to female ratio. Intentional illicit drug overdose was the most prevalent cause of poisoning, heroin being the most frequent substance found in 35.4% (n=230) of cases, followed by cocaine overdose at 6% (n=39). Significant correlations were found between heroin poisoning and asthma (F=20.29, DF=1, p=.0001), cocaine poisoning and hypertension (F=33.34, DF=1, p=.0001), and cocaine poisoning and cardiovascular disease.

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Fluropolymer (FPs) are present in commercial products such as ceramic tile/gROUT
sealants, fabric and upholstery protectors, leather impregnation sprays, and boot
sealants. These products typically contain a low percentage of FP in petroleum-
baseD solvents and/or propellants. FPs have low acute toxicity, with LC50’s greater
than 20 g/m3 in rats. However, there are reports dating back to the 1980’s of “outbreaks”
of human respiratory symptoms (including some fatalities) associated with the
use of sprays containing FPs, and in some cases the FPs have been implicat-
ed as the causative factor. To evaluate the toxicological merits of these claims, we
conducted a weight-of-evidence evaluation of the relevant epidemiological and ani-
mal toxicity literature. We found more than 20 case reports of mass or single out-
breaks of respiratory injury following the use of FP-containing sealants, but in each of
these reports, either the FP in the commercial product was not identified, the
product label or the material safety data sheet (MSDS) for the product did not list an
FP as an ingredient, or toxicity could not specifically be assigned to the FP (e.g., in
formulations containing multiple ingredients in addition to the FP), or ingredients
other than the FP were thought by the authors to be responsible for the reported ill-
nesses. Similar limitations were present in the animal exposure studies involving
FP-based sealants. For example, in some studies the FP concentrations in the test
material and/or the test chambers were never characterized or even measured.
In other studies, the exposures were too high to represent a realistic commercial use
scenario. In short, there are currently no animal studies that indicate that FP in a
commercial product, or even the FP product itself, is capable of causing respiratory
injury in humans under normal use conditions. We conclude that further research
would be necessary to establish a link between exposure to FP-containing sealants
and serious respiratory problems in humans.

1208 EVALUATION OF FLUOROPOLYMER TOXICITY FROM OUTBREAK REPORTS AND ANIMAL STUDIES.


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According to the automated air quality monitoring system, the critical air pollu-
tants in Mexico City are ozone and particulate matter < 10µm (PM10) and <2.5
µm (PM2.5). Particulate matter increases during the dry season (winter) and de-
creases during the rainy season (summer). PAHs adsorbed in respiRable blood lymphocytes from non-smoking young adults (n=98) obtained during one winter and the following summer. PAH-DNA adducts were analyzed using a chemiluminescence immu-
noassay with antisera elicited against DNA modified with 7,12-dihydroxy-7,9,10-
trimethylbenz(a)anthracene and 9,10-dimethyl-1,2-benzanthracene. Chromatographic separation of the adducts was achieved in methanol using reversed-phase high performance liquid chromatography and tandem mass spectrometry.

1210 POLYCYCLIC AROMATIC HYDROCARBON (PAH)-DNA ADDUCTS, CHROMOSOMAL ABERRATIONS, AND CYP1A1 AND GSTM1 RISK VARIANTS IN PERIPHERAL BLOOD LYMPHOCYTES FROM YOUNG ADULTS LIVING IN MEXICO CITY.

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1CDI Section, National Cancer Institute, NIH, Bethesda, MD, 2Carcinogenic Ambientes,
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Perchlorate is most widely known as a solid oxidant for missile and rocket propul-
sion systems and has been detected in drinking water, fruits, vegetables, and milk
throughout New Jersey and most of the U.S. In New Jersey, the FDA detected per-
chlorate in romaine lettuce (3.70-21.60 ppb), tomatoes (0.38-6.48 ppb), spinach
(6.02-40.90 ppb), broccoli (6.00-6.96 ppb), and whole milk (3.40-4.85 ppb); these
levels were below the U.S. average. Water sampling by the EPA found that numer-
ous sites in New Jersey exceeded the Minimal Reporting Level of 5.00 ppb with lev-
els ranging from 5.00-21.00 ppb. Perchlorate interferes with the uptake of iodine
into the thyroid and may interfere with the development of the skeletal system and
central nervous system of infants who are exposed to perchlorate through breast
milk. Increased perchlorate in breast milk leads to a decrease in iodine uptake by
the infant, which is why iodine supplementation is encouraged among lactating
women. The main objective of this study is to estimate total perchlorate exposure
for lactating women and infants based on perchlorate levels in maternal milk and
urine. Over 100 lactating mothers have provided consent to participate in this
study. Demographic data shows that over 90% of the subjects are Hispanic and
range in age from 18-38. The primary sources of drinking water are filtered, mu-
icipal tap water (54%) and bottled water (30%). Further analyses of the data will
be done within urine, water, and breast milk samples are analyzed. Subjects are asked
to provide a urine and breast milk sample on three separate occasions along with a
sample of their primary source of drinking water. At each sample collection, the
subjects are required to complete a 24-hour dietary recall. Perchlorate, thiocyanate,
nitrate, and iodide excretion in breast milk and urine will be analyzed using ion
chromatography and tandem mass spectrometry. [Funding: NJDEP Contract #
SR06-044]
The samples taken during the ‘fireworks’ period. Ongoing trace element analysis will dose-dependent differences in ROS production between the 2 sampling sites and completed. Particles were tested for toxicity and reactive oxygen species (ROS) prior to the Olympics and rose to pre-Olympic levels after the Olympics were completed. Concentrations of BC, primary emissions and after the Olympics when pollution emissions returned to previous levels. Olympics when interventions significantly reduced point source and traffic emissions, there were strong negative correlations between HR and BC in the early morning and positive correlations in the late afternoon. Ongoing trace element analysis will provide data to correlate source components with changes in cardiac endpoints.

## 1213 TOXICITY OF AMBIENT PM COLLECTED IN BEIJING AND TIANJIN DURING AND AFTER THE 2008 OLYMPICS.

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Understanding the toxicity of complex ambient pollutants presents challenging research issues. Intervention of air pollution sources can present a unique opportunity to study how changes in air pollution affect human health. In this study, we collected site-segregated ambient PM at 2 locations which were close in proximity to 2008 Olympic events: Tianjin and Beijing. Coarse, fine, and UFP PM fractions were collected with a 900 LPM ChemVol sampler at both locations during the Olympics when interventions significantly reduced point source and traffic emissions and after the Olympics when pollution emissions returned to previous levels. Additional samples were collected during the 48 hr period surrounding National Day which is a time of celebration and numerous firework displays. In general, ambient PM2.5 and NO2 concentrations during the Olympics were half of what they were prior to the Olympics and rose to pre-Olympic levels after the Olympics were completed. Particles were tested for toxicity and reactive oxygen species (ROS) production in human-derived BEAS-2B airway epithelial and HMEC-ST1.6P pulmonary vascular endothelial cell lines. Clear particle size differences in ROS production were observed in both cell lines. Additionally, there was some evidence of dose-dependent differences in ROS production between the 2 sampling sites and the samples taken during the ‘fireworks’ period. Ongoing trace element analysis will provide data to correlate source components with differential oxidative stress outcomes in both epithelial and endothelial cell lines.

## 1214 USE OF MULTIPLE LINES OF EVIDENCE FOR EVALUATING A VAPOR INTRUSION SCENARIO – CASE STUDIES

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A multifaceted approach was used to evaluate vapor intrusion scenarios at two distinct sites (A and B). Site A is an oil refinery, with elevated levels of methane and gasoline vapors beneath adjacent residential and commercial properties. Site B is a former chemical manufacturing facility with a chlorinated volatile organic compound (VOC) plume extending from beneath a commercial building (located on Site B) to offsite areas immediately adjacent to residential properties. Preliminary review of subsurface data suggested that residents/workers in these buildings may be exposed to VOCs emanating from the subsurface. A step-wise approach was taken to determine if mitigation measures were warranted to prevent indoor air exposures: (1) Shallow soil vapor data from residential and commercial buildings were compared to health-based screening criteria to identify, for further study, candidate buildings with the potential for subsurface vapor intrusion; (2) Indoor and outdoor air monitoring and/or subsurface soil vapor data were collected from candidate commercial and residential buildings; and (3) Potential for other sources of organic vapors (e.g., household products, building materials, background ambient air) as confounding variable(s) were assessed using multiple data analysis techniques. Based on the multiple lines of evidence from these analyses, it was determined that subsurface vapor intrusion did not have a significant effect on indoor air quality in the residential and commercial buildings at Site A. At Site B, soil gas, sub-slab vapor, and indoor/ambient air data indicated that VOCs had migrated from the samples by site, site of the months and a breakdown in commercial composition, but not into the surface area. Vapor intrusion was not of concern in residences located immediately adjacent to Site B.

## 1215 THE ROLE OF PM SIZE AND COMPOSITION ON THE CELLULAR TOXICITY INDUCED BY SAMPLES FROM AN ARID REGION CITY.

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Background: Discrepancies on the role of PM composition and specific health outcomes, particularly in relation to coarse particles exist. PM2.5 represents a more homogenous mixture, than PM10, probably accounting for its more consistent association with mortality worldwide. Nevertheless, neither can be considered harmless. Objective: Provide toxicological evidence on the role of PM size/composition using PM from Mexicali; a city where soil dust (aluminum-silicates) has a toxic potential and contributes importantly to air pollution. Methods: Semi-urban (SU) and urban (U) PM2.5 & PM10 were collected during six months. Pooled samples by site, site of the months and a breakdown in commercial composition, but not into the surface area. Vapor intrusion was not of concern in residences located immediately adjacent to Site B.

## 1216 HUMAN BIOmonitoring IN THREE AGE GROUPS, RESULTS OF THE FLESH ENVIRONMENTAL & HEALTH SURVEY (FLESH 2002-2006).

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The Flemish Centre for Environment & Health (Belgium) has organized a human biomonitoring campaign which included the recruitment of 1196 mothers and their children (958 adolescents, 589 children aged 7, 583 adults aged 25 to 64 years). Recruitment followed a stratified sampling scheme in eight geographical areas. We tested the hypotheses whether living in different areas has a measurable impact on the pollutant profile in the body and whether this was associated with signs of early health effects. We measured biomarkers of exposure and effect and collected health
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The study was commissioned, financed and steered by the Flemish Government (Department of Economics, Science and Innovation; Flemish Agency for Care and Health; and Department of Environment, Nature and Energy) next cycle of the Environmental Health Survey (2007-2011) more pollutants will be measured and we will focus on monitoring in areas at risk, so called "hot spots". The study was commissioned, financed and steered by the Flemish Government (Department of Economics, Science and Innovation; Flemish Agency for Care and Health; and Department of Environment, Nature and Energy) next cycle of the Environmental Health Survey (2007-2011) more pollutants will be measured and we will focus on monitoring in areas at risk, so called "hot spots". The study was commissioned, financed and steered by the Flemish Government (Department of Economics, Science and Innovation; Flemish Agency for Care and Health; and Department of Environment, Nature and Energy) next cycle of the Environmental Health Survey (2007-2011) more pollutants will be measured and we will focus on monitoring in areas at risk, so called "hot spots".

1219 THE ESTROGENIC EFFECT OF ORGANIC EXTRACT FROM SOURCE WATER OF X CITY IN CHINA.

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Water pollution by endocrine disruptors is one of the most critical problems concerning drinking water resources and environmental protection of water bodies. Up to now, the uterotropic assay is still considered as the "gold standard" and is an essential component when testing for estrogenicity since it incorporates the effects of metabolism and pharmacokinetics. The present study was conducted to explore the estrogenic effect using an estrogenic extraction from water source of x city in the southern China by the typical uterotropic assay. Furthermore, expression of proliferating cell nuclear antigen (PCNA) was detected by immunohistochemistry. The results showed that the changes of uterine wet weight and uterine gland number were insignificant among different doses, while uterine epithelial cell height increased significantly with increase of dosage. The results also showed the expression of PCNA was strong in positive control and mid dosage group, while its expression was negative or weak in negative control and low dosage group. It suggested that water samples detected had estrogenic activity. Taken together, the data presented in current study suggest that incorporation of morphologic and biochemical responses as end-points in the rodent uterotrophic bioassay did not show the high sensitivity of the test when assessing mixture and it therefore needs to be further improved in identifying potential environmental toxicants. Other endpoints and new methods need to be employed in order to increase the sensitivity, efficiency and reliability in the screening test.

1220 AQUATIC SPECIES AS SENTINELS FOR HUMAN HEALTH: COMPARATIVE TOXICOLOGY OF METALS, NANOPARTICLES, AND PCB’S.

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Aquatic toxicity has important implications for human health including using animals in aquatic environments as sentinels for both human health and the health of the aquatic environment and as models for human disease. Metals, nanoparticles, and organic compounds pose emerging and persistent concerns for public and aquatic health, but their full impacts are poorly understood. Given the current understanding of contaminants in the aquatic environment and the lessons that can be learned for human health it is important to address these issues. The panel of experts will highlight the emerging data that chromium may be a major pollutant in the marine environment with consideration that marine mammals are a sentinel species for human health, and also discuss potential novel adaptations in these whales to these exposures that may have implications for human health. The potential impact of metal nanoparticles using a fish model for development will be utilized to inform on how to rapidly determine which types of nanoparticles are more potent than others. The toxicity of nanoparticles will be compared and considered in human cells compared to cells from aquatic species to show how the response to these agents can be very different for various species. The impact of mercury will also be considered and its effects on development using a zebrafish model. It will consider how organochlorines impact the immune system of whales and humans and how whales may be sentinels for human health. Insight will be offered to researchers and risk assessors on the potential impacts of these chemicals, but even more importantly it reveals theories underlying these effects and thus suggests future directions in research for aquatic toxicology and aquatic models and how they relate to human health. For those currently engaged in nanotoxicology, metal toxicology, aquatic toxicology, risk assessment, regulatory management, occupational health, ecotoxicology, immunotoxicology, developmental toxicology, and toxicology education, this should be a particularly informative session.
1221 WHALES AS SENTINELS FOR HUMAN HEALTH: A GLOBAL ASSESSMENT OF CHROMIUM POLLUTION.

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Hexavalent chromium (Cr(VI)) is known as a human lung carcinogen. Exposure to Cr(VI) occurs in both occupational and environmental settings. However, despite the fact that substantial amounts of Cr are released into the environment and that marine waters favor the hexavalent form, few studies have considered Cr exposure in marine settings or the consequences of exposure to marine species. The goal of this project is to investigate the impacts of Cr exposure in the marine environment and its potential implications for human health. We are investigating the levels of Cr in both toothed and baleen whales using the sperm whale (Physeter macrocephalus) and North Atlantic right whale (Eubalaena glacialis), and we are comparing these to those Cr levels reported in lung tissue of occupationally exposed workers who died of Cr(VI)-induced lung cancer. In addition, the mean Cr skin level in both species was 23-28 times higher than the level reported for humans without occupational Cr(VI) exposure. Both particulate and soluble Cr(VI) were genotoxic to cell lines from both species after a 24 h exposure. However, the amount of damage in whale cells wassignificantly lower than the amount induced in human cells. These data suggest that whale cells may have evolved novel mechanisms to reduce the toxic effects of Cr.

1222 RELATIVE TOXICITY OF METAL NANOPARTICLES TO DEVELOPING ZEBRAFISH.

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Nano-sized metals and metal oxides have unique properties that will prove useful for applications in electronics, healthcare, remediation, imaging, sensor, catalysis, electronics, optics, construction and engineering industries. Rapid discovery and development of novel uses for nanoparticles will undoubtedly increase the probability of human and environmental exposure. Of concern are recent reports of adverse biological effects from metal nanoparticle exposure. Since nanoparticles may interact with biological systems in unexpected ways, evaluation of nanomaterial toxicity should cover a broad range of cell types, tissues, organs and systems. This study employed embryonic zebrafish as a whole animal assay to delineate metal nanoparticles that are biologically active from those that are not. The embryonic zebrafish model is dynamic in vivo system that offers the power of whole-organism investigations (e.g. intact organism, functional homeostatic feedback mechanisms and intercellular signaling) with the convenience of cell culture (e.g. cost- and time-efficient, minimal infrastructure, small quantities of nanomaterial solutions required, rapid evaluation of interactions between nanoparticles and integrated living systems). We investigated the effects of nanoparticulate metal oxide dispersions, biologically-synthesized Au and Ag nanoparticles, a small congeneric library of gold nanoparticles, and a small congeneric library of zinc oxide nanoparticles. Information gained from model systems such as the embryonic zebrafish, can be used to inform the development of safer nanomaterials and nanotechnologies.

1223 COMPARATIVE TOXICITY OF SILVER NANOPARTICLES IN HUMAN, MARINE MAMMAL AND FISH CELLS.

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Industrial application of silver nanoparticles has increased dramatically in recent years making them a new and emerging potential environmental contaminant. Many of these applications have the potential to release silver nanoparticles directly into the water stream. However, the toxicity of silver nanoparticles is unknown. The goal of this project is to determine the mechanisms of silver nanoparticle-induced cytotoxicity and genotoxicity in humans and aquatic species. We used cells from sperm whales (Physeter macrocephalus), North Atlantic right whales (Eubalaena glacialis), and modified zebrafish (Oryzias latipes) as model aquatic species. The data show that silver nanoparticles are cytotoxic to all species in a concentration-dependent manner. However, the potency was greater in the whale cells and greatest in the fish cells. Silver nanoparticles were not genotoxic to human cells, but were significantly genotoxic to fish cells, with again the whale cells exhibiting a response in between the two. Most nanoparticle toxicity work so far focuses on human health effects with much less study of the effects in aquatic species. These data indicate that while nanoparticles can be of limited toxicity to human cells, they can still have dramatically more toxicity in cells from aquatic species. These observations raise important issues for how risk assessors consider the full potential impact of nanomaterials as merely considering rodent and human effects is likely to be insufficient.

1224 ZEBRAFISH MODEL FOR UNDERSTANDING METHYLMERCURY DEVELOPMENTAL NEUROTOXICITY.

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Methylmercury (MeHg) affects the developing nervous system, and the children of women that consume high quantities of wild-caught fish during pregnancy may be at risk for neurological impairments. Data suggest that certain maternal dietary components (selenium and others) may reduce the embryotoxic effects of MeHg. The goal of this project is to identify neural and molecular biomarkers as predictors of MeHg exposure and deficits in specific neurobehavioral endpoints, and to explore the effect of Se on toxicity. Specifically, we are investigating the effects of sublethal MeHg exposure (either direct or via maternal diet of wild-caught fish) on developing zebrafish by analyzing early life stage (ELS) toxicity, changes in global gene expression by microarray analysis, and neurobehavioral dysfunction. Developmental exposure to MeHg results in ELS toxicity and neurodevelopmental alterations persist into adulthood as visual and learning deficits. Interestingly, selenium co-treatment reduced the uptake of methylmercury, ameliorated the resultant ELS toxicity, and improved the performance of exposed individuals in our visual assay. Gene expression analysis in exposed embryos was performed using an 8,000 element zebrafish cDNA microarray and revealed changes in the expression of a number of genes primarily associated with neurological structure and function, mitochondrial function, and immune system function, consistent with our findings of learning and neurobehavioral deficits. These results show that maternal exposure to moderate levels of MeHg can have dramatic developmental and behavioral effects in their offspring by disrupting numerous molecular pathways involved in the growth and development of multiple organ systems and that selenium may reduce some of the neurotoxicological effects of developmental methylmercury exposure.

1225 IMMUNOTOXICITY OF PCBs, AN EXAMPLE OF MARINE MAMMALS AS SENTINELS FOR HUMAN HEALTH?

D. De Guise, University of Connecticut, Storrs, CT.

This talk will discuss the recent advances in comparative immunotoxicology of PCBs, and how they apply to human and environmental risk assessment. A new approach will be examined, using in vitro systems to qualify and quantify toxicity in species such as humans and marine mammals, for which traditional in vivo approaches present significant logistical and ethical problems. Current efforts to translate in vitro findings to assessment of risk at the population level will be presented. The concept of marine mammals as potential sentinels for early detection and quantification of the likely effects of ubiquitous environmental pollutants on ecosystem and human health will be discussed.

1226 MAMMALIAN RETROTRANSPOSITIONAL ELEMENTS: EPIGENETIC REGULATION, SPECIES DIFFERENCES, AND POTENTIAL ROLES AS MEDIATORS OF CELLULAR RESPONSES TO TOXIC STRESS.

B. D. Storeg and C. Qin. Laboratory Sciences and Investigative Toxicology, Merck Research Laboratories, West Point, PA.

Epigenetic regulation of gene expression is being extensively investigated. However, approximately 38 to 47% of the mouse and human genomes respectively are composed of mobile elements (DNA transposons and retrotransposons) whose potential roles in susceptibility to toxicity and disease upon epigenetic dysregulation have...
not been fully explored. The retrotranspositional elements are the most numerous and complex, having promoter/enhancer activity, protein coding ability, and mutagenic potential and are subject to epigenetic control. Due to their structure and location, often within, or proximate to genes and their regulation by DNA methylation, these elements can modify gene expression and serve as epigenetic mediators of phenotypic variation in a species and strain-specific manner. In addition, some classes of retrotransposons remain active as mobile elements with the potential to create new somatic mutations involved in cancer and germline mutations with the potential to drive genome evolution and modulate disease susceptibility. In human, long interspersed nuclear elements (LINE-1) and Alu elements are the two classes of retrotransposons that remain active (mobile) while the less numerous endogenous retrovirus (ERV) LTR retrotransposons have mostly lost this capacity. In contrast, in mice, members of the class of retroviral LTR retrotransposons, in particular intracisternal A particles (IAPs), have retained retrotransposon activity. Germline IAP transpositions in certain mouse strains such as in the Agouti and Axin genes have provided fascinating examples of phenotypic variation mediated by epigenetic mechanisms, namely diet- and/or chemically-induced changes in DNA methylation. Given the large number of these genetic elements, and the species differences in their sequences, activity, and distribution in the genomes, further investigation of their potential role(s) in mediating disease processes and cellular responses to endogenous chemicals and xenobiotics is warranted.

1227 MAMMALIAN L1 RETROTRANSPORTS ARE POTENTIAL MUTAGENS IN HUMANS.

H. H. Kazazian. Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, PA. Sponsor: C. Qin.

LINE-1 (L1) sequences are present in 520,000 copies and make up 17% of the human genome. They constitute 20% or more of other mammalian genomes. Most of these sequences are truncated or otherwise defective, but the human genome has ~7000 full length 6 kb elements and the average human being carries about 90-100 active L1s. These L1s have an internal promoter, two ORFs, and an end in a poly A tail. The first ORF has nucleic acid chaperone activity, and the second ORF has reverse transcriptase activity. Reverse transcription and integration of L1s are coupled. The endonuclease nicks genomic DNA, and the reverse transcriptase uses the free 3' end at the nick as a primer and the L1 RNA as template. Both ORFs are critical for retrotransposition in cultured cells. L1s have contributed further genetic variation by providing the reverse transcriptase for Alu, SVA (another small retrotransposon), and processed pseudogene mobility. These other sequences, mostly Alus, make up ~10% of the genome. L1 has driven genome evolution in many other ways besides through their own mobility and that of other sequences. L1 can transduce or carry sequences from either its 5' or 3' end to other genomic locations, thus providing the possibility of exon shuffling. Homologous recombination can occur between mismatched L1s leading to deletion or duplication of sequences between the L1s. L1l can alter gene expression by providing splice sites and poly A signals in introns, and producing transcriptional pausing. In our recent work, we found that the bulk of L1 retrotransposition occurs in early embryonic development, not in the germ line, suggesting that L1 mobility may be even more frequent than has been previously thought. L1s are also developing genomic techniques to locate all new L1 insertions in any human genome. These techniques should provide important new insights into L1 biology, including the frequency and timing with which retrotransposition occurs in the human genome.

1228 MECHANISMS OF EPIGENETIC REGULATION OF L1 ELEMENTS IN HUMAN AND MURINE CELLS.

K. S. Ramos, D. E. Montoya, T. Kalfeljusch, V. Sriribinuk, I. Tengur and M. E. Lacy. Department of Biochemistry and Molecular Biology, University of Louisville School of Medicine, Louisville, KY.

Long interspersed nuclear element-1 (L1) is an abundant retrotransposon in mammalian genomes that remains silent under most conditions and is reactivated by cellular stress signals. Of relevance is the ability of benzo(a)pyrene (BaP), a genotoxic carcinogenic hydrocarbon, to increase L1 retrotransposition rates in human and murine cells. Increased retrotransposition in BaP-treated cells is mediated by up-regulation of L1 RNA levels, increased L1 cDNA synthesis, and stable genomic integration. Thus, mutagenesis and genomic instability in human populations exposed to genotoxic hydrocarbons may involve epigenetic activation of mobile elements dispersed throughout the human genome. Recent evidence has established that E2F-Rb family complexes participate in L1 epigenetic regulation via nucleosomal histone modifications and recruitment of histone deacetylases (HDACs) and 1 and 2 zinc finger proteins. Here we have examined the interactions between histone modifications on the L1 promoter and other epigenetic marks. We have also extensively regulated by “danger signals”. Assembly of this complex, termed the inflammasome, relieves the autoinhibitory state of caspase-1 and facilitates the proteolysis of IL-1β, IL-18 and IL-33 into secreted forms capable of orchestrating innate and adaptive immune responses. In support of these roles, asbestos fibers and silica, contact sen-

1229 GENETICS AND EPIGENETICS OF ENDOGENOUS RETROVIRUSES.

D. Mayer1, Y. Zhang1,2 and D. Reiss1,2. Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada. Sponsor: R. Storer.

Mammalian genomes are littered with sequences related to retroviruses. These endogenous retroviral elements (ERVs) are likely the result of ancient retroviral infections of the germ line that are now inherited as normal constituents of chromosomal DNA. Similar fractions of the human and mouse genomes are derived from ERVs, but there is a striking difference in the current activity of these elements in the two species. While there is little evidence for recent ERV activity in humans, several families of ERVs are active in inbred mice and cause 10% of all spontaneous mouse mutations through insertional mutagenesis. The first part of the presentation will compare human and mouse ERVs and will summarize our genome-wide studies to document polymorphic mouse ERVs. The remainder of the talk will focus on epigenetic studies of ERVs. Stochastic epigenetic silencing of some ERV insertions in mice has been shown to cause phenotypic variation between individuals. However, the prevalence of this phenomenon as well as its temporal dynamics are unknown. We have studied ERV DNA methylation patterns in mice and have observed that DNA methylation goes from stochastic to stable as insertions age. Variable methylation is frequent among randomly chosen recent ERV insertions, whereas older insertions are stably and heavily methylated. In contrast, our studies on one human ERV family have shown that the DNA methylation pattern for a given insertion is stable, but can vary widely between copies depending on genomic context. A model will be presented to explain these observations.

1230 INTRACISTERNAL A-PARTICLE (IAP) GENES: DISTRIBUTION IN THE MOUSE GENOME AND POTENTIAL ROLES AS SPECIES-SPECIFIC MEDIATORS OF CELLULAR RESPONSES TO TOXIC STRESS.

C. Qin and R. D. Storer. Laboratory Sciences and Investigative Toxicology, Merck Research Laboratories, West Point, PA.

Mice and rats have been the species of choice for the evaluation of chemical toxicity. However, different species and strains often respond differently. This has complicated human risk assessment for species and strain-specific effects. To explore the basis of these species- and strain-specific effects, we examined the distribution of intracisternal A particles (IAP) sequences, endogenous retroviral (ERV) elements, in the mouse genome and explored the possibility that IAP sequences embedded in genes may serve as mouse-specific genetic susceptibility factors for expression of disease phenotypes. We concluded that IAPs with LTR subtypes 1 and 1a are a class of young and active IAP elements. We found them located within, or in close proximity (<10kb) to, 550 genes in the C57BL/6 mouse genome. Functional (gene ontology) analysis revealed highly statistically significant associations of subsets of these genes with defined biological processes, most notably cell growth and signaling. For instance, 85 genes are involved in cell signaling (p<2.33E-32), 50 genes in cell death or apoptosis (p<2.02E-17), 38 genes in cancer (p<2.40E-16), and 47 genes in the immune response (p<2.13E-15). T o test the activity and inducibility of IAP LTRs embedded in the genes, LTRs were placed in front of luciferase reporter plasmids. All were transcriptionally active in transient transfection assays, with enhanced activity with the LTRs in the unmethylated versus methylated state. Consistent with the presence of a glucocorticoid response element in the LTRs, dexamethasone treatment modestly enhanced activity. Different inbred strains and outbred CD-1 mice had different IAP insertion patterns for 3 genes tested. Similar strain polymorphisms have been reported for IAP insertions by other investigators. These data suggest that these genetically regulated IAP elements have the potential to affect cellular responses to xenobiotics and disease processes in a species- and strain-specific manner.

1231 INFLAMMASOME ACTIVATION IN PULMONARY DISEASE.

M. E. Paynter. University of Vermont, Burlington, VT.

Microbial products and endogenous molecules elaborated following tissue damage can activate cells through pattern recognition receptors. While Toll-Like Receptor (TLR) agonists can induce the expression of IL-1β, IL-18 and IL-33, these cytokines require further proteolytic processing by caspase-1 to be secreted. Caspase-1 is activated when in a multi-protein complex containing intracellular pattern recognition receptors of the NOD-like family, in particular Nalp3, which are stimulated by “danger signals”. Assembly of this complex, termed the inflammasome, relieves the autoinhibitory state of caspase-1 and facilitates the proteolysis of IL-1β, IL-18 and IL-33 into secreted forms capable of orchestrating innate and adaptive immune responses. In support of these roles, asbestos fibers and silica, contact sen-
sitters, and the adventitious aluminum hydroxide all activate the inflammasome in their mechanisms of action. Inhibition of a TLR ligand (LPS) and a Nalp3 ligand (ATP or N-methyl d-aspartate) induces IL-1β and IL-33 mRNA expression, IL-1β cleavage, and the release of mature IL-1β protein. In vitro studies demonstrated that inhibition of caspase-1 or reactive oxygen species generation prevents IL-1β secretion. The role of the inflammasome in a model of nitrogen dioxide (NO2)-promoted allergic sensitization will be discussed.

1233 INTRODUCTION OF PROINFLAMMATORY AND IMMUNOSUPPRESSIVE IMMUNE RESPONSES BY CIGARETTE SMOKE ALDEHYDES.

B. M. Freed, School of Medicine, University of Colorado, Aurora, CO.

The effects of cigarette smoke on pulmonary immunity are complex. The acute effects of smoking are largely restricted to the lung, although chronic smoking is also a significant risk factor for autoimmune diseases, such as rheumatoid arthritis. Cigarette smoke suppresses acquired immunity by inhibiting gene expression associated with T cell and B cell activation, but induces innate immunity by up-regulating Cox-2, MCP-1 and IL-8. The immunotoxic effects of cigarette smoke can be reproduced by a single unsaturated aldehyde, acrolein. Acrolein inhibits expression of IL-2 by adding arginine and cysteine residues in the DNA-binding domain of NF-kB p50, preventing it from binding to the IL-2 promoter. Saturated aldehydes, such as acetaldehyde, do not react with these residues and do not inhibit DNA binding. In vivo, cigarette smoke activates IL-8 by activating NF-kB p65. Current research is focused on testing the hypothesis that NF-kB serves as a biological smoke detector that switches pulmonary innate immunity on and acquired immunity off.

1234 EXACERBATION OF PULMONARY TOXICITY BY PRO-INFLAMMATORY CYTOKINE HMGBI IN HYPEROXIA-INDUCED ACUTE LUNG INJURY.

J. L. Mantell, St John's University College of Pharmacy, Queens, NY/The Feinstein Institute for Medical Research, North Shore-Long Island Jewish Health System, Manhasset, NY.

Supraphysiological concentrations of oxygen (hyperoxia) are routinely used to prevent or treat hypoxemia and acute respiratory failure. However, prolonged exposure to hyperoxia can result in tissue damage in many organs, especially lungs, and lead to the development of lung injury and suppressed innate immunity. Hyperoxia-induced lung injury is a product of direct oxygen toxicity and the indirect effects from activated resident and recruited inflammatory cells. HMGBI, an emerging key player in systemic inflammation, mediates the recruitment of inflammatory cells and suppresses innate immunity in response to bacterial infection under hyperoxic conditions.

1235 INHALED CARBON MONOXIDE AS THERAPEUTIC MODALITY IN HUMAN INFLAMMATORY LUNG DISEASES.

A. K. Choi, Brigham and Women's Hospital, Harvard University, Boston, MA. Sponsor: K. Sarlo.

Carbon monoxide (CO) arises endogenously from heme degradation, catalyzed by the heme oxygenase enzymes. In cell culture, CO exerts potent anti-inflammatory, anti-apoptotic and anti-proliferative effects by modulating intracellular signaling pathways. In vivo, CO confers tissue protection in animal models of lung disease, including those with oxidative and inflammatory lung injury and ischemia/reperfusion injury. Whether CO offers potential therapeutic avenues in the treatment of lung inflammatory disorders will be discussed.

1236 THE GOOD, THE BAD, AND THE UGLY OF TOXICANT-INDUCED PULMONARY INFLAMMATION.

L. L. Mantell1 and J. T. Zelikoff2, 1St. John's University College of Pharmacy, Queens, NY/The Feinstein Institute for Medical Research, North Shore-Long Island Jewish Health System, Manhasset, NY and 2New York University School of Medicine, Tuxedo Park, NY.

A well orchestrated lung inflammation induced by cytokines is critical to optimizing host defense capabilities, while avoiding or minimizing potential damage to lung tissues. Normally, pulmonary inflammation plays a pivotal role during positive immune responses against microbial and small particle pathogens. Unfortunately, certain inhaled toxins, such as cigarette smoke and prolonged hyperoxia, can non-specifically induce dysregulated chronic and acute inflammation within the respiratory tract. Therefore, it is important to examine the types of immunomodulatory events that occur in the lungs in response to environmental agents and to pathogens, and to demonstrate how similar effects induced by those disparate challenges can lead to distinctly different (i.e., helpful vs. harmful) outcomes. Our panel of experts will present cutting edge studies on the regulation of inflammatory responses by the inflammasome, the nervous system and carbon monoxide. An important outcome of this session is to have attendees be able to recognize the major putative mediators involved in induction of pulmonary inflammation, describe signaling pathways that mediate inflammatory responses to inhaled toxins and pathogens, better understand the regulation of inflammatory response and identify potential targets and therapeutic strategies for further development for the amelioration of acute lung inflammatory injury and chronic diseases.

1237 IMPROVED SAFETY BIOMARKERS FOR MONITORING KIDNEY INJURY.

F. D. Sistare1 and F. Dieterle2, 1Merck and Co., Inc., West Point, PA and 2Novartis Pharmaceutical AG, Basel, Switzerland.

A number of accessible biomarkers are becoming available to toxicologists and to clinicians as qualified tools that have demonstrated their ability to out-perform BUN and serum creatinine for monitoring the early onset of certain drug induced kidney pathologies. These tools are beginning to positively impact the development of drug candidates that may present with low grade kidney toxicities in first-in-human enabling GLP animal toxicology studies, especially when observed in a single species, and human irrelevancy cannot otherwise be adequately assured. The biomarkers are showing utility not only for monitoring drug safety, but also for interrogating kidney disease progression and regression. Animal toxicology studies designed to assess the biological performance of these new safety biomarkers are providing new insights into fundamental aspects of kidney function, and are supporting potential opportunities to establish a positive response to intervention where kidney disease is a target for new therapies. With the positive response received recently from the U.S. FDA and EMEA regarding the acceptability and utility of certain qualified renal safety biomarkers for targeted regulatory applications, the challenge now is to expand the tool box and to define broader uses. Therefore, we should begin by understanding the performance strengths and limitations of the more robust kidney safety biomarkers established across numerous animal toxicology studies, understanding the molecular, cellular, and anatomical bases for kidney biomarker responses observed to chemical toxicities, and describing the research progress to fill the critical research and regulatory gaps and questions that remain. By fully grasping this information we should be able to present the strategy and progress made to bridge from animal studies to human clinical trials where establishing the performance attributes of these biomarkers is far more challenging and finally present clinical data of biomarker responses to disease and drug-induced kidney injury matching the pre-clinical data and supporting the clinical qualification and utility of these and additional promising clinical biomarkers.

1238 ONE YEAR AFTER THE FIRST REGULATORY QUALIFICATION EFFORTS: LIMITATIONS, OPPORTUNITIES, ADVANCES AND IMPACT ON TOXICOLOGY AND TRANSLATIONAL MEDICINE.

F. Dieterle1, S. Chiboure1, E. Perena1, A. Cordier1, J. Vonderstricher2, G. Maurer1, J. Orez2, D. Gerhold2, S. Troth1, F. Sistare2 and P. Nephrotoxicity Working Group1. 1Translational Sciences, Novartis, Basel, Switzerland, 2Safety Assessment, Merck Research Laboratories, West Point, PA.

The Critical Path Institute's Predictive Safety Testing Consortium (PSTC) has submitted data on 7 renal safety biomarkers to the FDA/EMA in a Voluntary eXploratory Data Submission (VXDS) to seek a first-ever formal regulatory qualification decision on safety biomarkers appropriate for regulatory decision making.
These 7 urinary biomarkers -- Kim-1, Clusterin, Albumin, TFF3, Total Protein, Cystatin C and b2-Microglobulin -- were accepted by regulatory authorities for monitoring acute drug-induced tubular or glomerular injuries in rat GLP studies and on a case-by-case basis under specific conditions for monitoring renal safety in early clinical trials. The feedback of health authorities on this first-ever approval of biomarkers independent from the use of a specific drug will be reviewed, in particular, the lessons learned and best practices for the qualification and submission of data supporting the use of new safety biomarkers. It will be shown, how these new tools should be used to enhance safety assessment and to prepare an application to bring a drug candidate into human clinical trials to address cause for concern and guard kidney safety. This includes a discussion of the application of thresholds, appropriate statistical considerations, and the design of pre-clinical and early clinical studies. In addition, the most recent efforts will be presented, which are aimed at extending the currently approved upon for purpose qualification context by cross-qualifying the biomarkers, by conducting specially designed recovery studies, and by preparing the qualification of additional biomarkers such as serum Cystatin C, which can be shown to be a better functional biomarker than BUN and serum creatinine.

**W 1239 PRECLINICAL QUALIFICATION OF NOVEL URINARY MARKERS OF RENAL INJURY.**


Preclinical studies offer a unique opportunity for both identification and biologic qualification of markers of renal injury. Simultaneous evaluation of kidney histopathology and urinary marker changes in controlled preclinical studies provide an initial insight on the utility of novel markers identified in toxicogenomic studies. Immunohistochrometry (IHC) can be used to further characterize marker biology by identification of marker localization in normal and diseased kidney, and for segment-specific markers of renal injury, to confirm specificity of the novel marker for discrete manifestations of nephrotic injury. Finally, the use of histopathology and/or IHC as a reference standard provides a more robust or stringent assessment of diagnostic performance of novel markers than current markers of renal injury such as serum creatinine and urea. In addition to phenotypic anchoring of novel markers through morphologic characterization, biologic qualification requires clarification of the relationship between changes in novel markers and onset, progression or resolution of renal injury, generally achieved through use of time-course and recovery studies. Furthermore, characterization of preanalytic variables such as inter- and intra-individual variability, and knowledge of effects of age, sex, diet or circadian rhythm are critical for biologic characterization and integrated assessment of diagnostic performance of novel urinary markers in preclinical studies. Morphologic and biochemical data from preclinical studies will be presented to illustrate these perspectives on biologic qualification of novel urinary markers of renal injury.

**W 1240 INTEGRATING ANIMAL WITH CLINICAL DATA TO BETTER UNDERSTAND KIM-1 AS A KIDNEY SAFETY BIOMARKER.**

J. V. Bonventre. Brigham and Women's Hospital, Harvard Medical School, Boston, MA. Sponsor: E. Santare.

The gene encoding kidney injury molecule-1 (Kim-1/TIM-1/HAVCR-1) is conserved across species in zebrafish, rodents, dogs, primates and humans. Urinary Kim-1 has been shown to be a sensitive and early diagnostic indicator of renal injury across a variety of acute and chronic preclinical and clinical kidney injury models. In a number of rodent models of nephrotoxicity urinary Kim-1 elevation has been shown to be much more sensitive and specific than other biomarkers and standard methods of detecting nephrotoxicity. The FDA and EMEA have worked together to encourage the submission of Kim-1 results during animal studies of new drugs. The utility of KM-1 has also been shown in a number of clinical studies in humans with acute kidney injury associated with ischemia and/or sepsis in adults and children, subjects administered nephrotoxic drugs such as cisplatin and gentamicin, and in transplant recipients that develop graft dysfunction. Levels of urinary Kim-1 can predict regression of proteinuria in patients with diabetes mellitus and can predict outcome in a critically ill population. Urinary Kim-1 levels correlate well with histological and functional injury in patients with a variety of kidney diseases. Urinary Kim-1 levels have also been shown to be elevated in a subpopulation of patients who were previously thought to have no active kidney dysfunction and this has important implications for patient selection for drug studies as well as therapeutic intervention. We have recently found that Kim-1 is a phosphatidylinositol receptor which is very active in taking up apoptotic cells as well as oxidized lipids in the damaged kidney. In conclusion clinical data comparing Kim-1's performance against a number of other urinary biomarkers in a number of populations support the conclusions from animal toxicology studies. The use of Kim-1 as a urinary biomarker has the potential to transform safety monitoring and the design of clinical trials of therapeutic agents.

**W 1241 ACUTE KIDNEY INJURY NETWORK (AKIN): ADVANCING CLINICAL APPLICATIONS OF PROMISING RENAL BIOMARKERS.**

R. L. Mehta. Medicine, University of California San Diego, San Diego, CA. Sponsor: E. Santare.

As acute kidney injury (AKI) is associated with a 40-60% mortality rate, there has been an explosion of research into finding ways to improve care for patients with this condition. AKIN is an international multidisciplinary collaborative research network focused on improving outcomes from AKI. AKIN's activities are designed to facilitate the acquisition of evidence through well designed and conducted clinical trials, dissemination of information via multidisciplinary joint conferences and publications and improve the translation of knowledge from pre-clinical research. AKIN has developed a consensus recommendation for the terminology, diagnostic criteria and staging of AKI and has suggested a research agenda for AKI. One of the research priority areas is the discovery, assessment and validation of unique renal biomarkers that can characterize the course of a patient with AKI. It is anticipated that renal biomarkers would significantly modify our ability to identify patients with AKI early in the course, define the nature and extent of damage, provide guidance for targeted intervention and provide prognostic information on renal recovery and development of progressive kidney disease. Additionally, it is necessary to distinguish between renal functional and structural biomarkers from those identifying the injury and repair process. While preclinical studies have identified several potential candidates, no single biomarker has all these attributes. Several biomarkers are now being assessed in clinical studies. This presentation will provide an overview of the conceptual framework for AKI, describe the novel serum and urinary biomarkers currently being evaluated in clinical settings and provide a framework of reference for the translation of preclinical data to human application.

**W 1242 IMPROVED SAFETY BIOMARKERS FOR MONITORING CLINICAL RENAL INJURY.**


There has been tremendous progress made in understanding the molecular mechanisms of renal diseases, in particular, acute kidney injury. However, translation of these findings to diagnostic and therapeutic use in clinical practice remains a challenge. Despite significant advances in therapeutics, the morbidity and mortality rates associated with acute kidney injury remain significantly elevated. These rates have not appreciably improved over the last few decades in light of these advances. The lack of significant progress in the prevention and management of acute kidney injury has been attributed, in part, to the failure to identify suitable physiologic surrogate endpoints for use in research study testing and the efficiency of new interventions. In fact, very few acute kidney injury studies have demonstrated a beneficial effect on the most commonly used physiologic surrogate endpoints, the serum urea nitrogen and creatinine concentrations. The diagnosis of acute kidney injury is typically based on either the elevation of serum creatinine or the detection of oliguria. Our goal is to identify thresholds on several pre-identified biomarkers using a classical nephrotoxic agent, IV contrast dye, in hospitalized diagnostic cardiac catheterization patients who develop nephrotoxicity compared to those who do not. This information will then be validated in a maintenance study with an agent known to cause renal tubular injury, cisplatin or gentamicin. Clinically current renal injury may be present with little to no significant change in serum creatinine making this marker imprecise. Therefore our current gold standard surrogate marker is far from perfect. Our current clinical needs mandate the assessment of these newer biomarkers and how they perform in this translational setting. With the positive response from the FDA and EMEA recently received and the efforts of the PSTC regarding the acceptability and utility of certain qualified renal safety biomarkers for targeted regulatory applications, the challenge for further research is now to define and support expanded use from prospective outcome based clinical trials.

**W 1243 OXIDATIVE STRESS AS A REGULATOR OF NORMAL FUNCTION AND MEDIATOR OF TOXICANT-INDUCED DAMAGE WITH IMPACTS ON REPRODUCTION AND DEVELOPMENT.**

S. P. Darnay. ORD, U.S. EPA, Research Triangle Park, NC.

Exposures to certain chemicals and environmental factors are known to induce oxidative stress, either directly or indirectly, resulting in complex responses at the molecular level in cells and tissues. With respect to reproduction, high levels of en-
The major thiol/disulfide couples, consisting of thioredoxins, GSH/GSSG and cysteine/cystine (Cys/CySS), are maintained at stable non-equilibrium conditions in cells, organelles and extracellular fluids. The lack of equilibration between these central redox couples illustrates the concept of oxidative stress as a global imbalance. The thioredoxins are more reduced than GSH/GSSG pools and the latter are more reduced than the Cys/CySS pools. The compartmental redox states vary from most reduced in mitochondria to sequentially more oxidized in nuclei, cytoplasm, secretory pathway and extracellular space. Accumulating evidence shows that each of these systems differentially regulates different cell functions, presumably through specific redox control of different proteins. Methods are now available to measure oxidative stress in specific compartments and in terms of oxidation of specific proteins. Results indicate that disruption of specific thiol redox signaling and control circuits underlie much of the pathophysiology attributed to oxidative stress. Importantly, thiol systems involved in the disruption of specific thiol redox signaling and control circuits underlie much of the pathophysiology attributed to oxidative stress. Importantly, thiol systems and non-radical species remain poorly defined. However, the emerging evidence indicates that long-term, low level oxidative stress may impart Leydig cell function with consequent decreases in testosterone secretion, thus contributing to declines in reproductive function with aging. To convey this message recent research findings on the relationship between oxidative stress and reproductive function with emphasis on specific reproductive and development toxins that are toxic to testicular tissue on animal models will be presented. Additionally, the disruption of abnormal sperm. Supported by NIH, CIHR, The Cole Foundation.

Multiple chemicals are known to destroy ovarian follicles in rodents and humans, with adverse impacts on reproductive success. However, the mechanisms are not yet fully understood. Our recent data suggest that the mechanisms of follicular destruction differ depending on the stage of follicular development. Studies in cultured neonatal rodent ovaries show that multiple reactive chemicals and ionizing radiation induce double-stranded DNA breaks in oocytes and induce loss of primordial follicles via a caspase-independent pathway. In vivo exposures support such a mechanism of toxicant-induced primordial follicle loss. In the adult ovary, most spontaneous follicular atresia occurs in antral follicles and appears to be driven by apoptotic death of the granulosa cells. Using cultured antral follicles and a human granulosa cell line, we have shown that an increase in reactive oxygen species is an early event in the initiation of apoptosis induced by gonadotropin withdrawal, several chemical toxicants, and ionizing radiation. Depletion of the antioxidant glutathione sensitizes antral follicles and granulosa cells to these apoptotic stimuli. Supplementation of glutathione and other antioxidants or overexpression of the rate-limiting enzyme in glutathione synthesis prevents the rise in reactive oxygen species and protects against apoptosis induced by these stimuli. Accumulating evidence from several laboratories also suggests that the anti-apoptotic effects of gonadotropin hormones in antral follicles are mediated in part by upregulation of antioxidants. These results suggest that reactive oxygen species mediate apoptotic destruction of antral follicles by various stimuli. In contrast, de-

Mammalian spermatozoa are redox-regulated cells. The generation of low levels of reactive oxygen species (ROS) is a key component of capacitation, a post-insensmaturation event that prepares spermatozoa for union with the oocyte. If ROS production is prevented, then capacitation is suppressed and fertilization cannot occur. Despite the physiological significance of these ROS intermediates, the excessive production of ROS is extremely detrimental to spermatozoa. The main reasons for this are that these cells possess a limited repertoire of antioxidant enzymes as a consequence of the restricted volume and distribution of their cytoplasmic space and these cells are replete with substrates for free radical attack including abundant unsaturated fatty acids and DNA. Exposure to oxidative stress results in a loss of sperm motility mediated by lipid peroxidation and oxidative DNA damage, reflected by an increase in the DNA base adduct, 8-OH 2'-deoxyguanosine. Since the presence of DNA damage in spermatozoa is associated with poor fertility, rates of miscarriage and increased incidence of disease in the offspring, determining the causes of oxidative stress in the male germ line is a matter of some urgency. A variety of environmental factors have been implicated in this process including electromagnetic radiation (heat and radiofrequency EMR), redox cycling xenobiotics (quinones) metabolites (catechol estrogens) metals (iron copper, manganese) and compounds that target the mitochondrial electron transport chain including un-terified, unsaturated fatty acids and pharmaceuticals. Susceptibility to oxidative stress in the germ line may be dictated by polymorphisms in key metabolic enzymes such as the cytochrome P450s and glutathione-S-transferases. Attention is also being given to the use of antioxidants as a means of ameliorating oxidative damage to spermatozoa. Although initial results are promising, some antioxidants were found to damage sperm DNA via a cross linking mechanism to which these cells are uniquely susceptible, given the unusually compacted nature of their chromatin.

Oxidative stress, defined as an imbalance between free radical generation and antioxidants, has been linked to adverse reproductive outcomes. The causes of oxidative stress in the male germ line is a matter of some urgency. A variety of environmental factors have been implicated in this process including electromagnetic radiation (heat and radiofrequency EMR), redox cycling xenobiotics (quinones) metabolites (catechol estrogens) metals (iron copper, manganese) and compounds that target the mitochondrial electron transport chain including un-terified, unsaturated fatty acids and pharmaceuticals. Susceptibility to oxidative stress in the germ line may be dictated by polymorphisms in key metabolic enzymes such as the cytochrome P450s and glutathione-S-transferases. Attention is also being given to the use of antioxidants as a means of ameliorating oxidative damage to spermatozoa. Although initial results are promising, some antioxidants were found to damage sperm DNA via a cross linking mechanism to which these cells are uniquely susceptible, given the unusually compacted nature of their chromatin.
Data show that the cellular transition from a proliferating to a differentiating state coincides with an oxidation of the intracellular biotin, glutathione (GSH), while other couples, such as thioredoxin (Trx), are not affected. Independent regulation of particular redox couples may provide rationale for the specificity of redox control during differentiation and also relate to oxidant-induced malformation. In human mesenchymal stem cells, undifferentiated cells are more reduced than differentiated (osteocytes or adipocytes) cells. With oxidant exposure, redox couples, both GSH and Trx, rebounded more quickly and to a greater degree and were less disposed to undergo apoptosis in undifferentiated cells than in differentiating cells, suggesting that the susceptibility to oxidative insult and redox misregulation is greater in differentiating than in undifferentiated/proliferating cells. In organogenesis-staged embryos, redox changes occur concomitantly with mitochondrial maturation, an increased need for oxygen and a subsequent increase in reactive oxygen species generation. Interestingly, antioxidant enzyme activities, such as GSH peroxidase, GSSG reductase, superoxide dismutase (SOD) and catalase increase as development progresses and thus provide a compensatory mechanism capable of correcting redox balance during periods of oxidative stress. The natural oxidative shift in GSH and the regulation of Trx in a reduced state suggest that these couples are tightly regulated and may control different aspects of differentiation. Thus, unbalanced oxidative stress and misregulation of specific couples may disrupt redox-sensitive pathways to cause dysmorphogenesis.

1249 PESTICIDES AND PARKINSON'S DISEASE: IMPLICATIONS OF NEW EPIDEMIOLOGY AND EXPOSURE DATA TO RISK ASSESSMENT.

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Numerous animal and epidemiological studies have suggested a relationship between pesticide exposure and the development of Parkinson's Disease (PD). Frequently, scientists and the media question whether regulatory processes are sufficiently protective of this potential risk factor. However, there has been relatively little attention paid to the exposure side of the risk equation in the research to date. Furthermore, there is a tremendous need for an improved interface between toxicology and epidemiology. Thus, it is important that we focus our attention on new epidemiology and animal research with an emphasis on the exposure question and the implications that the findings have for human health risk assessment. To adequately address these topics, speakers from government, academia and industry with knowledge of different aspects of pesticide risk assessment: toxicology, epidemiology, neurology, pharmacokinetics, and exposure assessment. The session will present new results of two important epidemiological studies on Parkinson's Disease that evaluate exposure of humans to pesticides, and an animal study that uses PBPK modeling to relate animal models of PD to estimated human exposure levels. The two epidemiological studies that will be presented are the Honolulu-Asia Aging study (HAAS) and the Farm and Movement Evaluation (FAME) Study of the Agricultural Health Study (AHS). In addition, the results of the Farm Family Exposure Study, which focused on exposure assessment to pesticides, will be discussed in relation to the practice of exposure assessment in agricultural worker epidemiologic studies. This session will end in a panel discussion that will be stimulated by two discussions representing industry and government who have specialized expertise in pesticide exposure assessment and have an understanding of the risk assessment process.

1250 A NEUROLOGIST'S BIRD'S EYE VIEW OF KEY RISK FACTORS OF PARKINSON'S DISEASE.

J. Langston, Director and CEO, Parkinson's Institute and Clinical Center, Sunnyvale, CA. Sponsor: A. Li.

Although there is no known cause of Parkinson's Disease, there are a number of risk and protective factors that have been suggested based on analytical epidemiological studies. Proposed risk factors include environmental exposures (i.e., pesticides and certain metals), genetic (i.e., mutations in the genes for alpha-synuclein, parkin, and LRRK2), dietary (i.e., milk, iron), hormonal (i.e. low estrogen status), occupation and place of residence. There are also protective factors that have been reported, such as cigarette smoking, coffee consumption, and non-steroidal antiinflammatory drugs (NSAIDS). Broader perspective on the relative contributions of these different factors and the strength of the scientific evidence supporting the association with PD will aid in developing new hypotheses, prioritizing research, and designing policy that consider interactions among multiplicative and interactive approaches (i.e., genetics, molecular biology, epidemiology, pharmacology and toxicology) involving both scientists and clinicians is needed to make progress toward the overall goal of understanding the natural history of Parkinson's disease, finding its cause and developing better treatments.

1251 HONOLULU-ASIA AGING STUDY (HAAS): RELATIONSHIP OF ORGANOCHLORINE LEVELS WITH PARKINSON'S DISEASE RISK.

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The Honolulu Heart Program / Honolulu-Asia Aging study (HAAS) is a community based, long term, prospective study of dementia and Parkinson's disease (PD) in a cohort of 8000 Japanese-American men living on the island of Oahu and born between 1900 and 1919. Interest in the association of neurotoxicants and PD was stimulated by the fact that many of the HAAS participants worked on sugar and pineapple plantations in Hawaii and would have been exposed to numerous agricultural chemicals. In order to further explore the possible relationship of pesticide exposure with PD risk, organochlorine levels were obtained on previously frozen occipital lobe brain samples from 445 cohort decedents. Twenty-one organochlorines were measured using gas chromatography. The presence of incidental Lewy bodies (i.e. found in brains of decedents without a history of PD during life) is thought to represent a preclinical stage of PD in persons who died before the classic motor features of the disease were manifest. New data on the association of brain organochlorine levels with neuropathologically confirmed Parkinson's Disease and with the presence of Lewy bodies in the substantia nigra or locus coeruleus will be presented.

1252 FARMING AND MOVEMENT EVALUATION STUDY (FAME): PARKINSON'S DISEASE IN PESTICIDE APPLICATORS AND THEIR SPOUSES.

F. Kamel1 and C. M. Tanner2, 1National Institute of Environmental Health Sciences, RTP, NC, and 2Parkinson's Institute and Clinical Center, Sunnyvale, CA.

FAME is a case-control study of PD nested within the Agricultural Health Study (AHS), a cohort of ~89,000 pesticide applicators and their spouses recruited in North Carolina and Iowa in 1993-1997. Suspect PD cases from the AHS were evaluated with medical records, an in-person neurologic exam, and a videotaped movement evaluation, and diagnosis was verified by consensus of two neurologists (N=115). Controls were a stratified random sample of the remaining cohort, frequency matched to cases by age, gender, and state (N=384). We evaluated exposure based on questionnaires completed at enrollment in the AHS; extensive telephone interviews conducted for the FAME study; and biological and environmental samples, including blood samples collected for measurement of persistent organochlorine pesticides. The FAME interviews collected complete occupational histories including detailed information on cumulative lifetime use of 31 pesticides previously implicated in PD by human, experimental, or mechanistic studies. We are presently evaluating associations of PD with pesticide exposure based on these multiple sources of information. Previous studies have indicated that farmers report pesticide use accurately and that exposure assessment based on the AHS questionnaires is consistent with data obtained from environmental and biological measurements made based on intensive sampling during pesticide application. Thus the FAME study has more detailed and reliable information on pesticide exposure in verified PD cases and matched controls than any study to date. Further, the focus of the study on an agricultural cohort, which is likely to have more frequent and greater pesticide exposures than the general population, will allow us to better characterize exposures associated with PD.

1253 FARM FAMILY EXPOSURE STUDY: BIOMONITORING AND EXPOSURE ASSESSMENT IN AGRICULTURAL POPULATIONS.

B. H. Alexander. Division of Environmental Health Sciences, University of Minnesota School of Public Health, Minneapolis, MN. Sponsor: A. Li.

The Farm Family Exposure Study evaluated pesticide exposure in ninety-five farm families in Minnesota and South Carolina to compare self-reported and observed exposure metrics to biomarkers of exposure. Exposure was based on 24 hour urine samples collected 24 hours before through 96 hours after a pesticide application that was part of the usual farm operation. Three widely used chemicals were new and glycophotis, and chlorpyrifos, the subject of more factors in the study include pesticides with differing chemical and physical properties. Urine concentrations of the chemical or metabolite were used to determine exposure from the application. Application practices and potential exposure to family members were ascertained by direct observation and questionnaire. The background and application related
exposure varied by chemical, the individual, and the potential for direct contact with the chemical. Background concentration was minimal for glyphosate but universal for chlorpyrifos. Compared to glyphosate and chlorpyrifos, the magnitude of 2,4-D exposure was much higher. Applicators and children assisting in the application had the most pronounced exposure. Application practices were inherently related to the exposure intensity of different chemicals and were not reliable predictors of exposure for the single application. Exposure to spouses and children not present during the application was low, but did vary by the measured exposure in the applicator. This study demonstrates the complexity of measuring pesticide exposure in agricultural populations. Modeling of pesticide exposure for epidemiologic investigations of diseases with long latency, including Parkinson’s disease, may be improved if chemical-specific information and potential routes of exposure are considered. Studies relying on individual recall should consider these sources of exposure uncertainty and their influence on inter- and intra-individual exposure variability over time.

**W 1254 PESTICIDE RISK ASSESSMENT AND ANIMAL MODELS OF PD.**

A. A. Li 1, G. L. Keddie 1, S. Warren 1 and L. J. McIntosh 1. Health Sciences, Exponent, San Francisco, CA. 2Independent Consultant, Chapel Hill, NC. 3Exponent International Ltd, Moorestown, NJ and 4Health Sciences, Exponent, Menlo Park, CA.

Whole animal models of Parkinson’s Disease (PD) using pesticides can contribute to our understanding of underlying mechanisms of degeneration that may be relevant to PD or other neurologic diseases. Human health risk assessment based on these pesticide animal models of PD requires evaluation of the relationship between routes and levels of exposures used in these animal studies with estimated human exposures. Qualitative and quantitative comparisons between animal doses used in animal models of PD and estimated or allowable human exposures levels will be presented. In one representative case study, a mouse PBPK model for paraquat (PQ) will be presented based on C57Bl/6 mouse blood and brain data following different routes of exposure. Specifically, blood and brain levels of PQ were measured at 6 timepoints (n=3 per timepoint) within 24 hours of a single i.p. (10 mg/kg bwt), oral (10 mg/kg bwt), dermal (40 mg/kg bwt) or intranasal (3 mg/kg bwt) exposure. Using appropriate human and chemical-specific parameters, this PBPK model was used to extrapolate experimental mouse exposures to human oral, dermal and inhalation exposures. Field studies of exposed workers have shown that the predominant route of exposure is dermal. Utilizing worker exposure conditions described in the UK risk assessment for PQ (UK draft assessment report, 1996), the human PQ PBPK model was used to estimate the brain dosimetry of PQ in farm workers and gardeners exposed under various conditions. Model simulation of dermal exposure of workers to PQ under field conditions to PQ indicated that the internal dose of PQ to the brain corresponds to an IP dose of PQ in mice that is 6-8 orders of magnitude below the dose used in PQ or PQ/Maneb animal models of PD. The PBPK study was sponsored by the UK Department of Environment, Food and Rural Affairs.

**W 1255 SAFETY OF HIGH-INTENSITY SWEETENERS: BITTERSWEET CONTROVERSY.**

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The quest for the perfect high-intensity sweetener with a clean, sweet taste, no off-flavor, non-nutritive, and no adverse health effects continues. To date, the U.S. Food and Drug Administration (U.S. FDA) has approved five artificial sweeteners: aspartame, acesulfame-K, neotame, and sucralose, in addition to saccharin. Currently, cyclamate is pending FDA approval/re-appraisal. The agency regulates high-intensity sweeteners as food additives, which must be approved as safe for their intended use before they can be marketed. Although these approved sweeteners have whetted the palates of millions of Americans over the years, the one problem common to all of them has been the controversies over their safety, which have been anything but sweet. Saccharin has been marketed for more than 100 years and represents a good example of how the shifting requirements of the law and the progress of science can change a substance’s status from ‘safe’ to ‘unsafe.’ Both the products’ manufacturers and the FDA maintain that the currently approved intense sweeteners are safe for their intended uses. Nevertheless, there are accusations in both the scientific literature and in the popular media about risks posed by these sweeteners. Is there really a cause for concern? To fully understand the issues, it is important to provide the current ‘state of the science’ as it relates to safety of the approved sweeteners; discuss the safety of certain sweeteners currently in development and/or approved outside the U.S., explore the evolving regulatory requirements for safety testing of sweeteners. The session will begin with a brief overview of the basic toxicological requirements for sweeteners in general, followed by presentations on specific controversial issues, lessons learned from previously approved sweeteners, concerns related to obesity, and a look at some possible future sweetener developments.

**W 1256 WHY IS THERE CONCERN ABOUT ALTERNATIVE SWEETENER SAFETY?**


A decrease in caloric intake is a prime mode of dealing with the explosion of obesity in the United States. Nearly one third of adults in a recent survey admitted to being on a diet. The popularity of alternative sweeteners, particularly intense sweeteners, to replace the calories from sugar in a variety of foods and beverages is great. In 2007, nearly 200 million adults used low-calorie, sugar-free foods and beverages. Despite this popularity and the fact that the FDA has approved 5 such compounds as food additives, and their approval is seconded by a variety of professional groups, doubts are repeatedly raised over their safety for a variety of reasons. First, human carcinogenic risk is inappropriately extrapolated from high-dose animal studies, as in the case of saccharin and bladder cancer. Second, poorly designed experimental designs are used to provide grist for anti-sweeteners’ mills (e.g., the recent European Ramazzini Foundation studies), Third, media reports often exaggerate the relevance and meaning of individual studies, and the concepts of dose and duration of exposure are ignored. Fourth, the concept that “natural” compounds are by definition more wholesome than anything produced in a lab is widely accepted, and is a valuable marketing tool. Finally, for some activists, proclaiming the supposed “dangers” of such products yields media attention and perhaps financial support from consumers. The common thread in all these types of attacks is a lack of attention to sound science.

**W 1257 TOXICOLOGY AND PHARMACOLOGY OF REBAUDIOSIDE (REB A).**

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Rebaudioside A (Reb A) and stevioside (S) (a closely related structural analogue) are steviol glycosides (SG) identified as the principal sweeteners in S. rebiana (Bertoni). They are natural non-caloric, high intensity sweeteners (– 200 x sucrose sweetness equivalence). A resurgence in interest in SG has been driven by the search for an alternative natural high intensity sweetener. Rebiana is the common name for high purity Reb A (> 97% Reb A), and has been determined GRAS as a general purpose use sweetener. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) (JECFA, 2008) allocated a permanent Acceptable Daily Intake (ADI) of 0.4 mg/kg bw/day, expressed as steviol, for SG. Reb A and S demonstrated a lack of genotoxic activity for SG in vitro and in vivo. The metabolism and pharmacokinetic profile for SG has been reported. Rat and human metabolism studies using high-purity Reb A and S confirm their metabolic equivalence. Studies conducted with S support the safety assessment of rebiana. Orally, SG are not readily absorbed from the upper small intestine of the rat or human. Human digestive enzymes are not capable of hydrolyzing β-glycosidic bonds, hence, SG are expected to escape digestion in the upper GI -tract. Bacteria of the Bacteroides family (predominantly Bacteroides) transform Reb A and S to steviol in the large intestine in the rat and human. There are no concerns for rebiana with respect to allergenicity or toxicity associated with metabolites or impurities. The safety of Reb A is supported by a number of published studies using purified SG, including rebiana, consisting of studies of metabolism, pharmacokinetics, and animal toxicity. Human studies with Reb A and S assessed glucose homeostasis in subjects with type 2 diabetes, and hemodynamics in subjects with normal to low-normal blood pressure. SG are safe and well-tolerated in normotensive or hypertensive individuals and subjects with type-2 diabetes following acute- or long-term consumption at doses of up to 1.5 g/day (~ 5 mg/kg bw/day; ~ 10 mg steviol equivalents/kg bw/day).

**W 1258 CONTROVERSIES SURROUNDING ASPARTAME.**

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The safety of aspartame has been questioned in both the lay and scientific press, despite hundreds of studies on the low calorie sweetener. The major controversies of aspartame safety continue to be focus on allegations of neurotoxicity and carcinogenicity. Recently a group of experts assessed all available data on aspartame, including premarket toxicology studies, post-market in vitro and animal studies, human clinical reports, and epidemiological studies. In addition, current consumption of aspartame in the US was evaluated. The findings from these assessments with specific focus on the neurotoxicity and carcinogenicity studies that fuel the controversy illustrate that the consumption of aspartame remains well below levels shown to...
have no adverse effects in well designed studies. Poorly designed studies and inappropriately extrapolation of data play a major role in anti-aspartame messages, creating an unwarranted concern regarding the safety of this well studied sweetener.


Genetics has experienced dramatic progress in recent years and genome sequencing has tremendously helped in the understanding of the sweet taste reception. A diverse range of chemical substances can induce sweet taste; even things that are a single sweet taste receptor. Current health and wellness concerns related to obesity are leading consumers and manufacturers to search for great-tasting foods and beverages with reduced caloric content. The current state of knowledge on sweet taste chemoreception and its role in obesity will be discussed. Notably, sweet taste receptors have been found in the intestine as well as in taste buds on the tongue. It remains to be determined whether a diet high in artificial sweeteners contributes to sweetness liking and preference with the same result as a diet high in sugar. The effects of high potency sweeteners on ingestive behavior in humans are extremely complex and will be discussed. The role of these findings in the developments of new sweeteners from safety and obesity perspective needs to be addressed.


Controversy often surrounds sweeteners in both the scientific community and in the popular media. Often there are challenges to FDA’s decisions, but time and again the agency’s original judgments have successfully withstood these challenges over the years. This presentation provides a look into FDA’s safety evaluations of a number of sweetener applications filed over the years in the U.S., with historical reflections on some of the more notable policy decisions. This is will frame the current FDA safety requirements for new sweeteners, including chemical identity, purity and specifications; estimates of probable consumer daily intake; toxicological information (usually built around an array of animal feeding studies) and other information of potential public health significance. Given the controversies that often surround safety judgments about sweeteners, it is helpful to understand, for example, how FDA approached the body-weight-gain decrement effect in test animals fed sucrose; the arguments FDA used for not requiring further carcinogenicity testing on the aspartame-K hydrolysis product, acetoacetamide, relative to potential thyroid tumorigenesis; and issues surrounding the safety of aspartame. These and other sweetener issues will be discussed in the context of FDA’s time-tested safety assessment methods for new sweetener approvals.

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Bromate is a common byproduct of ozone disinfection of water containing bromide, and is rapidly eliminated from the body of rats. These kinetic data are part of an ongoing project to evaluate the dose-response characteristics of bromate for eventual use in risk assessment of bromate in drinking water supplies. This work was supported by the Aswa RF 4042, IOA, MWD, NWRI, Calleguas Water, Long Beach Water, SNWA, and the Georgia Cancer Coalition.

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TK and toxicity data from a range-finding one-generation study were used to determine appropriate doses for an extended one-generation dietary toxicity study of 2,4-D in rats (based on Cooper et al., Crit. Rev. Toxicol. 36:69, 2006). Groups of 10 male (M) and female (F) CD® rats were treated with 0, 100, 200, 400, 600, 800, 1200 and 1600 ppm 2,4-D in diet from pre-breeding through weaning (M, 71; F, 95 days). A satellite group was included to evaluate 2,4-D effects from gestation day (GD) 0–17. Adult F given ≥1200 ppm 2,4-D had significantly decreased body weights/body weight gains and feed consumption during the pre-breeding period. Satellite F showed decreased body weight gain though body weight was measured to GD 17, which was compensated by GD 21 in main study animals. Feed consumption in parental F was markedly decreased throughout lactation at 800, 1200 and 1600 ppm. Litter size was decreased at concentrations ≥1200 ppm and pup body weights were significantly decreased at concentrations ≥800 ppm during lactation until termination on PND 55. Dams in the 1600 ppm group and litters in the 1200 and 1600 ppm groups were removed from study at or before lactation day (LD) 21 due to excessive toxicity. Adult test day (TD) 71 M had slight kidney histopathology at doses ≥400 ppm. TD 95 F had slight kidney histopathology at higher doses (≥800 ppm) there. There were no effects on reproduction, fertility, gestation length, litter size at birth, birth weights or pup sex ratio. There was no evidence of increased susceptibility in pups. The NOAELs were 100 ppm (M) and 400 ppm (F and pups). If the high dose level for the definitive study was selected based only on traditional toxicity endpoints, this dose would clearly have been in the nonlinear range with respect to TK. (Sponsored by the Industry Task Force II on 2,4-D Research Data)

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Both Ma Huang and Ephedrine (EPH), found in Ma Huang are incorporated in a number of dietary supplements containing caffeine, which have been shown to cause cardiac toxicity in rats following a single dose at lower concentrations than each component administered singly. Whether caffeine and ephedrine interact at the pharmacokinetic level or the pharmacodynamic level is not known. This study was conducted to compare the pharmacokinetics of ephedrine administered alone, or in combination with caffeine, and Ma Huang alone or in combination with caffeine. Jugular-vein cannulated male Fischer 344/N rats were administered EPH by i.v. injection (6.25 mg/kg), p.o. (6.25, 12.5, and 25 mg/kg), EPH + caffeine by gavage (5.0 mg/kg, equiv. to 12.5 mg/kg EPH) and Ma Huang + caffeine by gavage (12.5 and 30 mg/kg). Blood was collected at 5, 10, 15, 30 min, 1, 2, 4, 8, and 12 h following dosing. EPH, pseudocaffeine, and Ma Huang were measured using an LC-MS/MS method. Pharmacokinetic parameters were determined using non-compartmental analysis. The mean elimination half-life of ephedrine on i.v. dosing was 42 min, whereas after gavage, the mean t½ ranged from 132 – 165 min for ephedrine alone. EPH bioavailability was 48%. The area under the curve (AUC) increased disproportionately in a sublinear manner with dose of EPH. In combination with caffeine, EPH AUC increased slightly (1.2-fold). EPH in Ma Huang was 90% bioavailable. EPH AUC in Ma Huang increased was approximately 20% higher compared with Ma Huang alone. This study indicates that the administration of caffeine in combination with Ma Huang or EPH has only slight impact on the pharmacokinetics of EPH, and suggests toxicity of caffeine in combination with Ma Huang or EPH may result from pharmacodynamic interactions.
1264 METABOLISM OF DE(2-ETHYHEXYL)PHthalate (DEHP) IN NEONATAL MALE RHESUS MONKEYS AFTER INTRAVENTRICAL AND ORAL DOSING.


DEHP, the long chain plasticizer used in multiple polyvinylchloride medical devices, has been clearly established as a male reproductive toxicant in rodents, particularly when exposure occurs in developing animals. High exposures, primarily via the yolk sac, of male infants in neonatal intensive care units (NICU) have thus raised concern, but existing human data are inadequate to resolve the issue. Oral dosing of juvenile or adult nonhuman primates (NHP) with doses higher than those used to produce adverse effects in rodents has not caused testicular toxicity. However, there are no data available on NHP exposed to DEHP during the sensitive perinatal period. We monitored the formation of DEHP metabolites in blood samples over 48 hours in male rhesus monkeys after single iv or oral doses at 1, 5, 9, and 10 weeks of age. The hypophalamic-pituitary-testicular axis is active in this time period and the design attempts to mimic the human NICU exposure. Deuterated DEHP was utilized due to high and variable background levels of DEHP and was administered at 5 mg/kg body weight in 10% Intralipid emulsion. The analyses, both aglycon and glucurononated, monitored by liquid chromatography-tandem mass spectrometry included DEHP, mono(2-ethylhexyl)phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl)phthalate (MEHHP), mono(2-ethyl-5-oxo-hexyl)phthalate (MEOH), and mono(2-ethyl-5-carboxypentyl)phthalate (MECPP). Detailed data analyses are on-going, but it is clear that MEHP and its oxidized metabolites are formed throughout the dosing period after both iv and oral dosing and that a substantial proportion of these metabolites circulate in the aglycone form. Metabolite patterns are similar in rhesus monkeys of Indian and Chinese origin, and comparison with urinary metabolite profiles reported from NICU infants suggests that this is a relevant model for the evaluation of DEHP toxicity. Supported by Interagency Agreement 224-07-007 between FDA and NIEHS.

1265 PBPK MODELING OF BENZENE METABOLITES IN BONE MARROW OF HUMANS WITH VARIED WORKPLACE EXPOSURE PATTERNS AND CYP2E1 ACTIVITY.


A 6-compartment physiologically based pharmacokinetic (PBPK) model of benzene in the mouse was modified to include a bone marrow compartment and calibrated to provide good fit with human studies of benzene excretion via breath. The resulting human PBPK model was then used to determine whether differences in exposure rates (intermittent peak vs. uniform) for similar cumulative workday benzene exposures of 8, 80, or 800 ppm-hr produced biologically significant differences in benzene metabolism in persons with and without CYP2E1 induction. The predicted bone marrow dose of benzene and its major metabolites (benzene oxide, phenol) within 24 hours did not vary appreciably with exposure rate or induction status at 8 ppm-hrs, consistent with expected impacts of physiologic damping on deep tissue compartments. At 80 ppm-hrs the predicted bone marrow dose showed no appreciable impacts of exposure rate on major metabolites, but a paradoxical trend of disproportionately higher benzene fraction and a lower fraction of hydroquinone, catechol, and benzoic acid was observed at the highest peak exposures. Similar trends, but of greater magnitude, were seen at 800 ppm-hr for all exposure rates, and induction tended to attenuate the trend where uniform exposures corresponded to the highest bone marrow dose. This paradoxical trend is explained by model-predicted metabolic saturation leading to higher blood benzene levels, enhanced exhalation losses of unchanged benzene, and a consequent lower fraction of metabolite production that prominently affects benzoquinone precursor metabolites, hydroquinone and catechol. The model predictions suggest that it may be important to consider interactions between benzene exposure patterns, CYP2E1 induction, and capacity-limited metabolism when evaluating dose-response trends for benzene hematotoxicity.

1266 IMPROVED ANALYSIS OF TRICHLOROETHYLENE (TCE) METABOLITES BY GAS CHROMATOGRAPHY (GC).

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TCE and several haloacetic acids [e.g., dichloroacetic and trichloroacetic acid (DCA and TCA)] are common contaminants of drinking water in the U.S. Our objective was to develop a GC method of analysis of these and other TCE metabolites in biological samples, with improved separation of GC peaks, simplicity and sensitivity. Measurement of DCA and TCA by headspace analysis required their esterification with a mixture of distilled water, concentrated sulfuric acid and alcohol (6:5:1). Methanol, ethanol, 1- and 2-propanol, t-butanol and 1-octanol were evaluated for their ability to provide good separation with a short retention time. Ethanol proved to be superior. TCE, chloroform and trichloroethylene can be analyzed concurrently with DCA and TCA in blood, tissue and urine samples. The LOD and LOQ for these analytes were 5 ng/ml and 20 ng/ml respectively. Toxicokinetic and metabolism studies of low levels of TCE, perchloroethylene and related haloacarbons can be conducted using this procedure. Supported in part by U.S.DOE cooperative agreement DE-FC09-02CH111090.
and hepatotoxicity, whereas activation of Nrf2 enhances detoxification of NAPQI by Nqo1 and elimination of AA-GLUC via Mrp3. (Supported by NIH grants ES09716, ES07079, ES013714, ES09649, and RR021940).

**1269 USE OF PHARMACOKINETIC MODELING TO EVALUATE GENETIC POLYMORPHISMS IN CARISOPRODOL METABOLISM.**

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The muscle relaxant carisoprodol is one of the most commonly abused drugs in the U.S. and other Western Countries. We previously presented a pharmacokinetic model of carisoprodol metabolism based on first order rates of absorption, metabolism and elimination. The model was able to accurately predict plasma concentrations of carisoprodol and its primary metabolite meprobamate in individuals displaying the metabolic profile observed in the general population. We now present a revised model to address individuals who are poor metabolizers of carisoprodol due to a CYP2C19 polymorphism (Brannames et al., 2005). The revised model is able to correctly predict blood concentrations of carisoprodol and meprobamate in these individuals. To do so, the model's first order rate constant for carisoprodol metabolism (km) is reduced from 0.95 to 0.24 per hour and the percentage of carisoprodol metabolized to meprobamate (consistent with a shift in metabolic pathways) is increased from 0.63 to 0.79. We found it was also necessary to reduce the absorption rate constant for carisoprodol, suggesting that GI uptake of this drug may be saturable in these individuals even in the therapeutic dose range. We have also developed an alternative pharmacokinetic model that describes carisoprodol metabolism as a saturable Michaelis-Menten (MM) process. Our intent is to use the MM model to predict the time course of blood concentrations in overdose situations. Preliminary analyses show the MM model fits the available data with a Vmax value of 1850 mmol/hr and a km value of 28mM. The presentation will also contrast the fit of the two models to blood concentration data obtained at therapeutic and toxic doses.

**1270 COMPARATIVE TOXICOGENOMIC ANALYSIS OF PCB 126 REPS DERIVED FROM PRIMARY HUMAN AND RAT HEPATOCYTES.**

F. Carlson1, T. R. Sutter2, M. Colin1, S. Goodwin2 and J. Silsworth2.

Relative potency (REP) measurements of individual “dioxin-like” compounds (DLCs), such as co-planar polychlorinated biphenyls (PCBs), are often estimated using the toxic equivalency factor (TEF) approach. An international World Health Organization committee has established the PCB126 REP for human cells/cell lines is at least an order of magnitude lower than the rodent-derived PCB126 TEF. The current microarray study sought to extend these findings by investigating dioxin dose response changes in gene expression induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and PCB 126 in primary hepatocytes from either human donors or female Sprague-Dawley rats. Nonlinear mixed-effects models, which directly estimated PCB126 REP, were generated for 47 unique human and 79 unique rat genes using an automatic model selection scheme. Only five orthologs were commonly modeled in both species. The geometric mean maximum likelihood estimates of the PCB126 TEF for all human and rat genes were 0.002 and 0.06, respectively. This species difference in sensitivity was also extended to significantly enriched categories for gene ontology and KEGG pathways. Closer examination of the data revealed that while many rat genes possessed PCB 126 EC50s below -8 logM, the PCB 126 EC50s for most human genes were above -8 logM. In addition, the human genomic response was found to be less sensitive to TCDD exposure compared to rats. Thus, to be accurate, any standardized risk value based on animal studies but used for human health risk assessment must account for species differences in sensitivity to both the reference TCDD and the DLC. Overall, the current study raises serious questions about the suitability of rat-derived REP estimates for use in human health risk assessment.

**1271 PAH ANALYTICAL TECHNIQUES AND IMPACTS ON RISK ASSESSMENT.**


Polycyclic aromatic hydrocarbons (PAHs) constitute a large group of chemicals that are formed during incomplete combustion of organic substances. They can be anthropogenic in origin – burning of coal, oil & gas, tobacco – or form naturally in the environment – volcanoes, forest fires. Due to the numerous sources, PAHs are ubiquitous in the environment. PAHs are often found at contaminated sites. They are a contaminant of concern at 749 National Priorities List (NPL) sites (approximately 60% of sites) and are ranked sixth on the CERCLA priority list of hazardous substances. A number of standard laboratory techniques are available to determine the composition, distribution, and abundance of PAHs in various environmental media including EPA method 8270C which uses gas chromatography/mass spectrometry. However, more sensitive techniques for measurement of PAHs have also been developed including high-resolution isotope dilution mass spectrometry (ID/MS). We have conducted a study to compare a standard analytical method (EPA 8270C, often used in the environmental industry) to a more sensitive method (ID/MS) for monitoring PAHs in soil. Our results indicated that the frequently used standard laboratory method (EPA 8270C) consistently underestimated PAH concentrations in soil as compared to the more sensitive ID/MS technique which was found to be more accurate and more precise. Using the benzo[a]pyrene toxic equivalency (BaP TEF) scheme, it was found that the BaP TEF values generated using the current standard method were significantly greater than those from the more sensitive technique. These findings may have important implications for contaminated soil risk assessment. Risks associated with exposure to PAHs in soil (via inadvertent ingestion, dermal contact and inhalation of soil/dust) are directly proportional to measured PAH concentrations. Any error in the determination of PAH concentrations will consequently impact the calculation of risk. Thus, use of more sensitive laboratory techniques for PAH measurement will reduce uncertainty and provide for a more accurate understanding of site conditions and liability.

**1272 SPECIES DIFFERENCES IN HUMAN AND RAT RELATIVE POTENCY VALUES FOR DIOXINS, FURANS AND PCBs ARE CONGENER-SPECIFIC.**


In 2005, Silsworth et al reported that the EROD-based REP value for PCB126 in human hepatocytes was 100-fold less than the REP value in rat hepatocytes. To confirm the lower REP value of PCB126 in other human cells and to determine whether this observation held for other dioxin-like compounds with 0.1 TEFs, we tested normal human epidermal keratinocytes (NHEKs) and calculated EC50-based REP values for CYP1A1 mRNA induction for PCB126, TCDD, 2,3,7,8-tetrachlorodibenzofuran (TCDF), 1,2,3,6,7,8-hexachlorocyclohexane (HxCDD) and 1,2,3,6,7,8-hexachlorobenzodioxin-p-furan (HxCDF). Chemical concentrations were analytically determined. Our results demonstrated that in NHEKs, PCB126 had a REP value of 0.002, compared to 1 of TCDD. This value is 50 times less than the TEF value of 0.1 assigned to it by WHO2005 and the same as the value reported earlier for human hepatocytes. The REPs for TCDF and HxCDD were 0.07 and 0.19, respectively, similar to their assigned value of 0.1. HxCDD had a REP value of 1.19, which is 12 times its assigned value. For direct comparisons to responses in rats, the same stocks of TCDD, PCB126 and HxCDF were used to treat the rat cell line, H4IE, resulting in REP values of 0.06 for PCB126 and 0.05 for HxCDF, similar to the assigned value of 0.1, confirming our chemical concentrations. Importantly, the EC50 value for TCDD in H4IE cells was 20 times less than the value obtained in NHEKs, as has been shown in other human cells. In conclusion, our preliminary data indicate that the differences in REP values between rat and human are congener-specific. A complete study of congeners in human cells is warranted and these results should be incorporated into TEFs by risk assessors.

**1273 APPLICATION OF NONLINEAR DOSE-RESPONSE METHODS BASED ON MODE OF ACTION FOR POLYCHLORINATED BIPHENYLS (PCBs ).**

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A mode-of-action (MOA) for PCB-promoted rat liver tumors that fulfills the Hill Criteria and the comprehensive requirements of strength, consistency, specificity, temporality, dose-response and biological plausibility, was used as the basis for deriving a set of preliminary Tolerable Daily Intakes (TDIs) for four different PCB mixtures using a nonlinear dose-response model. Nonlinear approaches are suitable for investigating HCAs because none of the MOA elements shows linearity at low doses. The derived TDIs were then compared to allowable intake levels calculated using the linear default extrapolation approach as currently used by EPA. The statistical dose-response analysis was conducted using EPA Benchmark Dose Software, which was initialized to employ a dichotomous multi-stage procedure to model dose
response in the observable region of the data in order to extrapolate to a point-of-departure (POD) at or near the fringe of the observable region. In this preliminary evaluation of a nonlinear dose response model for PCBs, the application of conservative toxicological uncertainty factors (UFs) was used as the simplest method for extrapolating below the POD to the threshold dose anticipated by the MOA. This resulted in the determination of a preliminary set of TDIs, one for each PCB mixture, that were approximately 3,000 to 5,000 times greater than the corresponding values for the risk-specific dose (10⁻⁶). This nonlinear dose-response assessment indicates that much higher levels of PCBs can be tolerated without subjecting individuals to unreasonable excess cancer risks. Further refinements to the nonlinear approach may include the application of probabilistic methods to estimate the changing shape of the dose-response curve as it flattens from the POD to the observable region where it approaches a threshold consistent with the PCB MOA.

**PL 1274**  
**WEIGHT OF EVIDENCE EVALUATION OF THE MODE OF ACTION FOR PCB-PROMOTED RAT LIVER TUMORS USING THE HUMAN RELEVANCE FRAMEWORK.**

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While it is well established that sufficient exposure of rodents to PCB mixtures (i.e., Aroclors) can promote liver tumors the mode of action (MOA) has been elusive. A recent publication described the likely MOA of hepatic tumors in Aroclor-dosed S-D rats. Numerous biochemical measurements taken over the course of a chronic bioassay with Aroclors 1016, 1242, 1254 and 1260 showed that liver tumors were closely, consistently, and predictably correlated with the net hepatic cytosolic activity of redox-cycling quinones (RCQ) acting as catalysts for the production of reactive oxygen species (ROS). This indicated a tumorigenic MOA whereby (1) tissue PCB/TEQ accumulations induce expression of mixed function oxidases (MFOs) and (2) MFOs convert endogenous substrates to RCQs, eventually resulting in ROS-mediated promotion via H2O2 mitotic signaling of spontaneously initiated liver cells. These findings are corroborated by the results of other bioassays with TCDD, individual PCB congeners and binary PCB mixtures based on the results of bioassays conducted by the National Toxicology Program (NTP). Because a well-defined MOA can be described for PCB-promoted rat liver tumors, the totality of the data can be used in a systematic weight of evidence (WOE) evaluation of the relevance of these tumors to human health and for risk assessment. This evaluation follows EPA’s Guidelines in conjunction with the methodology of ILSI/IPCS which provides a decision-logic based approach for determining the relevance of PCB-promoted liver tumors in animal studies to humans. The WOE supporting the postulated MOA fulfills the Hill Criteria and the comprehensive requirements of strength, consistency, specificity, temporality, dose-response and the key criterion of biological plausibility. Since the MOA fulfills the rigorous ILSI/IPCS Human Relevance Framework and none of the elements show linearity at low doses a non-linear approach is justified for cancer risk assessment of rodent liver tumors for humans.

**PL 1275**  
**THE RELATIONSHIP BETWEEN THE IC50, TOXIC THRESHOLD, AND THE MAGNITUDE OF STIMULATORY RESPONSE IN BIPHASIC (HORMETIC) DOSE-RESPONSES.**

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Hormesis is a dose-response relationship characterized by a biphasic (U- or inverted U-shaped) response. We present the results of a study designed to assess the relationship between toxic potency (as measured by the IC50) and the magnitude of the hormesis stimulation. We describe a new parameter (ΔX), which we define as the difference between the concentration (or dose) that inhibits 50% of the growth of the organism under study (IC50), and the concentration (or dose) of the respective toxicological threshold (e.g., benchmark dose (BMD) or zero equivalent point (ZEP)). Our analysis includes a subset of data from a previously published report describing a National Cancer Institute study that exposed 13 strains of yeast to over 2,100 putative anticaner agents (representing over 29,000 dose-responses). The toxic threshold used in this Calabrese et al. (2008) paper was the BMD5 (Crump, 1984). Thus, the ΔX in this presentation (in μM) is defined as: ΔX = IC50 – BMD5. We have found that the ΔX and the magnitude of stimulation above the control response are inversely related. For example, the Calabrese et al. (2008) paper presents model mean estimates for the aggregate response of yeast growth below the BMD5. When the mean estimate of response for were segregated into 5 approximately equal quintiles (n = 79, mean responses of 99, 102, 104, 111, and 151%) of the control response, quintiles 1-5, respectively, and compared to the mean ΔX values (50, 40, 36, 36, and 21 μM, quintiles 1-5, respectively), it was observed that as the magnitude of stimulation (or evidence of hormesis) increases, the ΔX decreases. These findings describe the first known relationship between toxic potency and the magnitude of hormetic response and warrant further inquiry.

**PL 1276**  
**HEALTH RISK ASSESSMENT OF MELANINE IN DRINKING WATER.**


Acceptable levels of melamine in drinking water were determined based on NSF/ANSI 61 Annex A (2007a). Melamine is detected in the extract water of products that convey drinking water, since it is used as an ingredient in some materials used to make these products. In Fall 2008, melamine was implicated in as many as 50,000 acute renal failure cases in infants that consumed contaminated milk in China. The complex of melamine and cyanuric acid was implicated in many cases of renal failure resulting from renal obstruction with melamine cyanurate crystals in domestic dogs and cats that consumed contaminated pet food in the U.S. in Spring 2007. Limited quantitative data for melamine exposure in humans were identified, such as data from the milk adulteration. Both renal and bladder precipitation of melamine crystals were observed in domestic and laboratory animals after dietary melamine intake. Although crystals were generally seen in both organs, species-specific differences were observed with respect to the predominate organ. Renal precipitation of melamine predominates in non-rodent species, such as dogs and cats, while bladder precipitation predominates in rodents. Most of the data in dogs and cats were from a mixture of melamine and cyanuric acid, which likely affected the target organ. Repeated dietary melamine intake was associated with bladder stones with a male predominance in rodents. Upon chronic dietary exposure, these stones progressed to bladder tumors in male, but not female rats or male or female mice. The bladder tumors were considered to arise from a threshold mechanism secondary to urinary melamine precipitation that lead to bladder stone formation. Kinetics data support the lack of melamine metabolism. Since insufficient quantitative data were available to identify a NOAEL for renal or urinary calculus formation in non-rodent species exposed to melamine alone, the RID of 0.1 mg/kg/day melamine was based on the 10% benchmark dose level of 38 mg/kg/day for urinary calculus formation in laboratory rats. The RID of 0.1 mg/kg/day was used to determine the Total Allowable Concentration in drinking water of 0.9 mg/L.

**PL 1277**  
**ARSENIC BIOAVAILABILITY IN SOIL: EVIDENCE FROM IN VITRO AND MICROPROBE STUDIES.**

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Bioavailability of arsenic in soil is a continuing issue for appropriate human health risk assessment associated with arsenic exposure. Recent results of in vitro and microprobe analysis of arsenic at a former secondary lead smelter and two former mining/milling sites provide some additional perspective on this issue. In vitro analysis of arsenic bioavailability at a former lead smelter site was entirely consistent with low or zero assessability of arsenic in soils from non-contaminated areas, and increasing content of highly assessable arsenic in contaminated soils closer to the former location of the smelter stack. Results of the in vitro assay between two independent laboratories were consistent, demonstrating the reproducibility of the methods. Estimates for possible remediation goals for arsenic at this site for a given risk target were perhaps an order of magnitude higher than typical default values, when bioavailability information was taken into account. In vitro analysis of arsenic bioavailability at two former mine/mill sites suggested that, regardless of soil contaminations, the amount of available arsenic was small. Results indicate that potential arsenic-related exposure may be only a fraction of exposure estimated using default assumptions. Electron microprobe analysis of soils indicated that arsenic was observed within the soil matrix and in forms typically non-assessable in the in vitro assay. Iron content of soils at these sites was high (25,000 mg/kg or higher), and arsenic was likely associated with insoluble iron species. The current results reconfirm that knowledge of information on bioavailability of arsenic is critical to risk assessment at sites with arsenic soil contamination. Quantitative use of information on bioavailability of arsenic may provide the risk manager with addition options for remediation and/or mitigation.
current understanding of cancer as a multifaceted process. A HESI Committee is developing a framework for the use of DNA adduct data in risk assessment. The approach includes: 1) defining a preliminary framework for data organization, 2) assessing available methods for quantitative measurement of adducts; 3) using case studies to refine the framework and draw inferences. Conclusions to date include: DNA is not pristine; DNA adducts cannot be used in isolation to determine mode-of-action (MOA); and DNA adducts (biomarkers of exposure) are not equivalent to mutations (biomarkers of effect). The framework stresses the consideration of a variety of adduct data in context with traditional toxicology data to inform MOA characterization. Conclusions on the relevance and applicability of DNA adduct data to quantitative risk assessment will be further refined by subsequent review of selected case studies. The strategy for selection and evaluation of case studies of known DNA-reactive carcinogens is discussed. A complementary effort summarizes the methods used to identify and quantify DNA adducts, focusing on reliability and validation. That effort describes sensitivity and specificity as key factors for method selection, as is sample type. The reliability of oxidative DNA damage measurement techniques is reviewed. Assay limitations are discussed along with issues that have an impact on results, i.e., sample collection, processing, and storage. This framework is a critical effort to aid understanding of issues underlying DNA adducts as biomarkers and to improve the context for data inference and application in cancer risk assessment. (This abstract does not reflect U.S. EPA policy.)

PL 1279 THE TRANSLLOCATION AND FATE OF CHRYSTOLITE ASBESTOS IN COMBINATION WITH FINE PARTICLES FOLLOWING EXPOSURE IN A BIOPERSISTENCE STUDY.

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To evaluate the translocation, fate and clearance of chrysotile asbestos that was used as a component of a joint-compound in an inhalation biopersistence study was performed using chrysotile in combination with the sanded reformulated joint-compound. A positive control of the amphibole asbestos amosite was also evaluated. Groups of Wistar rats were exposed 6 h/d for 5 d to either 7RF3 chrysotile and the aerosolized sanded joint-compound or to amosite asbestos. A control group was exposed to filtered-air. The animals were followed through 1 year post exposure. The initial results through 30 days after cessation of exposure are reported. The chrysotile used in the Ready Mix joint compound is rapidly removed from the lung. In the chrysotile & sanded exposure group the T1/2 of fibers L>20 μm was 4.8 days. The WT1/2 for amosite for fibers L>20 μm was 266,000 days. Histopathological examination showed that the chrysotile & sanded material exposed lungs had the same appearance as the filtered-air controls. The amosite exposed group showed an intense inflammatory response and developed interstitial fibrosis within 30 days post exposure. The lungs from sub-groups of rats were evaluated by confocal microscopy with the size and distribution of fibers in airways, parenchyma and macrophages quantified at each time point. In addition, a separate time course study addressed mechanisms of cell-associated transport of amphibole amosite and chrysotile fibers through parietal pleural lymphatics and evaluated any associated inflammatory cell reaction. The initial results of this study confirm that the chrysotile and sanded joint compound is quickly cleared from the lung, that there is no sustained inflammatory response. In contrast, the amphibole amosite following short-term bronchial clearance remains in the lung, produces an intense inflammatory response leading quickly to interstitial fibrosis.

PL 1280 VASCULAR EFFECTS OF A SUBCHRONIC INHALATION EXPOSURE TO CONCENTRATED AMBIENT AIR PARTICLES IN ATHEROSCLEROSIS SUSCEPTIBLE MICE.


Several studies have reported the adverse effects of particulate air pollution on cardiovascular function and disease. The causal physical properties of particles and their mechanisms of action/injury remain unknown. This study examined the vascular effects in 15 wk old male ApoE knockout mice fed normal chow and exposed by inhalation for 3 months (Nov. 2007 – Feb. 2008, 5d/wk, 6 hr/day) to either filtered air (FA), fine (F) (ave. conc. 219 μg/m3; range 55 – 780 μg/m3; ave. size -680 nm), or ultrafine (UF) (ave. conc. 365 μg/m3; range 46 – 680 μg/m3; ave. size -95 nm) RTM concentrated ambient air particles (CAPs). Mice were examined for vascular effects at 0 hr, 72 hrs and 1 month post-exposure. Histopathology performance at 72 hrs post-exposure showed a lack of significant pulmonary inflamma-

PL 1281 VASCULAR LIPID PEROXIDATION AND INDICES OF PLAQUE REMODELING ARE PROMOTED BY INHALATION EXPOSURE TO VEHICULAR EMISSIONS, BUT NOT HARDWOOD SMOKE OR A SIMULATED COAL COMBUSTION ATMOSPHERE.

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The source emissions that contribute to cardiovascular morbidity and mortality have not been compared in a head-to-head manner. Using an established animal model of hypercholesterolemia and atherosclerosis, we conducted 7- and 50-day whole body inhalation exposures to emissions from a gasoline engine, a diesel engine, hardwood combustion, and coal combustion. To address the long range transport of coal combustion emissions, this atmosphere was modified by addition of several gases (NOx, SOx). Additionally, we produced a “traffic” atmosphere with combined gasoline and diesel engine emissions. For the 50-day exposures, apolipoprotein E-null mice were exposed to one of four levels of each pollutant atmosphere, or a high level with particles removed by filtration. Following exposures, aortas from exposed mice were harvested and probed for indices of lipid peroxidation and mRNA for matrix metalloproteinases (MMPs), endothelin-1 (ET-1) and several other markers. While strong responses to gasoline and diesel engine emissions were observed (along with the combined emissions), minimal to no effect could be determined from the woodsmoke and coal atmospheres. Furthermore, removal of particles from the whole exhaust atmospheres resulted in no difference from the responses to the whole exhaust, indicating that the gaseous portion of emissions contained the driver(s) of these effects. In 7-day studies, results were consistent with those from 50-day studies, and we found that several changes in PCR markers could be recreated by exposures to nitric oxide and carbon monoxide. However, lipid peroxidation could not be replicated by any individual gas, and was only upregulated by gasoline and/or diesel engine emissions. These studies confirm that complex pathophysiological responses are likely to arise from exposure to complex mixtures. Supported by the National Environmental Respiratory Center with funds from multiple government and industry sponsors.

PL 1282 MECHANISTIC INSIGHTS INTO THE RELATIONSHIP BETWEEN LUNG AND VASCULAR RESPONSES TO AMBIENT PARTICULATE MATTER (PM).


The mechanisms by which inhaled ambient PM induces vascular response are not well understood. We examined lung and aortic response of rats to three ambient PM. Chemically characterized PM10 and PM2.5 from the north and PM10 from the south of Mexico City were tested for in vivo acute lung and vascular toxicity. Male Wistar Kyoto rats (16 weeks) were intratracheally instilled with either saline or one of three PM at 3 mg/kg. All three PM induced inflammation (day 1-3 day) as evidenced by the increases in bronchoalveolar lavage fluid (BALF) protein and neutrophils with small differences between PM types. Gene expression for markers of inflammation, oxidative stress, transcription factors and cytokines, and receptors which recognize oxidatively modified proteins and lipids (RAGE and Odfb1) were analyzed in the lung and aorta. Pulmonary HO-1, TNF-α, MIP-2, tissue factor and PAI-1 but not tPA mRNA increased with all three PM. Maximum response occurred at day 1 but diminished by day 3. Endothelin-1 was induced
slightly at day 3 but not MMP-2. The most remarkable induction occurred with iNOS in the lung at day 1. RAGE expression decreased in lung at day 1, while Oldd-1 increased slightly at day 3. Although the effects on the aorta were milder than what was observed in lung, the response was significant and occurred at day 3 with most markers. Aorta MMP-2, HO-1, endothelin-1, iNOS and iNOS inducement up to 3-fold by all three PM at day 3, PAI-1 and tPA were not induced consistently or significantly. Unlike lung, both Oldd-1 and RAGE expression were increased in the aorta. We provide the evidence of delayed vascular effects from ambient PM exposure that are reflective of oxidative stress, vasoconstriction, matrix abnormalities and increased thrombosis likely mediated via upregulation of RAGE and Oldd-1. (Does not reflect US EPA policy). Supported by EPA SEE Program, and EPA/UNC co-op #CR83546301.

**PI 1283 ARSENIC INDUCES ENDOTHELIAL ACTIVATION, INFLAMMATION AND Atherosclerotic Lesion Formation.**

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Exposure to arsenic increases cardiovascular disease, especially atherosclerosis in humans and experimental animals. However, little is known about the biochemical mechanisms by which arsenic exacerbates atherogenesis. In the present study we show that in vitro, sodium arsenite (5-20 μM), significantly (P<0.05) induces the surface expression of adhesion molecules ICAM-1, VCAM-1 and increases the expression of MT-1 MMP in vascular endothelial cells. Incubation of endothelial cells with sodium arsenite (5-20 μM) increased the transmigration of activated mononuclear (THP-1) cells in a concentration dependent manner and anti-MT-1 antibodies (10 μg/mL) attenuated the arsenic-induced transmigration of monocytes. Moreover, we also observed that in bone marrow derived macrophages, sodium arsenite (5 μM) increased the foam cell formation by 1.8 fold and expression of the mRNA of cytokine, IL-6 by 7 fold. In vivo, sodium arsenite (1,4.9 and 49 ppm) feeding to three week old apoE-/- mice for 13 weeks increased the lesion formation in the aorta. Further, we also observed that in bone marrow derived macrophages, sodium arsenite (5-20 μM) increased the transmigration of activated mononuclear cells. We provide the evidence of delayed vascular effects from exposure to ambient PM exposure that are reflective of oxidative stress, vasoconstriction, matrix abnormalities and increased thrombosis likely mediated via upregulation of RAGE and Oldd-1. (Does not reflect US EPA policy). Supported by EPA SEE Program, and EPA/UNC co-op #CR83546301.

**PI 1284 EXPOSURE TO PARTICULATE AIR POLLUTION IN CHILDHOOD INCREASES SUSCEPTIBILITY TO DIABETES/INSULIN RESISTANCE DEVELOPMENT IN ADULTHOOD.**

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Background: Obesity has become a leading cause of preventable morbidity and mortality worldwide, and its rapid increase in genetically stable population points to the importance of environmental factors, such as fine particulate matter (diameter <2.5μm, PM2.5). We hypothesized that exposure to PM2.5 during childhood increases the susceptibility to type 2 diabetes mellitus (T2DM) and insulin resistance (IR) development in adulthood. Methods: Male C57BL/6 mice at 3-4 weeks old were randomly exposed to either concentrated ambient PM2.5 or filtered air (FA) in a whole-body exposure system “OASIS-1” in Columbus, OH for 10 weeks (6 hours/day, 5 days/week). During the exposure period, mice were fed either normal feeding to three week old apoE-/- mice for 13 weeks increased the lesion formation in the aorta. Moreover, circulating levels of soluble ICAM-1 and MCP-1 were also significantly higher in the arsenic-fed mice. These data suggest that exposure to arsenic stimulates endothelial activation, inflammation and exacerbates plaque formation.

**PI 1285 INTERSTRAIN VARIATION IN CARDIAC AND RESPIRATORY ADAPTATION WITH ACUTE PARTICULATE MATTER (PM) AND OZONE (O3) EXPOSURES.**

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OBJECTIVES: Increased ambient PM and O3 are associated with adverse cardiac and respiratory events in humans. However, the mechanisms by which PM and O3 affect cardiac function remain uncertain. It is accepted that physiological adaptation to acute O3 exposures involves the cardiac and respiratory systems. We hypothesize that adaptation to PM and O3 is strain dependent in mice. In addition, the adaptation to O3 is an integrated response involving cardio-respiratory coordination. STUDY DESIGN: Heart Rate (HR), HR variability (HRV), respiratory rate (RR), and core temperature were assessed by implanted telemeters and plethysmography in 3 inbred mouse strains; C57BL/6J (B6), C57/HF (HeJ) and C57/HFouJ (OuJ). Mice were exposed for 3 consecutive days to either filtered air (FA), carbon black particles (CB), or O3 and CB sequentially (O3CB). RESULTS: Time-dependent changes in response to O3CB and FAHFA exposures were observed in each strain relative to FAHFA. These changes were most prominent in HeJ and OuJ mice. For example, HeJ and OuJ mice demonstrated dramatic increases in HRV with reductions in HR during O3CB exposure. In contrast, depressed HR occurred in B6 mice with only slight changes in HRV. Moreover, each mouse strain show distinct adaptation profiles to repeated O3CB exposures. The B6 mice were either unresponsive or rapidly adaptative. In contrast, HeJ mice generally showed marked responsiveness and adapted after 2-days of exposure; and the OuJ mice showed marked responsiveness with only modest adaptation over the 3-day O3CB exposure. Likewise, HeJ and OuJ mice demonstrated rapid breathing with O3CB exposure, whereas B6 mice showed no change in RR. CONCLUSIONS: These findings suggest that genetic factors determine the magnitude of the response to acute co-pollutant exposures, which may determine susceptibility to air pollutant morbidity and mortality. Moreover, physiological adaptation to O3 exposure may involve a coordinated integration among the cardiovascular and respiratory systems.

**PI 1286 RAT MODELS OF CARDIOVASCULAR DISEASE DEMONSTRATE DISTINCTIVE PULMONARY GENE EXPRESSION FOR VASCULAR RESPONSE GENES: IMPACT OF OZONE EXPOSURE.**


Comparative gene expression profiling of tissues from rat strains with genetic diverse cardiovascular diseases (CVD) can help decode the transcriptional program that governs organ-specific functions. We examined expressions of CVD genes in lungs of rat models at baseline and following ozone exposure to reveal mechanisms of susceptibility. Gene lists for heart failure, obesity, hypertension and stroke were compiled from literature. Each list was distinct with a maximum overlap of 19% between two lists. Using rat 230A Affymetrix GeneChips, lung expression was measured for male 12-14 week Wistar Kyoto (WKY, healthy), Spontaneously Hypertensive (SH), Stroke-Prone SH (SHRSP), Heart Failure SH (SHHF) and Diabetic (JCR) rats following air or 1.0 ppm ozone exposure for 4 hrs. Principle component analysis for each gene list identified clusters for each of the rat strains. For hypertension genes there were three clusters with similar gene expressions: 1) JCR; 2) WKY, SHR, SHRS, and 3) SHHF strain. Those regulating vasosconstriction were induced in JCR but down-regulated in other strains. Vasodilation genes were suppressed in JCR but upregulated in other strains. For heart failure and stroke genes there were three clusters: 1) JCR; 2) WKY; and 3) SHR, SHHF and SHRS strain. Those for obesity, there were three clusters: 1) JCR; 2) SHR, SHRS, and WKY; and 3) SHHF strain. The similarity of expression response among the SHR, SHRS, and WKY rats could be attributed to known similarity among their genomes. Ozone caused minimal pulmonary response (few genes changed) in JCR but marked response in other strains (WKY>SHRS>SHR>SHHF). Based on analysis of functional categories, heart (5-20%) and lung (70-80%) indicated most affected by ozone in JCR, SHRSP and WKY, while stroke genes were altered in the SHR. Our study demonstrates how pulmonary expression patterns are influenced by cardiovascular disease and provides insights into the mechanisms of ozone susceptibility differences among disease models. (Does not reflect US EPA policy.)
1287 A SINGLE TRANSITION METAL-RICH PARTICULATE INHALATION EXPOSURE ELICITS CONCENTRATION-DEPENDENT CARDIOVASCULAR TOXICITY IN HYPERTENSIVE RATS.

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Recently, investigators in the CALFINE study demonstrated an association between the fine particulate matter (PM)–associated metals, Ni and Fe, in ambient air in nine California counties and increased cardiovascular mortality (Ostro et al. 2007). Residual oil fly ash (ROFA), a waste product of fossil fuel combustion from boilers, is rich in the transition metals Fe, Ni, and V, and when released as a fine particulate particle, is an important contributor to ambient fine particulate air pollution. We hypothesized that a single acute inhalation exposure to transition metal-rich particulate matter will cause concentration-dependent cardiovascular toxicity in Spontaneously Hypertensive (SH) rats. Rats were exposed once by nose-only inhalation for 4 hours to 3.5 mg/m³, 1.0 mg/m³ or 0.45 mg/m³ of a synthetic particulate matter (dried salt solution) consisting of Fe, Ni and V sulfates and that is similar in composition to a well-studied ROFA sample. Twenty-four hours after exposure, only the high dose caused an increase in plasma Angiotensin Converting Enzyme (ACE), an enzyme that stimulates vasoconstriction and increased blood pressure, and serum myoglobin, a muscle protein released by injured cardiac myocytes. Thus, metal particulate exposure increased circulatory markers of cardiac injury and disease in rats with hypertension suggesting that metal components of PM may be acutely toxic in individuals with pre-existing cardiovascular disease. Heart rate, core body temperature, activity, and electrocardiogram data are currently being analyzed to assess the physiological effects of metal sulfate particle exposure (This abstract does not reflect EPA policy).

1288 RESVERATROL (TRANS-3, 5, 4‘-TRIHYDROXYSTILBENE) PROTECTS THE FETUS FROM THE IMMUNOTOXIC EFFECTS OF TCDD FOLLOWING PERINATAL EXPOSURE BY BLOCKING AhR ACTIVATION.

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TCDD, an environmental pollutant that causes thymic atrophy and immunotoxicity, severely affects both maternal and fetal immune system during pregnancy. TCDD mediates its toxic effects primarily through activation of aryl hydrocarbon receptor (AhR). Resveratrol, a natural phytoalexin present in plant products including grapes and nuts, exhibits anti-cancer, anti-inflammatory, and anti-inflammatory properties. It is also known to act as an AhR antagonist. In the current study, we investigated whether resveratrol when administered into pregnant (C57BL/6) mice can reverse the immunotoxicity of TCDD on mother and the fetus. Resveratrol at lower concentrations (1-5 µM) acted as an AhR antagonist and blocked TCDD-induced upregulation of Fas and FasL expression in T cells and consequent apoptosis in T cells. Moreover, resveratrol protected the pregnant mothers and their fetuses from T cell mediated thymic atrophy, priming and chronic changes and apoptosis in thymocytes. There was significantly reduced expression of CYP1A1 in thymus and liver when resveratrol was used in vivo post-TCDD exposure. Significant reduction in luciferase expression under the control of CYP1A1 promoter was observed when resveratrol was added in culture with TCDD. Together, these studies demonstrate that consumption of resveratrol, a natural plant product during pregnancy can protect fetuses from TCDD-mediated immunotoxicity (This work was supported in part by NIH grants P01AT005961-01A1, R01AI053703, R01ES09098, R01AI059800, R01DA016543, and R01HL058441).

1289 ROLE OF NF-κB/REL PROTEINS IN MODULATING THE 3′IgHRR BY LPS AND TCDD.

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Transcriptional regulation of the immunoglobulin heavy chain (IgH) gene involves several regulatory elements including the 3′ IgH regulatory region (3′IgHRR). The 3′IgHRR is composed of at least four enhancers (hs3A; hs1,2; hs3B; hs4) and contains binding sites for several transcription factors including NF-κB/Rel proteins. The dioxin responsive element (DRE) may also contribute to 3′IgHRR regulation. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), a known disruptor of B-cell differentiation, induces binding of the aryl hydrocarbon receptor (AhR) to a DRE motif within both the hs1,2 and hs4 enhancers. Interestingly, in transient luciferase reporter studies TCDD profoundly inhibited the activation of a 3′IgHRR by the B-cell activator lipopolysaccharide (LPS) but enhanced LPS-induced activation of the hs4 enhancer. Within the hs4 enhancer, the DRE overlaps an NF-κB/Rel binding site (KB) and site-directed mutational analysis demonstrated a cooperative interaction between proteins binding to these motifs. The objective of the current studies was to evaluate the expression of NF-κB/Rel proteins including their negative regulator IkBα following LPS stimulation and TCDD treatment and to compare these results to 3′IgHRR activity under the same treatment conditions. For these studies we utilized the CH12.LX B-cell line and its variant, CH12.IKBeαA, which expresses an inducible IkBeα super repressor (IkBeαA). Western blot analysis of LPS-stimulated CH12.IKBeαA cells demonstrated a decrease in endogenous IkBeα with a reciprocal increase in nuclear RelA which appeared to be diminished when IkBeαA was expressed. Utilizing transiently expressed luciferase reporters, induction of IkBeαA expression partially attenuated both LPS-induced activation of the 3′IgHRR as well as h4v activation following an LPS and TCDD co-treatment. Also, co-immunoprecipitation (Co-IP) assays with the CH12.LX cells have demonstrated an interaction between the AhR and NF-κB/Rel. These results suggest that NF-κB/Rel proteins, perhaps through an interaction with the AhR may be at least partially responsible for 3′IgHRR modulation by LPS and TCDD. (Supported by NIH/NEIHS R01ES014676)

1290 GENERATION OF NOVEL “REGULATORY” DENDRITIC CELLS VIA AhR ACTIVATION.

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Dendritic cells (DCs) are key antigen presenting cells governing T cell activation and differentiation. TCDD, the prototypical AhR ligand, has been shown to affect the differentiation of murine bone marrow-derived dendritic cells (BMDCs). Previous studies have also demonstrated that TCDD and 3-formylindolo[3,2-b]carbazole (FICZ), an endogenous AhR ligand, can induce Tregs or Th17 cells, respectively. Induction of indoleamine 2,3-dioxygenase (IDO) has been implicated in AhR-mediated immune suppression. However, the effects of AhR activation on two DC subpopulations - inflammatory (GM-CSF-derived) DCs and steady-state (Flt-3-derived) DCs - have not been investigated. We hypothesize that AhR activation induces novel regulatory DCs from both inflammatory and steady-state DCs. To test this hypothesis, inflammatory or steady-state BMDCs were generated in the presence of vehicle, TCDD, or FICZ. Both TCDD and FICZ decreased expression of CD11c but increased MHC class II and CD80 expression on inflammatory BMDCs. TCDD increased levels of both IDO and IDO2 mRNA in inflammatory BMDCs by 18 and 24-fold, respectively. TCDD also upregulated LPS- and CpG-induced TNF-α and IL-6 production by these BMDCs. Previous studies have shown that AhR activation can alter NF-κB signaling. TCDD downregulated LPS- and CpG-induced p65 levels and induced a trend towards upregulation of RelB levels in inflammatory BMDCs. In contrast, steady-state DCs treated with TCDD expressed lower levels of CD11c but increased CD86 expression. Following stimulation with CpG or LPS, TCDD decreased TNF-α and IL-6 production by steady-state BMDCs. TCDD induced a 7-fold increase in IDO mRNA levels in steady-state DCs but had no effect on IDO. Overall, our results suggest that AhR activation differentially affects inflammatory and steady-state DCs thereby contributing to the generation of regulatory DCs that may ultimately direct the production of Tregs or Th17 cells. This research was supported by grants from NIEHS (ES015784) and NCRR (RR17670).
and were unchanged in null mice; thymus weights were unaffected in both wild-type and null mice and liver weights were elevated in both. The role of elevated glucocorticoids in immunosuppression was addressed by exposing adrenalectomized (adx) and sham-operated female C57BL/6 mice to 7.5 or 15 mg/kg PFOA in drinking water for 10d. Mice were immunized and bled as in experiment 1 with PPARα null mice. Suppression (p < 0.05) of IgM titers was similar in sham-operated and adx mice. These studies suggest that the mechanisms by which PFOA affects TDAR are not mediated by PPARα receptor activation or by excess corticosterone release. Additional work with T cell-independent antibody responses and with adrenalectomized PPARα null mice are planned to further elucidate the mechanism of PFOA-induced antibody suppression (This abstract does not reflect EPA policy and was supported by UNC/EPA Cooperative Training Agreement CTR29472).

1292 MECHANISMS OF ETHANOL MEDIATED INHIBITION OF TOLL LIKE RECEPTOR SIGNALING IN MACROPHAGES.

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The mechanisms underlying the effects of alcohol on host defense are still poorly understood. Previous studies indicate that ethanol interferes with LPS induced TLR4/CD14 complexes formation and receptor clustering, thus inhibiting TLR signaling. TLR3 is not known to cluster or to be recruited to lipid rafts like TLR4. As ethanol still seems to inhibit the poly I:C-induced cytokine production by macrophages, it may act by other mechanisms. In an ELISA-type assay, binding of poly I:C to TLR3 protein was tested. No inhibiting effect of ethanol on poly I:C binding to TLR was found, but butanol seemed to increase binding, suggesting that inhibition of ligand binding is not a mechanism of ethanol action. ELISA assay studies on cell culture supernatants indicated that alcohols from methanol through pentanol were tested for their ability to inhibit the LPS and poly I:C induced cytokine response. The macrophage like cell line RAW 264.7 was activated with 50 ng/ml LPS, or with 50 μg/ml poly I:C. It was found that the efficiency of the tested alcohols in decreasing the cells pathogen induced TNF production positively correlates with their carbon chain length. Interestingly, the effect of isopropanol was in between the effects of ethanol and 1-propanol. The mechanism behind these phenomena will be subject to further evaluation. To determine whether the effects of ethanol on TNF production is mediated at the post-transcriptional rather than the transcriptional level, RAW 264.7 cells were treated with the translation inhibitor cycloheximide together with LPS or poly I:C challenge. Cells were treated with 5 μg/ml cycloheximide, and at the same time point with 50 ng/ml LPS, or 50 μg/ml poly I:C. The TNF response completely vanished. Even when applied 20 min after challenge with LPS or poly I:C, cycloheximide almost completely abrogated the TNF response. IFN protein had been stored in the cell, this would not have occurred. This work was funded by grant AA009505 from NIAAA.

1293 5D-PGJ2-G, A PUTATIVE METABOLITE OF 2-ARACHIDONYL GLYCEROL, ACTIVATES PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR γ (PPARγ) IN M1 MACROPHAGES.

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Previous published results from our laboratory demonstrated that 2-arachidonyl glycerol (2-AG), an endocannabinoid, suppressed IL-2 secretion in activated T cells by the activation of peroxisome proliferator activated receptor (PPARγ) independent of the cannabinoid receptors, CB1 and CB2. 2-AG is structurally similar to arachidonic acid and a comparison of the eicosanoid pathway of arachidonic acid and 2-AG predicts that the potential metabolites of 2-AG are glycerol esters of various prostaglandins and thromboxanes produced from arachidonic acid. One of the putative metabolites of 2-AG is a glycerol ester of a prostaglandin, 15d-PGJ2-G. Treatment of Jurkat T cells with increasing concentrations of 15d-PGJ2-G followed by activation with phorbol ester and ionomycin demonstrated that 15d-PGJ2-G suppressed IL-2 secretion in activated T cells in a concentration-dependent manner. Since one of the known ligands for PPARγ is 15d-PGJ2-G, it was investigated if 15d-PGJ2-G served as a ligand for PPARγ. Co-transfection of HEK293T cells with PPARγ-LBD plasmid and pPR-hc reporter plasmid followed by treatment with 15d-PGJ2-G demonstrated that 15d-PGJ2-G transcriptionally activated PPARγ-LBD in a concentration-dependent manner. In addition, the increase in luciferase activity caused by increasing concentrations of 15d-PGJ2-G was inhibited in the presence of a PPARγ antagonist, T0070907. We hypothesize that 15d-PGJ2-G is a metabolite of 2-AG and is responsible for IL-2 suppression by acting in a CB1/CB2 independent manner through the activation of PPARγ. (Supported in part by NIH ROI DA12740)

1294 SHORT-TERM SODIUM TUNGSTATE EXPOSURE REDUCES THE QUANTITY OF CYTOTOXIC AND HELPER T-CELLS IN C57BL6 MICE AFTER IMMUNE CHALLENGE.

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Tungsten as the water-soluble tungstate (WO4-) has been identified as a water contaminant and can be absorbed by ingestion. In this study, we examined the effect of 28-day sodium tungstate (Na2WO4·2H2O) exposure in drinking water on murine leukocytes after immune challenge. Drinking water included weight adjusted tungstate concentrations of 0, 62.5, 125 and 200 mg/kg/day. Additionally, mice were given a specialized molybdenum free diet (0 ppm). After 28 days of tungstate exposure, and 24 hr prior to sacrifice, mice were given (ip) saline, lipopolysaccharide (LPS) (5 mg/kg), or Staphylococcus aureus enterotoxin B (SEB) (20 μg). Following sacrifice, spleen and blood were isolated cells and were surface stained with a panel of lymphocyte (CD3, CD4, CD8, CD19, CD71) and myeloid (CD11b, Ly-6C, Ly-6G, F4/80) markers. Flow cytometry revealed that cytotoxic T-cells (CD3+CD8+) and helper T-cells (CD3+CD4+) from spleens had a dose dependent reduction in activation/proliferation as measured by CD71 positivity. In SEB treated mice the CD71 positive cytotoxic T-cells in 0 and 200 mg/kg/d treatment groups were 12.87% ± 2.05% SE vs 4.44% ± 1.42% (P<0.001) respectively. Helper T-cells were reduced 5.85% ± 1.23 vs. 2.76% ± 0.51 (P<0.001) respectively. There was also a trend toward reduced numbers of CD71 positive NK cells (CD3+CD16+1cells) in LPS treated mice. The effects of tungstate were only observed in the spleen and not blood. Myeloid cells appeared not to be affected by tungstate exposure. Reduced numbers of activated/proliferating cytotoxic and helper T-cells in response to challenge may result in susceptibility to infections. These data suggest that tungstate exposure may have a detrimental effect on the adaptive immune response.

1295 ACTIVATION OF THE TRANSCRIPTION FACTOR, NUCLEAR FACTOR ERYTHROID 2-RELATED FACTOR 2 (NRF2), INHIBITS IFN GAMMA PRODUCTION BY MURINE T CELLS.

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Nrf2 is a transcription factor that upregulates numerous cytoprotective genes in response to electrophilic and oxidative stimuli. Although best characterized for its role in the regulation of detoxification and antioxidative genes, Nrf2 also plays an anti-inflammatory role in macrophages and other myeloid cells. The purpose of the present studies was to determine the expression and function of Nrf2 in murine murine T cells. Nrf2 mRNA is detectable in mature CD3+ T cells and is upregulated by the Nrf2 activator, tert-butyldihydroquinone (tBHQ). In addition, tBHQ induces expression of the Nrf2 target genes, Ho-1, Nqo1, and Gclc. Moreover, the current studies functional in CD3+ T cells as evidenced by the Nrf2-dependent upregulation of IFN-γ upon IFN-γ transfection, which is likely due in part to decreased binding to the AP-1 and NFκB response elements in wild-type CD3+ cells, but only modestly inhibits AP-1/NFκB transfection in CD3+ T cells (NRF2), INHIBITS IFN GAMMA PRODUCTION BY MURINE T CELLS.
TRICLOSAN GLUCONURIDATION AND SULFONATION IN CHANNEL CATFISH LIVER FRACTIONS.

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The personal care product and antibacterial agent Triclosan has been found in wastewater and sediments from sewage treatment plants. As a lipophilic compound with log P of >4, Triclosan would be expected to bioaccumulate in exposed fish. However, the phenolic hydroxyl group in Triclosan’s structure suggests the possibility that this substance will be glucuronidated or sulfonated, resulting in reduced bioaccumulation. We therefore examined microsomal glucuronidation and cytoxic sulfonation of Triclosan in channel catfish liver using 14C-UDPGA or 35S-PAPS to follow the conjugation reactions. Subcellular fractions were prepared from livers of five control fish and assays were conducted at 20°C. To obtain kinetic parameters for each pathway, linear conditions for product formation were established with respect to protein concentration and incubation time, then incubations were conducted with varying concentrations of triclosan and saturating concentrations of UDPGA or PAPS. Reaction rates were fit to the Michaelis-Menten equation or the equation for substrate inhibition, as appropriate. Glucuronidation followed classic Michaelis-Menten kinetics: the Km for hepatic glucuronidation was 41 ± 15 μM (mean ± S.D., n = 5 samples) and Vmax was 968 ± 385 pmol/min/mg protein. Sulfonation exhibited substrate inhibition at triclosan concentrations above 10 μM. The Km was 3 ± 1 μM Vmax was 831 ± 190 pmol/min/mg protein and Ki was 86 ± 48 μM. Compared with other halogenated phenolic xenobiotics, Triclosan was efficiently sulfonated and glucuronidated in catfish liver, suggesting this compound will be rapidly eliminated from exposed fish.

ENDOCRINE DISRUPTION IN A NORTHERN CALIFORNIA WATERSHED: THE RESPONSE OF A RESIDENT FISH SPECIES.

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Endocrine disrupting compounds (EDCs) are widespread in the environment and are known to damage the reproductive systems of fishes. Deleterious effects of EDCs include the production of female reproductive proteins and malformed or tumorous testes in adult male fish, reduced egg production in adult females, and altered mating behavior in both sexes. Recent findings suggest that these effects may extend beyond individual fish to influence population declines. Research has also shown that water samples from a number of locations in and near the San Francisco Bay (SF) / Sacramento-San Joaquin (SSJ) watershed in Northern California produce estrogenic responses in adult male rainbow trout. However, rainbow trout are not found throughout this entire estuary and thus may not be representative of the many threatened and endangered native estuarine species that are found across a range of salinities in this watershed. To address this gap in knowledge, our work utilizes a ubiquitous euryhaline resident fish species (Midasichthys berylina) as an indicator of exposure to reproductive contaminants. Investigations include determining whether specific sites within the SF Bay / SSJ Delta estuary that differ in contaminant inputs (treated wastewater effluent and/or pyrethroid pesticide run-off) cause male M. berylina to express chorionin, an egg coat protein normally found only in females that is a well-established biomarker of endocrine disruption. Initial work focused on commercially available salmonid antibodies cross-react with M. berylina chorionin, permitting rapid assessment of exposure and its effects on plasma samples. Initial sampling will establish a pattern of EDC distribution at these sites and will be followed by confirmatory laboratory exposures to environmentally relevant concentrations of specific pyrethroid pesticides (e.g., the wastewater effluent component ethylendeitradial).
increases in flight times from pesticide exposure. Blood samples were collected and plasma ChE activity was measured over time following exposure to either chlorpyrifos at 1.0, 2.5, 5.0, and 7.5 mg/kg or aldicarb at 0.1, 0.25, 0.5, and 1.0 mg/kg. The results indicated that chlorpyrifos dosed birds exhibited a dose dependent decrease in activity at the initial time measurements after dosing with full recovery at all dosage levels by 48 hours. All aldicarb dosage groups showed extreme plasma ChE depression at the first post-dose measurement (1 hour) with full recovery by 24 hours. The time of peak inhibition would correlate with migratory flight activity after exposure to a contaminated food or water source. In total, the results of these studies show that low-dose exposure to carbamate and organophosphate pesticides can be detrimental to the flying ability of non-target avian species. These studies were funded in part by Nevada Agricultural Experiment Station Hatch Grant 0731.

**1301 HYPOXIA ACCELERATES TOXICITY OF AN ARRAY OF PAHs AND PAH MIXTURES.**

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Previous work in our laboratory revealed that hypoxia interacts with the polycyclic aromatic hydrocarbon (PAH) fluoranthene (FL) and the model PAH Naphthalene (NP) to induce pericardial edema and, in the case of FL, spinal deformities. This study examined whether PAH benz(a)pyrene was found not to induce overt pericardial edema. Additionally, interactions of PAHs with various thyroid hormones were examined. Embryos exposed to FL, NP, and PAH benz(a)pyrene were induced to hyperthyroidism (T3/T4) and hyperactivity. PAH benz(a)pyrene was also shown to induce pericardial edema and, in the case of FL, spinal deformities in the presence of hypoxia.

**1302 DEVELOPMENTAL AND GROWTH EFFECTS ASSOCIATED WITH ACUTE EXPOSURE OF EGGS TO BISPHELOAL A IN RAINBOW TROUT.**

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Effect of maternal exposure to xenosterogens on embryonic development and growth in fish is poorly understood. The objective of this study was to determine the effect of maternal exposure to Bisphenol A (BPA), a xenosterogen, on development and growth in rainbow trout (Oncorhynchus mykiss). Trout oocytes were exposed to 0, 30 and 100 micro grams/ml of BPA for 3 h in the ovarian fluid to mimic in utero exposure, and this was followed by fertilization. The BPA levels in the embryos showed a declining trend after exposure and was completely eliminated by 24 and 35 h post-fertilization in low and high BPA groups, respectively. Despite the elimination of the contaminant during embryogenesis, high BPA treatment delayed hatching, yolk absorption, time to first feed and growth rate compared to the other two groups. This BPA-treated developmental abnormality correlated with lower serum-like growth factor (IGF)-2 but not IGF-1 mRNA levels. The deleterious effects on growth rate post-hatching in high BPA group persisted for a longer time (400 days) and corresponded with a suppressed IGF-1, IGF-1 receptor and growth hormone receptor mRNA abundances. Also, juvenile fish from the BPA exposed group showed an abnormal cortisol and glucose response to an acute stressor exposure. Taken together, the results demonstrate that maternal exposure to BPA affects development and leads to growth and performance defects that persist even in juvenile trout.

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**1303 INFLUENCE OF ORGANOCHLORINE PESTICIDES ON STEROID HOMEOSTASIS AND REPRODUCTIVE INDICES IN LARGEMOUTH BASS (MICROPTerus SALMOIDES).**

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Organochlorine pesticide (OCP) exposure has been linked to altered plasma sex hormone levels and poor reproductive performance in largemouth bass (LMB). Possible mechanisms for disruption of hormone homeostasis include direct effects on gonadal steroid synthesis or altered hormone metabolism. In this study, effects of in vivo and ex vivo OCP exposure on ovarian steroidogenesis were investigated. Incubation of ovarian tissue with the polychlorinated biphenyl (PCB) toxaphene (TOX) decreased basal and human chorionic gonadotropin (hCG)-stimulated estradiol (E2) and testosterone (T) synthesis by up to 46 and 92%, respectively. Incubation with toxaphene (TOX) had no effect on basal E2 or T synthesis, but reduced hCG-stimulated synthesis by up to 65 and 74%, respectively. To investigate the influence of in vivo exposure on OCPs on gonadal steroidogenesis, E2 production was measured in ovarian explants from LMB after 2 months in a mesocosm containing OCPs. Basal and hCG-stimulated E2 production by control explants was 3.1 ± 0.4 and 4.3 ± 0.6 pg/mg tissue, respectively. Similar production was observed in tissue from exposed LMB (3.3 ± 0.3 and 4.8 ± 0.5 pg/mg, respectively). However, gonadal somatic index (GSI) was significantly lower in exposed LMB (38% of control). In further studies, LMB were administered TOX, MXC, p,p’-DDE or dieldrin in the diet for 2 months. While a reduction in ovarian E2 synthesis was observed only in LMB exposed to ethylestradiol (EE2, positive estrogenic control), curtailed GSIs were induced in females exposed to EE2 and TOX (25 and 53% of control) and p,p’-DDE exposure increased hepatosomatic index vs all other groups. Exposure to p,p’-DDE or dieldrin had no effect on hepatic estrogen sulfotransferase activity. These findings suggest that OCPs are capable of perturbing gonadal steroidogenesis directly, however compensatory mechanisms may ameliorate this effect when exposure occurs in vivo. These studies were supported by NIH RO1ES015449.

**1304 IMPACT OF THIAMETHOXAM ON GLYCOLYTIC AND GLUCOCENZIMES OF A FRESH WATER FISH, CHANNA PUNCTATUS.**


Thiamethoxam (TMX) is the first commercial neonicotinoid insecticide and is widely used both for foliar/soil applications. It provides excellent control of a broad range of commercially important pests. The recent studies reported that TMX is hepatotoxic and hepatocarcinogenic and induce tumors in mice liver. The present study investigated in vivo toxic effects of TMX on glucose metabolism in freshwater edible fish, Channa punctatus. The fish were exposed to sublethal concentration (38.26 ppm) of TMX for different time period (24, 48, 72 and 96 hours) to assess the levels of glycogen, glucose, pyruvate and lactate and specific activities of selected enzymes of glucose metabolism in liver, brain, gill, muscle and kidney tissues. The data show a decrease in glycogen and pyruvate levels and an increase in glucose and lactate levels. The specific activities of phosphoglucomutase (PGM), phosphofructokinase (PFK), glucose-6-phosphate dehydrogenase (G6PDH), pyruvate carboxylase (PC) and PEP carboxykinase (PEPC) were significantly increased while lactate dehydrogenase (LDH), isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH), and malate dehydrogenase (MDH) exhibited a decrease as consequence exposure to TMX. The data suggest that TMX enhanced the mobilization of glycogen through increased breakdown of glycogen and reduced the oxidation of glucose to meet the increased energy demands during induced toxic stress condition. The altered enzyme activities could be attributed to the differential effect of TMX resulting in the mobilization of glucose metabolism in tissues of fish. Ch. V. Purtushoth Reddy and C. Janahal Department of Biochemistry, Chaitanya Postgraduate College (Autonomous) Kishanpura, Hanamkonda-506 001 (A.P), INDIA.
Physiologically-based pharmacokinetic (PBPK) models are part of a broader continuum of increasingly data-informed approaches to dose response analysis ranging from default based on external dose to more biologically-realistic models. By facilitating the incorporation of dose measures of relevance to the mode of action of chemicals, and quantitative physiological scaling taking into account relevant chemical-specific physical chemical properties and biological constants, PBPK models provide a representation of biologically effective dose as a basis for conducting more informed extrapolations across studies, species, routes, and dose levels. Resultingly, they increase precision and reduce uncertainty in risk estimates. Despite the availability of PBPK models for a number of chemicals incorporating significant additional biological data over default and the potential of such models to contribute more broadly to the development of additionally informative testing strategies, their adoption in regulatory risk assessment has been limited. This limited uptake is being addressed in a project undertaken as part of the World Health Organization/International Programme on Chemical Safety project on harmonization. The initiative includes preparation of guidance and case studies on the characterization, documentation, evaluation, and communication of PBPK models for risk assessment. Aspects being addressed include the need for early and continuing communication between risk assessors and modellers, greater consistency in consideration of mode-of-action as a basis for relevant PBPK models and sufficiently transparent documentation of model development to support potential application. More consistent and transparent consideration of the basis for and output of PBPK models relative to default approaches in risk assessment is also being addressed.

Metabolomics is a demanding challenge, for which sophisticated analytical methods (e.g. GC/MS and LC/MS) are required. Currently, we hold mass spectra, retention indices, structures and links to external metabolic databases for a large number of compounds which are routinely screened by gas chromatography – time of flight mass spectrometry. In addition we develop and apply metabolomic and metabolic fingerprinting methods by high resolution LC/MS and by direct infusion – mass spectrometry. Metabolomics applications follow a two-tiered approach: they can be used for sample discrimination and classification (e.g. for clinical diagnostics or GMO substantial equivalence), or for biochemical and mechanistic studies (e.g. for understanding the onset and progression of human diseases, or for detailing regulatory modules in cells or subcellular compartments). Therefore, we use several biological models on organismal, tissue or cellular levels. Current applications include the linking of metabolic aberrations in fetal, newborn and young adult rat lungs to developmental effects. These biomarkers provide a consistent metric with which to measure safety through nonclinical and clinical development. This session will outline how Safety First will be implemented and how novel translational safety biomarkers are needed for its success.
disease. In addition to examining endogenous molecular changes in response to
stimuli, metabolomics can equally be applied to the examination of xenobiotic
metabolites, the footprints of cellular metabolism that are left on compounds for-
cognate to living systems. This presentation will discuss the application of UPLC-ESI
QTOFMS based metabolomics protocols to the reexamination of the metabolism
of Aminoflavone, areca alkaloids, PhEur, melatonin and acetaminophen.

S 1311 METABONOMICS IN PHARMACEUTICAL DISCOVERY
AND DEVELOPMENT.
D. Robertson, Applied and Investigative Metabonomics, Bristol-Myers Squibb,
Princeton, NJ.
Metabonomics has emerged as a key technology in pharmaceutical discovery and
development, evolving as the small molecule counterpart of transcriptomics and
proteomics. A critical component of any toxicology study is a thorough characteri-
dzation of the experimental models employed in our investigations and the bio-
chemical ramifications of the interaction of the employed model with the com-
 pound under study. Metabonomics engenders such understanding at a much
broader level than traditional toxicity endpoints. This presentation will focus on
recent developments in the pharmaceutical application of metabolomics technol-
ogy emphasizing its use in model characterization, mechanistic toxicology and bio-
marker discovery.

S 1312 METABONOMICS AND TRANSCRIPTOMICS: A
SYNERGISTIC APPROACH TO BIOMARKERS AND
MECHANISMS OF TOXICITY.
J. Lehman-McKeeman, Bristol-Myers Squibb, Princeton, NJ.
Metabonomic profiles can provide important leads for identifying key events un-
derlying toxicologic responses. Furthermore, as a likely phenotypic consequence of
altered gene expression, metabolic changes are important complementary data
for determining the systemic relevance or significance of induction or repression
of specific gene pathways. Although many investigations have utilized either metabo-
nomic or transcriptomic data to evaluate adverse effects or develop mechanistic hy-
potheses, the integration of both types of data has not been routinely accomplished.
Efforts to integrate these platforms indicate that each can inform the other and aug-
ment the identification of the major systemic effects of a xenobiotic. For example,
agents such as phenobarbital and dallysulfide, which induce numerous xenobiotic
metabolizing enzymes through activation of the constitutive androstane receptor
(CAR), affect hepatic transcriptional changes in rats of a wide variety of recognized
CAR-dependent genes. However, the urinary metabolomic profile indicated that
the major change in endogenous intermediates was a marked increase in the excre-
tion of glycine, a precursor in ascorbate biosynthesis. With evidence for altera-
tions in Vitamin C metabolism, the transcriptional profiles were re-evaluated, and
dose- and time-dependent transcriptional changes in enzymes regulating ascorbic
acid biosynthesis and utilization were identified. Although these transcriptional
changes did not represent those that changed to greatest extent, the urinary
metabonomic data confirm that they were the most significant with respect to sys-
temic effects on intermediary biochemistry. Similar analyses integrating transcrip-
tional profiles from skeletal muscle with metabolites in urine or tissue extracts have
helped to identify to possible biomarkers of skeletal muscle toxicity. Concerted ef-
forts to exploit the relationship between metabolomic and transcriptional profiling
data should be pursued in order to advance the use of these technologies for deter-
mining mechanisms of toxicity and human safety evaluation.

S 1313 INCORPORATING ‘OMICs IN THE STUDY OF
REPRODUCTION AND DEVELOPMENT.
S. J. Sumner and T. B. Knudsen, Metabonomics/Biomarkers, RTI International,
Research Triangle Park, NC, and National Center for Computational Toxicology, U.S.
Environmental Protection Agency, Research Triangle Park, NC.
In recent years, ground breaking research in genomic applications in the area of re-
productive and developmental toxicology have been successful in linking changes in
the expression of specific genes and their higher-level biological processes to ef-
fcts induced by drugs or chemicals in developing tissues. While gene expression
profiling has demonstrated the ability to provide mechanistic insight into the cellular
mechanisms of drug and chemical-induced effects, proteomics provides advan-
tages in areas beyond the genome. For example, post-translation modifications of
proteins that are known to be involved in cell-cell signaling cascades for develop-
mental pathways, the flux-balancing in signaling molecules themselves, or the me-
bolistic intermediates connecting to these pathways are important parts of our ability
to understand the pathogenesis of fetal malformations. These higher-level opera-
tions can be inferred, but not directly evaluated through measurement of mRNA or
dNA sequencing. In recent years, the application of proteomics in the study of re-
production and development has rapidly increased, while such studies that incor-
porate metabolomics approaches are at their infancy. A summary of the recent ad-
vances in genomic, proteomic, and metabolomic methodologies that demonstrate
the successful use of these technologies in the study of reproduction and develop-
ment will be provided. Finally, an illustration of how these data may be integrated
by multi-scale models of dynamical systems will be highlighted that can serve to
improve our understanding of reproductive and developmental toxicities.

S 1314 METABONOMICS IN THE STUDY OF REPRODUCTION
AND DEVELOPMENT.
Metabonomics/Biomarkers, RTI International, Research Triangle Park, NC.
Our group conducted studies in animal models to demonstrate the use of
metabolomics to draw correlations between early life exposures and reproductive or
developmental outcomes. Male and female CD rats were fed AIN76A (devoid of
genistein) or AIN76A with genistein added (250 or 500 ppm) through breeding.
Female rats were maintained on these diets through gestation (gd), lactation, and
weaning (postnatal day, pnd, 21). Offspring were maintained on the mothers’ diet
following weaning. Metabolomics of endogenous compounds in urine could differ-
entiate dams that received the three diets, and could differentiate the pups based
on gender, diet, and pre- or post-pubertal age. Studies were also conducted in pregnant
CD rats exposed daily during gestation (gd 14-20) to vehicle and effect or no effect
levels of butyl benzyl phthalate (BBP) and phenotypic anchor (e.g., anatomical
distance, retained nipples) were measured for pups during development. Metabolomics of
urine collected from dams (gd 18, pnd 21) and from pups (pnd 25) exhibited differences in levels of endogenous metabolites in urine based on age, gender, dose, and phenotypic anchor. In general, urine from BBP exposed dams
collected on gd 18 showed an increase (relative to controls) in Kreb’s cycle metabo-
lites, tyrosine, and tryptophan. Urine from BBP exposed dams on pnd 21 showed an
increase in nicotinamide, and decrease in trigonelline. Female pups born to
dams receiving BBP had increased levels of methyl nicotinamide, and decreased lev-
els of quinoline, tryptophan, and glutamate. Male pups born to BBP exposed
dams, showed a decrease in quinoline, tryptophan, and glutamate, but did not
show an increase in methyl nicotinamide. Urine from male (but not female) pups
had an increase in Kreb’s Cycle metabolites for both dose groups. Alanine,cynidine,
and aminolevulinate were decreased (relative to controls) for the male pups born to
BBP-exposed dams. At necropsy, the offspring males (but not the females) at the high
BBP dose exhibited reproductive tract malformations, as anticipated. This research
was supported by RTI International.

S 1315 APPLICATION OF TRANSCRIPTOMICS TO ASSESS
CHEMICALS WITH ESTROGENIC ACTIVITY.
J. M. Paciff and G. P. Daston, Procter & Gamble Company, Cincinnati, OH.
There is a growing concern about the potential adverse health effects to humans
and wildlife resulting from exposure to chemicals with the ability to interfere with
the normal function of the endocrine system. These chemicals have been referred to
as “endocrine disrupters”, of which the most abundant are chemicals with estro-
genic activity. Since these chemicals may interfere with the production or activity
of hormones of the endocrine system, and have the potential to affect multiple cellu-
lar pathways, the use of transcriptomics (global analysis of gene expression) for their
categorization offers a great opportunity to significantly improve the process of
assessing their safety and risk. We have used this approach with model chemicals, to
identify molecular fingerprints that are indicative of estrogenicity, both in females
as well as in the males. These molecular fingerprints show clear similarities in this
response between females and males, but also demonstrate gender-specificity of the
estrogenic responses. We have also determined that the transcriptional program
elicited by exposure to chemicals with estrogenic activity, at equipotent doses,
clearly indicate differences in the response of an organism at different life stages
(fetal vs. juvenile), can be detected very early on during the exposure phase (hours
vs. days), and is more sensitive than traditional assays (i.e. the uterotrophic assay).
Further, we have determined the genomic response of an estrogen-sensitive cell line
(Ishikawa cells) to estrogen exposure in a time and dose dependent manner.
Comparing the estrogenic response of this in vitro system versus the in vivo re-
response (human vs. rat), we determined that the Ishikawa cells are capable of
mounting a proper estrogenic response and they offer a robust in vitro system to
evaluate the potential estrogenicity of chemicals of interest. By determining quanti-
tative relationships between changes in gene expression and manifestations of estro-
geticity, our data clearly supports the use of transcript profiling as an endpoint in
quantitative assessments of estrogenicity for any given chemical.
**S 1316 IDENTIFYING MOLECULAR MECHANISMS OF GENE EXPRESSION IN MAMMALIAN GAMETES AND EMBRYOS USING FUNCTIONAL GENOMICS APPROACHES.**

E. Memili, Animal and Dairy Sciences, Mississippi State University, Mississippi State, MS. Sponsor: R. Carr.

Spermatogenesis and oocyte are produced through gametogenesis, and the union of these two haploid gametes at the fertilization stage sets the stage for the fascinating event of mammalian development. Gametogenesis and embryogenesis are both developmentally regulated through the synthesis and release of the initial set of transcripts and proteins. While mostly maternal RNAs and proteins support early embryogenesis, synthesis and proteins are synthesized soon after fertilization, a critical event known as Embryonic Genome Activation. Environmental toxicants, stress factors, and in vivo culture conditions are all known to affect the gamete and embryonic development, and fertility. Molecular dynamics of both transcriptome and proteome can be analyzed using high-throughput methods of transcriptomics and proteomics, generating hypothesis for mechanisms of gene regulation. This presentation will review the advances in functional genomics and its applications in identifying genome-wide analyses of bovine gametes and embryos, a review of applications in other mammalian species, and provide future prospects.

**S 1317 VIRTUAL TISSUE MODELS IN DEVELOPMENTAL TOXICITY RESEARCH.**

T. B. Knudsen1, I. Shah1, M. R. Rountree1, A. V. Singh2 and B. L. Kayeck1.


Prenatal exposure to drugs and chemicals may perturb, directly or indirectly, core developmental processes in the embryo (patterning, morphogenesis, proliferation, and apoptosis, and cell differentiation), leading to adverse developmental outcomes. Because embryogenesis entails a genomic program that orchestrates aggre-gate behavior across time and space, a challenge for systems biology is to integrate data and knowledge from the genomic sciences into multi-scale models of development that can be translated into a regulatory context. Computational models of embryonic systems can be used to understand how the core developmental processes are wired together to give rise to specific regulatory networks (GRNs) and cellular reaction networks (CRNs), and to progressively unravel the changes in these complex systems caused by toxicant exposure. The virtual embryo project (v-Embryo™) at the US EPA aims to build, over the long-term, a working computer model of an embryo through knowledgebase and simulation of toxicity pathways that are important for development (http://www.epa.gov/ncc5/v-Embryo). In the short-term, the technology focuses on specific virtual tissue prototypes to formulate hypotheses about how mechanisms at one scale (molecular) interact to produce higher level (tissue) phenomena, thus guiding research to build quantitative dose-response models that connect to the dynamics of the subnetworks GRNs and CRNs. The v-Embryo™ signs with high-throughput screening assays and systems biology research initiatives at the National Center for Computational Toxicology (NCCT), including ACToR, ToxCast™, and the Virtual Liver. (This work has been reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy).

**S 1318 INTERACTOMES AND THEIR APPLICATION IN TOXICOLOGY.**

L. N. Meyers, NSOE, Duke University, Durham, NC.

A major challenge in the analysis of microarray data in toxicology and other fields is taking full advantage of the large and often very complex datasets that are obtained. A variety of tools are available for such analysis. The use of interactomes as a particularly powerful systems biology tool applicable to the analysis of the transcriptomic response to toxicant exposure will be highlighted. Interactomes are networks of protein-protein, protein-DNA, and other interactions that occur in organisms. They are derived both from careful curation of decades of biological research on such interactions, and via high-throughput assays designed to detect such interactions (e.g., yeast two-hybrid screens). By overlaying gene expression data on these interactomes, it is possible to analyze large, complex microarray datasets in a statistically robust and biologically meaningful fashion. Our panel of experts, who are foremost researchers in toxicological research utilizing interactomes, will discuss both the important toxicological findings and applications of this cutting-edge technology.

**S 1319 SYSTEMS BASED APPROACHES FOR THE IDENTIFICATION OF CELLULAR RESPONSES TO TOXICANTS.**

J. Begley1, R. Cunningham1, A. George1, J. Rooney1, F. Joseph1 and A. Patil1.

Biomedical Sciences, University of Albany, SUNY, Rensselaer, NY and 2Biological Sciences, University at Albany, Albany, NY.

The toxic effects of chemical exposures can be dramatically influenced by the collection and coordination of cellular proteins and pathways. There are over 30,000 gene products found in human cells, thus allowing cells to initiate complicated protein-based responses to toxicants. Fast and cost effective approaches are therefore needed to mechanistically analyze the contribution that specific gene-products and the collection of gene-products (i.e. networks) make towards toxicity modulation. We have developed high throughput screening approaches using gene-deletion libraries from bacteria and yeast to identify conserved responses to DNA damaging agents. We have furthered our high-throughput output to computational mapping and homology based approaches, to identify functional pathways and protein networks that respond to damage, and to identify novel human proteins that coordinate translational responses to damage. Our high-throughput and computational pipeline is amenable for mass screening of drug libraries and the resulting data has great potential for both toxicity prediction and mechanistic studies.

**S 1320 PATHWAY MAPPING OF CHEMICAL-PERTURBED REGULATORY NETWORKS.**

J. Ruvin, Environmental Sciences and Engineering, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Signaling networks are altered, either permanently or transiently, in response to chemical exposure. Such alterations may occur on various scales and the activation of nuclear receptors and downstream networks and pathways is a good example of the complex responses to chemical exposure. In common among most nuclear receptors is that they transcriptionally regulate a number of proteins involved in xenobiotic metabolism and that their ligands are frequently causing liver cancer in rodents through non-genotoxic mechanisms. The genome-wide view of gene expression that microarrays offer is a suitable research paradigm to unravel the paucity of events evoked by activation of the nuclear receptors. Pathway mapping is now used frequently to provide more biological relevance to the analyses. This presentation will illustrate the utility of pathway-based interpretation of the genomics data in mechanistic toxicology by considering time- and dose-dependent effects of activators of PPARalpha and CAR. Key nodes responsible for the effects of these agents in rodent liver, the temporal shift in responses, and the concordance/differences between agents may be easily observed when networks, rather than individual genes, are compared.

**S 1321 IDENTIFICATION OF NEW BIOLOGICAL PATHWAYS AFFECTED BY TRANSITION METALS.**

J. H. Freedman, Laboratory of Molecular Toxicology, NIEHS, NIH, Research Triangle Park, NC.

Exposure to transition metals and metalloids (copper, cadmium, mercury, copper, zinc, silver, and arsenic) is correlated with the progression of a variety of human pathologies ranging from cancer to cognitive dysfunction. To avoid metal-induced cytotoxicity, sophisticated mechanisms for regulating the intracellular concentrations of metals and repairing metal-induced damage have evolved. Microarray studies have shown that metals affect the transcription of hundreds of genes that apparently have unrelated functions. We applied multi-species (yeast, C. elegans, mouse, and human) comparative genomics to identify novel pathways that are responsible for the activation or repression of transcription. Gene Ontology analyses of microarray data groups differentially expressed genes in to functional categories. Applying gene expression data to previously defined pathways and interaction networks using CytoScape, Ingenuity Pathway Analysis and GenMAPP, however, allows for the identification of metal responsive signal transduction pathways. Using this approach, microarray data from metal-treated C. elegans, yeast, HepG2 cells, and mice has been analyzed. In addition to the previously characterized metal responsive signal transduction pathways: NRF2, MAPK, NF-κB, new pathways have been identified. These pathways include those regulated by retinoic acid, calcium, HNF4α, and cAMP-dependent protein kinase. The consequence of inhibiting the expression of genes in these pathways using gene deletions or RNA interference technologies has provided insights into the role of metals in intermediate metabolism, translation, as well as development.
**S 1322** IDENTIFYING MOLECULAR PATHWAYS THAT MODULATE ENVIRONMENTAL AND GENETIC COMPONENTS OF COMPLEX DISEASES.

J. M. Gehle1, R. Thomas1, Y. Zhang5, M. C. Rosenstreet1, A. P. Davis1, C. Murphy1, L. L. Martin1, K. G. Becker3 and C. J. Portier1. Laboratory of Molecular Toxicology, NIEHS, Research Triangle Park, NC. Gene Expression and Genomics Unit, NIA, Baltimore, MD and Department of Bioinformatics, MDIBL, Salford, ME.

Identifying relationships between phenotype, genotype, and environmental factors using traditional experimental approaches is particularly challenging. Using novel bioinformatics approaches to synthesize information from genetic association studies and mechanistic environmental health research, we have developed an efficient method for the identification of molecular pathways associated with both genetic and environmental contributions to several complex diseases. Consistent with other work using protein interaction datasets, we have found signal transduction pathways versus highly conserved metabolic pathways are more often linked to disease phenotypes. For example, PPAR and adipocytokine signaling define a metabolic syndrome subnetwork and identify natural and synthetic retinoids, antiprosthetic medications, Omega 3 fatty acids, and a bisphenol A derivative as potential environmental modulators. To validate our predictions using independent datasets and methodology, we define transcription factor regulation from gene expression datasets for several environmental factors and phenotypes identified in the metabolic syndrome subnetwork. Through utilization of pathways as the unifying concept for linking current knowledge on environmental factors, genes and disease we have identified apoptosis, p53 signaling, PPAR signaling and steroid metabolism as key pathways associated with complex diseases having strong environmental components. Further validation of hypotheses generated will define disease associated targets with both environmental and genetic support, allowing for efficient screening of environmental factors, screening that could set priorities for further research and guide public health decisions.

**S 1323** IRON TOXICITY IN THE MITOCHONDRIA: UNDERSTANDING NETWORK RESPONSES IN FRIEDREICH'S ATAXIA.

B. Van Houten. Department of Pharmacology and Chemical Biology, Hillman Cancer Center, University of Pittsburgh, Pittsburgh, PA.

Frataxin, a conserved mitochondrial protein involved in iron homeostasis, is reduced in patients with Friedreich's ataxia (FRDA) due to a triplet repeat expansion in the first intron. Transcript profiling and DNA damage assays were performed on blood cells from FRDA patients. Altered expression patterns identified using gene set analysis. GOminER, DAVID, Ingenuity Pathway Analysis, included: immune response; signaling pathways; oxidative phosphorylation, proteins synthesis, transcription; apoptosis; and genotoxic stress response pathways. FRDA patients had significantly more mitochondrial and nuclear DNA damage than a control population. Frataxin mRNA levels correlated with age of onset and displayed unique sets of gene alterations involved in oxidative phosphorylation and protein synthesis. Thus analysis of blood in FRDA patients yields insight into the nature and progression of the disease.

**S 1324** TRANSCRIPTIONAL CHANGES IN IMMUNOTOXICOLOGY: TRANSCRIPTION FACTORS, SIGNAL TRANSDUCTION, AND EPIMETGENICS.

N. Kerkvliet1 and K. Nohr1. Environmental & Molecular Toxicology, Oregon State University, Corvallis, OR and Environmental Health Sciences Division, National Institute for Environmental Studies, Tsukuba, Ibaraki 305-0013, Japan.

Numerous pioneering studies have taken advantage of innovative genomics technology to demonstrate that various chemicals cause toxic effects by inducing transcriptional changes. These changes are induced by the activation or inactivation of various transcription factors by the direct binding of chemicals or indirectly through modulation of signal transduction pathways. Epigenetics has been recognized as another pivotal mechanism to regulate transcription particularly in development and cellular differentiation that has been found in response to environmental factors. The latest studies are shedding light on the relationship between transcription factor functions and epigenetic alterations. Particular focus will be given to several transcription factors or signal transduction pathways which are highly expressed in immune cells and/or closely related to immune suppression, such as the NF-κB family, E2F, AhR and p53-activating ATM and ATR kinases, and also on the epigenetic regulation of immune reactions. Taking a broad as well as an up-close view of these up-to-date studies on how chemicals induce transcriptional changes and cause adverse effects in immune cells will advance our understanding of the characteristics of immunotoxicity and give insights into the mechanisms of transcription-mediated toxicity in general.

**S 1325** THE E2F FAMILY IS A SENSITIVE TARGET OF ARSENITE IN THE THYMUS: A CHARACTERISTIC DOWN-REGULATION OF E2F-RELATED GENES REVEALED BY IMMUNOTOXICOGENOMICS.

K. Nohr1, Environmental Health Sciences Division, National Institute for Environmental Studies, Tsukuba, Japan.

A variety of environmental chemicals are reported to induce thymus atrophy and cause adverse effects on immune system. We adopted a toxicogenomics approach to identify the pathways that mediate the atrophy induced by arsenite. C57BL/6 mice were administered arsenite at doses that reduce thymus weight by approximately 30%, and gene expression changes in the thymus 24 hours after the administration were analyzed using microarrays. The microarray analysis showed that arsenite down-regulates a variety of E2F target genes, including Ccnb2 and Ccn2, that are involved in cell cycle progression. These changes were not detected in the course of thymus atrophy by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or estradiol (E2). Cell cycle analyses of the thymocytes from arsenite-exposed mice showed arrest in the G1 phase. Among various tissues, expression of Ccnb2 and Ccn2 was found to be especially high in the thymus of the control mice and relatively high in their spleen, and expression of these genes in the thymus and spleen was significantly suppressed by arsenite exposure. Thus, the arsenic-induced down-regulation of E2F target genes is a characteristic feature of lymphoid cells. Our further studies suggest that a kinetics change in a pocket protein by arsenite is key to regulate the E2F function. The results of these studies illuminate a unique mechanism of arsenite insults to immune system.

**S 1326** ROLE OF P53 AND ATM/ATR IN DMBA-INDUCED IMMUNOTOXICITY.

S. W. Burchiel1, J. Gao1, L. Mitchell1 and F. Laurell1. College of Pharmacy, University of New Mexico, Albuquerque, NM and 2Los Alamos National Lab, Los Alamos, NM.

We have shown that 7,12-Dimethylbenz(a)anthracene (DMBA) immunoxicity is CYP1B1 and microsomal epoxide hydrolase (EPHX1) dependent in C57BL/6 mice, suggesting that DMBA-3,4-dihydrodiol-1,2-epoxide, a known genotoxic agent, is responsible for immune suppression. ATM/ATR and p53 pathways are sensors of genotoxicity. In the present studies, we found that p53 was activated (phosphorylated) in WT mice. WT mice were immunosuppressed by DMBA, whereas p53 null mice were resistant. ATM, ATR, and phospho-ATM levels were increased by DMBA in both WT and p53 null mice, but not in CYP1B1 null or EPHX1 null mice. Therefore, ATM and ATR appear to detect DMBA metabolite-induced genotoxicity producing p53 activation and immunosuppression.

**S 1327** NF-KB PLAYS A MAJOR ROLE IN THE MATURATION OF DENDRITIC CELLS INDUCED BY CHEMICAL SENSITIZERS.


Application of low-molecular-weight chemicals (hapten) on the skin may result in allergic contact hypersensitivity. During in vivo immune responses, the role of antigen-presenting cells is played primarily by dendritic cells (DC) acting as initiators of the immune response. Considering similarities between immunity to simple chemicals and that to infectious agents, it is reasonable to speculate that hapten itself stimulates DC maturation. The establishment of human in vitro models of dendritic cell (DC) allowed the possibility of demonstrating that haptenes able to directly activate cultured human DCs derived from peripheral blood monocytes or from human CD34+ hematopoietic progenitors. Indeed, DC phenotype was extensively modified after addition of NIS04 or DNBC (dinitrochlorobenzene) with up-regulation of co-stimulatory molecules (CD86,CD80,HLA-DR) and cytokines (IL-12p40,IL-6,IL-8). Signal transduction pathways such as NFkB and Mitogen-activated protein kinase (MAPK) have been described to play a major role in DC maturation in response to Toll-like receptor agonists. We and others have shown that both DNBC and NIS04 activated MAPKs but only NIS04 was able to activate NFkB. NIS04 induced the degradation of IκB-alpha and the binding of the p65 subunit of NFkB to DNA. Using a specific pharmacological inhibitor of NFki, we found that this pathway was central to the maturation of DC by regulating CD40, HLA-DR, CCR7, IL-8, IL-6 and IL-12p40 expression. These results suggested a causal relationship between activation of NFkB by chemical sensitizers and alteration of DC phenotype.
Despite mounting evidence that prenatal and early post-natal environmental exposures adversely impact immune function later in life, our understanding of how this occurs is limited. The aryl hydrocarbon receptor (AhR) is an important modulator of the development and function of the immune system; however, the mechanism by which AhR regulates the immune system remains poorly understood. We have obtained evidence that developmental exposure to the AhR-specific ligand TCDD affects epigenetically-regulated events important for generating a response to respiratory viral infection. Specifically, developmental exposure to TCDD causes long-lasting defects in the offspring’s response to influenza A virus infection, including reduced expansion and differentiation of virus-specific CD8+ T cells and production of the important antiviral cytokine interferon (IFN). These changes occurred as a result of developmental exposures that did not affect overall cellularity of immune organs. Although there were defects in the offspring’s immune response, the exposed dams did not exhibit impaired immune function. Also, impaired CD8+ T cell responses were inherent to bone marrow cells of the developmentally-exposed offspring, as defects could be transferred to unexposed mice. Epigenetic mechanisms such as DNA methylation and histone modifications regulate IFNγ expression and T cell clonal expansion, supporting the idea that AhR modulates programming of the immune system via epigenetic mechanisms, and point to known epigenetically-regulated pathways as targets. These findings are of considerable interest because poorer CD8+ T cell responsiveness and reduced IFNγ production correlate with an impaired ability to combat viral infection and to detect and destroy tumor cells. Moreover, although epigenetic mechanisms are known to regulate gene expression in immune cells, the idea that inappropriate AhR activation during development causes permanent functional changes in lymphocytes via epigenetic regulatory mechanisms is a novel paradigm. Supported by NIH grants K02-Es012409 and R21-Es013863.

**S 1328 IMMUNE PROGRAMMING BY THE ARYL HYDROCARBON RECEPTOR.
**

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**W 1331 WORKSHOP OVERVIEW.
**

M. J. Kallman, Investigative Toxicology, Eli Lilly & Company, Greenfield, IN.

A brief review of the latest regulations on pediatric clinical requirements and associated regulatory expectations for nonclinical support will be presented. Reasons for the increased interest in providing pediatric evaluations for drug approval will be considered.

**W 1332 THE POSTNATAL DEVELOPING BRAIN: A TARGET OF TOXICITY.
**

G. D. Cappon, Department of Safety Sciences, Pfizer Global Research and Development, Groton, CT.

To design nonclinical safety studies evaluating the potential effects on the brain, an understanding of CNS development is needed. Juvenile toxicity studies are designed case-by-case. The timing of dosing in the animal should reflect the intended pediatric population such that the phase of growth and development of the organ system to be evaluated are similar. This presentation will provide an overview of postnatal brain development and a high level comparison across species.

**W 1333 ENDOCRINE ENDPOINTS: INTERACTIONS DURING POSTNATAL CNS DEVELOPMENT.
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D. R. Mann. Department of Physiology, Morehouse School of Medicine, Atlanta, GA. Sponsor: M. Kallman.

The endocrine system undergoes tremendous development during adolescence. Not only is this system a potential target for toxicities during this period, but because it plays a substantial role in CNS development, alterations in normal endocrine development can have a profound impact on CNS function. This presentation will provide an overview of postnatal endocrine development, its influence on CNS development, and potential consequences that could confound interpretations of juvenile toxicity studies.

**W 1334 TESTS TO EVALUATE COGNITIVE AND EMOTIONAL FUNCTION IN RODENTS.
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C. V. Vorhees. Division of Neurology, Children’s Hospital Research Foundation, Cincinnati, OH. Sponsor: M. Kallman.

Much discussion has occurred regarding the need for more sensitive tests of learning and memory for safety assessment for pediatric use of a pharmaceutical and how these compare to those employed for pre- and postnatal development studies. Furthermore, many regulatory agencies have raised concerns for potential effects on emotional development. This presentation will consider advantages/disadvantages of currently available tests for learning and memory as well as endpoints considered to reflect emotional behavior in rodents.

**W 1335 INDUSTRY PERSPECTIVE: START WITH THE END IN MIND.
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Many considerations are needed when designing a juvenile toxicity study to address concerns regarding potential effects on the developing CNS. These include dosing period, endpoints to evaluate, dose levels, and species. Behavioral response may be affected by systemic toxicity, motivation, activity levels, and stress which are often related to dose levels and design specifics. This presentation will discuss how study designs can impact risk assessment.

**W 1336 HISTOLOGICAL CHANGES IN ANIMALS: IMPLICATIONS FOR USE IN PEDIATRIC PATIENT POPULATIONS.
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R. Mellon. Division of Anesthesiology, Analgesia, and Rheumatology Products, Food and Drug Administration, Silver Spring, MD. Sponsor: M. Kallman.

Animal studies suggest that some drugs, such as anesthetic agents, have the potential to produce widespread neuropathosis of the brain during what appears to be the synaptogenesis period of development. Several of these animal studies also sug-
gest that the histopathological changes may be associated with possible cognitive sequelae. These observations in animals have raised concerns regarding the use of these agents in pediatric patients. This presentation will provide a historical overview that outlines the challenges faced by FDA on this topic in determining the potential clinical significance.

1337 TOXICOLOGY OF UNINTENTIONAL AND INTENTIONAL DISASTERS.

A. J. Harris1 and M. Orlingher.1 CTEH, North Little Rock, AR, and Office of Emergency Management, U.S. Environmental Protection Agency, Cincinnati, OH. There is no greater challenge in toxicology than making decisions concerning public health under extreme circumstances with limited data. Yet, with the ever increasing risk of terrorist activities, the occasional accidental release of chemicals during transport and manufacture, and the unpredictable occurrence of natural disasters such as volcanic activity, hurricanes and wild fires; toxicologists from a wide range of governmental agencies and companies are faced with making decisions that affect the health of impacted communities. Such responses to emergency situations is where the rubber meets the road in putting toxicological principles into public health practice. To address these issues tropical disasters will be used to initiate discussion about the challenges faced during catastrophic events involving toxic agents in order to develop better strategies to address the toxicological impact on public health. We will consider the need for toxicological assessment and support during unintentional and intentional disasters with examples from recent events including hurricanes Katrina and Rita, the California wild fires, and the collapse of the World Trade Center. A brief roundtable discussion will follow to identify the idiosyncrasies and commonalities of dealing with unintentional and intentional disasters.

1338 INTRODUCTION.

M. Orlingher. Office of Emergency Management, U.S. Environmental Protection Agency, Cincinnati, OH.

Environmental and industrial accidents or natural disasters may result in discharges of toxic materials into the environment in a wide variety of settings. Such events may create conditions that do not always fall within the existing regulatory context, although it may be necessary to provide information to the public about exposure hazards and health risks. The topics and speakers in this workshop are meant to illustrate recent disasters, and prompt a discussion of the issues which toxicologists face after these events and how to plan for future responses.

1339 DHS ROLE AND RESPONSIBILITIES UNDER THE NEW NATIONAL RESPONSE FRAMEWORK.


The role of the Department of Homeland Security is becoming increasingly involved in a variety of disaster response scenarios, be they intentional acts of terrorism, natural disasters, or industrial accidents. Under the new National Response Framework (NRF), DHS will be integrated into a full spectrum of government response beginning at the local and state levels and possibly involving multiple federal agencies. Appointing experienced physicians with emergency medicine and/or medical toxicology board certifications to senior level posts at DHS, and elsewhere in the government, recognizes the value of having such depth of expertise both in planning for, as well as, in responding to, and coordinating, such events. This talk will discuss DHS’ planning and contemplated response activities across a range of potential toxicological events.

1340 PUBLIC IMPACT OF VOLCANIC ACTIVITY.

P. Godd. CTEH, North Little Rock, AR.

Volcanic activity can produce air quality impacts from volcanic ash as well as volcanic smog (a.k.a. vog), a phenomenon that produces high ambient air concentrations of the respiratory irritant sulfur dioxide. The impact of vog on air quality in communities near active volcanoes, such as large parts of the State of Hawai‘i, is a public health concern that can vary greatly both in magnitude and severity of resulting health problems. Because of variations in the occurrence and duration of vog releases as well as variation in meteorological conditions, health departments and other authorities often struggle to warn communities of vog impacts before they reach human receptors. Unfortunately in many cases, public health impacts cannot always be predicted because vog releases and resulting areas of impact cannot always be predicted accurately. Consequently, because of the size of the area of potential impact, more broad public impacts from volcanic ash and vog may result from catastrophic volcanic eruptions, even though these events can be more predictable. Despite the advance of technologies available to assess ambient air quality impacts in real time, the recent threat of eruption of Mt. St. Helens in Washington State presented new challenges to emergency responders tasked with monitoring ambient air quality in areas impacted by volcanic ash and vog. First and foremost is the challenge presented by the immense size of the area of potential impact of a large eruption with regard to proper placement and maintenance of air monitoring equipment and manpower. Second is the logistical challenge presented by the timely collection and transmission of real-time air quality data, often in rural and remote areas, for use by communities, government entities, and industries impacted by volcanic ash and vog. Finally, determining how to divert resources based on changing meteorological conditions presents an extreme challenge given the vast area to be covered and limited resources. This presentation will address these issues and encourage discussion of public health impacts of volcanic activity and approaches to monitoring changes in ambient air quality in impacted areas.

1341 RISK ASSESSMENT AND PUBLIC HEALTH IMPLICATIONS OF WTC DUST CONTAMINATION TOPIC.

P. Godd. CTEH, North Little Rock, AR.

The collapse of the WTC resulted in unprecedented environmental contamination which directly impacted nearby buildings. Massive quantities of dust combined with emissions from the longest-burning commercial fire in US history created a complex mixture of chemical contaminants that were dispersed directly into severely damaged buildings. The nature and extent of contamination was initially unknown. Emerging data regarding the unique composition and morphology of WTC Dust precluded any reliable prediction of risks to the health of individuals potentially reoccupying such buildings in the absence of further study of conditions in affected buildings. This talk will address the extent and results of testing, and the implications of the presence of these contaminants to public health.

1342 MONITORING AND SURVEILLANCE OF DISASTER RESPONDERS: TRACKING THE HEALTH OF WTC CLEAN UP AND RECOVERY WORKER.


Hundreds of workers responding to the WTC disaster, participated in the clean up and recovery effort. Seven years after the cleanup operation came to a conclusion, the health of these workers continues to be of significant concern. Inhalation of the complex mixture of airborne contaminants at the disaster site has resulted in an “after disaster” for hundreds of workers who still suffer from the harmful effects of that exposure. The nature of the contaminant mixture and the harm it has caused continue to be the focus of the exposure assessment, epidemiological and clinic communities. This talk will address the questions that continue to be outstanding for these workers and discuss what should be done to prepare for a future event.

1343 TOXICOLOGICAL RISKS OF URBAN WILDFIRES: ANALYSIS OF FIRE ASH AND DEBRIS FOLLOWING THE SOUTHERN CALIFORNIA WILDFIRES.


The 2007 Southern California wildfires burned more than 350,000 acres in populated and open space areas, consuming some 3200 structures and 2000 vehicles. The destruction left in the wake of the fires resulted in the potential for wide-spread public exposure to toxic materials. Residents were warned of the risk of exposure to contaminants in burn ash and debris upon reentry to destroyed neighborhoods and properties. In addition, there were concerns of off-site migration of hazardous substances into sources of drinking water. Results from the sampling and analysis of ash show that concentrations of lead (mean = 1075 mg/kg), arsenic (mean = 11.4 mg/kg), cadmium (14.7 mg/kg), and copper (4458 mg/kg) were significantly above California background levels and exceeded both California and federal health-based screening levels. Also highlighted in the presentation will be recovery efforts that had a direct impact on reducing public health threats post-fire, efforts which are now regularly applied in the wake of California firestorms events.
The Chesapeake Bay Commission has evaluated alternative fuel development efforts in the Chesapeake Region. Already under stress from anthropomorphic factors, the Chesapeake Bay Region could be adversely impacted by the wide spectrum of use of the region for biofuels production, transport, storage, and combustion. This Regional presentation will characterize the potential adverse effects on public health and ecological degradation from the production and use of biofuels in the Bay, an uncertain and complex challenge. There are multiple types of biofuels that are derived from various feedstocks and production processes. The amount of land use devoted to biofuels in this region will vary tremendously in part by the biofuel stock in economic demand, the advances made in the growth rate and energy content of plant stocks, and whether it can be imported. Domestic corn planting in the Bay Region increased 11,000 acres from 2005 to 2006, primarily for use in ethanol production with consequences for decreased food availability, soil loss, and nutrient runoff. In contrast, a biodiesel production plant in Baltimore will import soybeans as the raw material due to economic incentives, thereby avoiding issues with domestic corn production. Potential human health effects will occur through exposure to the fuels, inhalation of combustion products, and fallout into water supplies. Algal blooms due to increased nitrogen deposition in the estuarine environment from biofuel production would impact public and environmental health. Air quality could be impacted from combustion, as could water quality through deposition of the fuel products into the Bay. Estuarine and marine organisms, some with commercial importance, could be adversely impacted. Ethical issues over the displacement of crops for food to energy for mobile sources have arisen and will be considered. Both the public health and ecological problems posed by the wide spectrum of biofuels being considered for use in the Bay will be addressed. [This abstract of a proposed presentation may not reflect US EPA policy.]

The increased use of biofuels within the Chesapeake Bay region will likely result in accidental releases to the Bay, atmospheric inputs at, and runoff from the extensive watershed. The composition of fuels influences the fate and effects of these mixtures in estuarine ecosystems. Biofuels are variable and also differ in hydrocarbon composition from fossil fuels and these differences can influence their fate and effects within the estuary. Long-term exposures and effects will depend both on the loading of chemicals to the Bay as well as the persistence of these chemicals. This presentation will examine these characteristics in comparison to those associated with conventional fossil fuels. In addition, because some viscous biofuels can exert adverse effects as a result of physical modifications of sediments, this aspect of exposure will also be examined. A conceptual model that illustrates the sources, loadings, and fate of biofuel constituents as these relate to the estuarine biota will be presented along with a framework for considering the variable toxicological implications of the various fuels.

The energy crisis has highlighted the emerging uses of biofuels for transportation. The sources of the biofuels range from corn to saltwater plants. Consequently, the toxicity of these biofuels is largely unknown and will be complicated by the numerous plant sources as well as the distillation techniques used for biofuel generation. This talk will focus on the health effect(s) of jet fuel exposure and extrapolate these findings to potential toxicity issues with biofuels.
cular dysfunction than larger particles of the same composition. However, it re- mains unclear if coronary microvascular endothelial function is affected to a similar degree. Rats were exposed to filtered air (control) or TiO₂ nanoparticles (primary particle diameter, ~21 nm) via inhalation at concentrations relevant to ambient air pollution (9.5 μg measured pulmonary deposition). Coronary arterioles (~150 μm in diameter) were isolated from the left anterior descending artery distribution and responses to flow (FID) (5-25 μL/min), acetylcholine (ACh, 10⁻³ - 10⁻¹ M), and the Ca²⁺ ionophore, A23187 (10⁻³ - 10⁻¹ M), were assessed. Endothelium-depend- ent FID was preserved in coronary artery stress by ~60%, compared to control rats. Conversely, a profound vasoconstriction (16±7 μg/rat. The spinotrapez- ium) resulted from cumulative additions of ACh in arterioles from rats exposed to nanoparticles, whereas control rats responded by vasodilation (73±6%, dilatation). Similarly, nanoparticle exposure impaired arteriolar dilation in both groups, indicating that vascular smooth muscle NO responsive- ness remains intact after nanoparticle exposure. These results suggest that nanoparticle exposure significantly impairs Ca²⁺ dependent microvascular re- sponses to ACh and A23187, whereas responsiveness to shear stress is preserved. It is probable that such disturbances in coronary microvascular function contribute to the cardiac events associated with particle pulmonary exposure. Support: NIH RO1-E0515022 and HEI #4730 (TRN)

1351 TIME COURSE OF SYSTEMIC EFFECTS FOLLOWING A SINGLE EXPOSURE TO CARBON NANOTUBES.

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Pulmonary exposure to carbon nanotubes (CNT), in a mouse model, causes aortic mitochondrial DNA damage and enhanced thrombogenicity, prerequisites of ath- erosclerosis. The link between endpoint systemic effects and pulmonary exposure is unknown. We hypothesized that blood cell gene expression and serum protein analysis will provide insight into the relationship between CNT-induced lung and cardiovascular effects. C57BL/6 mice were exposed to 40μg of multi-wall CNT (MWCNT) and sacrificed at 4hr, 24hr, 7d and 28d post single exposure. We eval- uated serum proteins and blood cell gene expression of biomarkers related to po- tential cardiovascular effects. Furthermore, the CNT effects on lung and cardiovas- cular tissues were characterized by a coordinated gene expression analysis. At 4hr, a marked systemic inflammatory response was evidenced by increased inflammatory serum proteins (e.g. IL-6, CXCL1) and upregulated blood cell gene expression of inflammatory mediators. Cardiovascular tissue, including heart and aorta, showed a generalized stress and inflammatory response. In addition, the expression of spe- cific endothelial related genes was elevated in the aorta (e.g. E-selectin). The sys- temic effects at 4hr were mostly a reflection of the ongoing lung response. At 24hr, inflammatory serum proteins and blood cell gene expression had returned to baseline and the systemic tissue response had diminished. In exchange, serum acute phase proteins (e.g. C-reactive protein, serum amyloid P) and accompanying liver gene expression were increased. Serotonin levels increased at both 4hr and 24hr increased serum levels of the prothrombotic protein plasminogen activator inhibitor-1 were found. The late blood response (7 and 28d) was characterized by increased serum osteo- ptenin levels in conjunction with increased lung expression of genes coding for a matrix metalloproteinase (e.g. arginase I, galectin-3, osteopontin). In conclu- sion, our data suggests a link between CNT-induced pulmonary toxicity and pulmonary nanoparticle exposure impairs endothelium de- pendent arteriolar dilation. Histological sections of the spinotrapezius muscle were prepared for intravital microscopy 24 hrs after exposures. Intraluminal infusion of the Ca²⁺ ionophore A23187 was used to evaluate endothe- lium-dependent arteriolar dilation. Endogenous microvascular NO production was measured with an electrochemical sensor. Oxidative stress in the microvascular wall was quantified via dihydroethidium fluorescence (O₂⁻, probe). Histological sections of pulmonary tissue revealed nanoparticle uptake by alveolar macrophages, and mig- ration to nearby lymph tissue. TiO₂ nanoparticles quenched spontaneous NO sig- nals (1.9 ± 0.9% in vitro by S-Nitroso-N-acetyl-D,L-penicillamine (550 mM)). As in previous experiments, A23187 produced dose-dependent arteriolar dilations (10- 69% of maximum response). Nanoparticle exposure robustly attenuated this to 6- 16% of the maximum response. Nanoparticle exposure also increased microvascu- lar oxidative stress and NO production. Inhibition of either myeloperoxidase (4-aminobenzenediazid, 10 μm) or NAPDH oxidase (apocynin, 10⁻³ M) partially restored NO production and general microvascular func- tion. These results indicate that in conjunction with microvascular dysfunction, nanoparticle exposure also initiates local oxidative stress and decreases NO bioavailability. Support: R01-E0515022 and HEI #4730 (TRN)


T. R. Nurkiewicz1, M. Donlin1, A. Hubbs1, A. Goodwill1, J. Frisbee1, B. Chen2, D. Frazer2 and V. Castranova2, 1Center for Interdisciplinary Research in Cardiovacular Sciences, West Virginia University, Morgantown, WV and 2National Institute for Occupational Safety and Health, Morgantown, WV.

Previous studies in our laboratory have shown that pulmonary nanoparticle expo- sure causes peripheral microvascular dysfunction. This dysfunction is characterized by impaired endothelium-dependent arteriolar dilation and venular leukocyte adhe- sion. The mechanisms that produce these effects remain poorly understood. The purpose of this study was to determine if neurological mechanisms and/or circulat- ing leukocytes play a fundamental role between pulmonary exposure and peripheral microvascular dysfunction. Rats were exposed to TiO₂ nanoparticles via inhalation (primary particle diameter ~21 nm) at depositions of 4-90 μg/rat. Some rats re- ceived a bolus dose of cyclophosphamide (200 μg/g, i.p.) 3 days prior to nanoparti- cle exposure to deplete circulating neutrophils. The spinotrapezius muscle and lung were prepared, and plasma was sampled from each rat for multi-plex analyses. Following nanoparticle exposure, plasma IL-1, 2, 13 and ICAM-1 were altered. Consistent with previous experiments, nanoparticle exposure significa- ntly limited arteriolar dilation (in response to A23187) to 0.7-7% of the normal maximum response. Co-incubation with the Fast Na⁺ channel antagonist tetrodotoxin (TTX, 10⁻⁴ M) restored dilation by as much as 54%. Neutrophil de- pletion similarly restored dilation by as much as 42%. These mechanistic data sup- port prominent hypotheses that suggest peripheral vascular effects associated with particle exposure are due to neurogenic and/or inflammatory mechanisms. Support: NIH RO1-E0515022 and HEI #4730 (TRN)

1354 CARdiovascular EFFECTS OF ULTRAFINE PARTICULATE MATTER ON SPONTANEOUSLY HYpertensIVE RATS.

K. Salisbury, G. Guokas, P. Willett, D. Meacher and M. T. Kleinman, Department of Medicine, University of California, Irvine, Irvine, CA.

Exposure to particulate air pollution has been associated with increased cardiovas- cular morbidity and mortality. A suggested mechanism for this association is the de- velopment of oxidative stress and inflammation with exposure to ambient particu- late matter. Exposure to ultrafine particles may result in greater oxidative stress and inflammation than exposure to larger particles due to a larger surface area to mass ratio and greater ability to translocate to other areas of the body. Spontaneously hy- pertensive rats, an animal model of arterial hypertension, were implanted with telemetry devices to measure heart rate and blood pressure. The rats were exposed to concentrated ultrafine particles or filtered air for 5 hours per day, 4 days per week for 12 weeks in Riverside, CA. Rats exposed to ultrafine particles showed greater serum levels of the inflammatory cytokines IL-1β, IL-6, IL-12, TNF-α, and IFN-γ, the anti-inflammatory cytokine IL-10, monocyte chemoattractant protein-1, and others, compared to control rats. Flow cytometric cell phenotyping showed higher levels of mononuclear cells and neutrophils in the ultrafine particle exposed rats. Analysis of telemetry data from exposed animals showed a depression in blood press- sure during exposure periods compared to non-exposure periods. In addition, rats exposed to ultrafine particles showed a decrease in heart rate compared to the con- trol animals. These results indicate a change in cardiovascular function with expo- sure to ultrafine particulate matter. Further analysis is needed to determine the mechanistic relationship between the increase in inflammatory biomarkers and change in cardiac parameters.

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FINE PARTICULATE MATTER EXPOSURE DEPRESSES PULMONARY FUNCTION IN THE DEVELOPING MOUSE LUNG.

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Evidence is increasing that ambient air pollution has chronic negative effects on pulmonary function in the developing lungs of children. In this study, a mouse model was used to test whether exposure to ambient particulate matter delays or arrests lung growth. Mice undergo a rapid period of growth between birth and 6 weeks of age. After lung remodeling and alveolarization is complete normal lung growth occurs from 4 weeks into adulthood. C57BL/6 mice were exposed to concentrated ambient fine particulate matter less than 2.5 μm or purified air for 8 weeks starting at either 4, 6, or 8 weeks of age. An invasive lung function measurement was utilized using parameters that are considered to be classical methods for accurate and specific determination of pulmonary mechanics (e.g., pulmonary resistance and dynamic compliance). Analysis of the data shows a depression of pulmonary function in particle-exposed animals compared to purified-air exposed animals, 1 wk post 8 wk exposure. At the end of their lung growth period particle-exposed mice had a significant decrease in dynamic compliance compared to purified-air exposed mice. The particle-exposed animals also displayed increased pulmonary resistance as well as a depression in minute inspiratory and expiratory flow compared to purified-air exposed animals. Histopathological analyses of lung tissues were performed to determine if the functional decrements are accompanied by morphological changes.

ACTIVATION OF ENDOTHELIAL CELLS AFTER EXPOSURE TO AMBIENT ULTRAFINE PARTICLES: THE ROLE OF NADPH OXIDASE.

Y. Mo, R. Wan, J. Tollerud and Q. Zhang.
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Ultrafine particles (UFPs) may pass from the lungs to the circulation because of their very small diameter. The direct effects of UFPs on vascular endothelium remain unknown. We hypothesized that exposure to UFPs leads to endothelial cells O2·− generation via NADPH oxidase and results in activation of endothelial cells. Our results show that ROS generation was inhibited when mouse pulmonary microvascular endothelial cells (MMPVEC) were pre-treated with the ROS scavengers, or the NADPH oxidase inhibitor, diphenyleneiodonium chloride (DPI), but not with the mitochondrial inhibitor, rotenone. This was further confirmed by studies showing that UFP-induced ROS generation in MMPVEC was abolished by p67phox siRNA and UFPs did not cause ROS generation in MMPVEC isolated from gp91phox−/− mice. ROS generation in endothelial cells was also determined in vivo by using lung imaging. G90phox−/− endothelial cells did not generate ROS when they were exposed to UFPs. We demonstrated by western-blot and immunofluorescence staining that MMPVEC treated with UFPs resulted in the translocation of cytosolic protein p67phox, p47phox and rac 1 to the plasma membrane. Our results demonstrate that NADPH oxidase in the pulmonary endothelium is involved in ROS generation following exposure to UFPs. To investigate the activation of endothelial cells due to UFP-induced oxidative stress, we determine the miogen-activated protein kinase (MAPKs) in MMPVEC after exposure to UFPs. Our results showed that ROS generation in MMPVEC to UFPs causes increased expression of phospho-p38 and phospho-ERK1/2 MAPKs. However, these increases were blocked by pretreatment with DPI and p67phox siRNA. Moreover, exposure of MMPVEC isolated from gp91phox−/− mice to UFPs did not cause increased expression of phospho-p38 and phospho-ERK1/2 MAPKs. Our findings confirm that UFPs can affect endothelial cells directly to generate ROS via activation of NADPH oxidase. This can further lead to up-regulation of phospho-p38 and phospho-ERK1/2 MAPK expression that may result in the endothelial dysfunction.

EXPOSURE OF NEONATAL MALE RATS TO 1359

1359 EXPOSURE OF NEONATAL MALE RATS TO 1359

1359 EXPOSURE OF NEONATAL MALE RATS TO ENDOCRINE DISRUPTER BISPHENOL A AFFECTS ONTOGENIC EXPRESSION PATTERN OF TESTICULAR STEROID RECEPTORS AND THEIR COREGULATORS.


Bisphenol A (BPA), an endocrine disruptor has the propensity to leach from various consumer products. Detection of BPA in human amniotic fluid, cord blood indicated its capacity to transverse the placental barrier thus highlighting a need to investigate its effect during critical ‘windows’ of development. Present study was undertaken to determine the effect of exposure of neonatal male rats to environmentally relevant dose of BPA on the testicular expression pattern of steroid receptors (ERα, ERβ, AR) and their coregulators/co-repressor viz. NCoR; coactivator viz. SRC-1, GRIP-1, p/CIP) during development. Neonatal male rats were s.c injected with 2.4μg/kg bw/day of BPA in sesame oil from postnatal day 1-5 and controls received vehicle. Animals were sacrificed on PND 15, 30, 45, 60, 75, 90, 125, and 150 days and tissues were dissected out. One testis from each animal were used for gene expression studies using Real Time PCR and another for protein expression profile using immunohistochemistry. Expression pattern of steroid receptors was studied at gene and protein level whereas expression of steroid receptor coregulators was studied only at the protein level. The results obtained were compared with their respective age matched controls. Significant decrease in the expression profile of ERα, ERβ and AR at both the gene and protein level was seen in the treated group. Coregulator viz. NCoR and SRC-1 showed a significant decrease in expression, whereas GRIP-1and p/CIP showed a significant increase in their protein expression.
profile in the treated animals. These results demonstrated that exposure of neonatal male rats to BPA lead to impairments in expression pattern of steroid receptors and coregulators which play a crucial role in modulating the steroid hormone action during critical period of differentiation and development of male reproductive organs and their function.

**1360**

**ESTRADIOL IS INVOLVED IN THE UPREGULATION OF GLUTATHIONE SYNTHESIS BY FSH IN OVARIAN FOLLICLES.**

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The antioxidant tripeptide glutathione (GSH) detoxifies electrophilic metabolites and reactive oxygen species. Our previous studies showed that treatment with gonadotropins enhances ovarian GSH synthesis. In the present study, we evaluated the effects of follicle-stimulating hormone (FSH) and estradiol (E2) on the expression of glutamate cysteine ligase (GCL), the rate-limiting enzyme in GSH synthesis, in ovarian granulosa cells (GC) and follicles. Primary GC or small antral follicles were isolated from ovaries of 25-day-old female E2-primed Sprague-Dawley rats and were cultured with 0 or 20mg/ml FSH plus or minus 100 ng/ml 17β-E2. In isolated GC, GSH concentrations increased significantly after 48h, but not 24h, of FSH treatment, and this effect was enhanced by E2. FSH alone increased GCL subunit protein levels at 48h without affecting mRNA levels. In contrast, FSH with E2 induced mRNA and protein expression of both GCL subunits as early as 24h; levels remained elevated at 48h. E2 alone had no effects. Luteinizing hormone receptor mRNA levels also increased after 48h of FSH treatment, consistent with GC differentiation. The PKA inhibitors, H89 and PKI, abolished the effect of FSH on GCLM protein, whereas only PKI blocked the effect on GCLC. These results suggest that the effects of FSH alone on GCL are mediated by PKA signaling. Small antral follicles cultured with FSH showed a 35-fold increase in FSH secretion at 24h versus controls. FSH-treated follicles had a 2-fold increase in total GSH and a 2-fold increase in the ratio of reduced to oxidized GSH versus controls at 24h, indicating that untreated controls were more oxidized. This coincided with a significant rise in GCL subunit mRNA and protein levels. Taken together these data show that FSH and E2 interact to maximally stimulate GCL subunit expression and GSH synthesis in GC. The data further suggest that E2 mediates the pronounced stimulation of GCL expression/GSH synthesis by FSH in small antral follicles. Supported by NIH ES09653.

**1361**

**HORMONAL SUPPRESSION RESTORES FERTILITY IN IRRADIATED MICE FROM BOTH ENDOGENOUS AND DONOR-DERIVED SPERMATOGENESIS.**


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Irradiation reduces sperm production and causes male sterility in rodents and humans. Suppression of testosterone was able to restore spermatogenesis in irradiated rats, but not in mice or humans. Recently, we showed that testosterone suppression with a GnRH antagonist, acyline, and flutamide (A+F), given to mice after irradiation, improved the recovery of endogenous spermatogenesis and enhanced colonization of transplanted stem spermatogonia. Here we further explore the restoration of fertility from endogenous or donor-derived germ cells in irradiated mice by hormone suppression. All mice were irradiated, with or without spermatogonial transplantation (ST) 3-4 wk later from GFP-marked donors, and followed by spermatogonial evaluation and fertility testing. For endogenous recovery, testes of mice were irradiated (10.5 Gy) and subjected to either 10-wk A+F or sham treatment. Hormone suppression enhanced recovery of endogenous spermatogenesis as indicated by increased tests weights (1.3-fold) and sperm count (2-fold) at 21 wk after irradiation. In the hormone-suppressed group 12 out of 15 mice recovered fertility at about 22 wk after irradiation, while all sham-treated mice remained infertile as of 30 wk. For donor-derived spermatogenesis, mice were irradiated (13.5 Gy) and were either treated with: 1) sham A+F & sham ST; 2) A+F & sham ST; 3) ST and sham A+F; or 4) A+F and ST. Tests weights were also significantly higher in mice treated with both A+F and ST than those in mice treated with either A+F or ST at 21 wk. Furthermore, in the transplanted, hormone-suppressed group, 2 out of 20 mice were fertile from donor-derived spermatogenesis at 18 wk after irradiation, but no mice from the other groups regained fertility within 28 wk. Thus hormone suppression successfully restores spermatogenesis and fertility from both endogenous and transplanted spermatogonial stem cells in irradiated mice. The combination of ST with hormonal suppression should be investigated to restore fertility in young men after cytotoxic therapy for cancer.

**1362**

**INSULIN RESISTANCE IN SPRAGUE-DAWLEY RATS CHRONICALLY TREATED WITH ETHANOL.**

R. M. Rutka1, M. Ferguson2 and M. J. Ronis2.

1University of Arkansas for Medical Sciences, Little Rock, AR, 2Pharmacology & Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR, and 3Physiology & Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR.

We have previously demonstrated that hepatic insulin signaling is disrupted in Sprague-Dawley (SD) rats fed EtOH-containing diets by total enteral nutrition. (To determine if whole body insulin resistance could be demonstrated in the TEN model, we conducted euglycemic-hyperinsulinemic clamp studies in adult (age 90 days) rats chronically (4 weeks) fed ethanol-containing diets (10-13 g/kg EtOH). Rats were fed either control diets formulated to meet the National Research Council recommended daily nutrient intake for rats, or an isocaloric diet containing 10-13 g/kg EtOH (EtOH calories replaced a portion of the carbohydrate calories). The glucose infusion rate (GIR) of rats chronically fed >10 g/kg ethanol was lower (P<0.05) than control rats receiving the same isocaloric diet without ethanol. Because blood ethanol concentrations (BECs) of rats in the TEN model are pulsatile (i.e., EtOH concentrations occur as large pulses over a 6 day period), insulin clamp studies were conducted at different stages of the pulse (i.e., different BECs), and preliminary data suggest that insulin sensitivity does not vary with differences in BECs. We conclude that chronic ethanol intake causes whole body insulin resistance and while consistent with previous data demonstrating disruption of hepatic insulin signaling, these data suggest that overall insulin sensitivity does not vary throughout the EtOH pulse and therefore does not drive EtOH metabolism as previously predicted. Supported in part by NIAAA008645.

**1363**

**LOW DOSE ETHANOL CONSUMPTION IMPROVES INSULIN SENSITIVITY IN RATS.**

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While chronic consumption of high doses of ethanol is well known to have adverse health consequences, intake of low doses have been reported to improve several markers of health outcomes. Published results from our laboratory using total enteral nutrition (TEN) in rats, in which ethanol-containing diets are infused directly into the stomach, have clearly demonstrated that chronic ethanol intake results in disruption of hepatic insulin signaling. Based upon previous literature reports we posited that using the same TEN model, but with chronic low EtOH doses, insulin sensitivity would increase. Young adult male Spraque-Dawley rats (age 60 days) were surgically fitted with an intragastric cannula and fed a high fat diet plus low dose EtOH (4 g/kg/d). After 4 weeks of this diet, rats had gained weight equal to control rats that were fed the same isocaloric diet (except EtOH calories were replaced with carbohydrate calories). Rats were fasted over night and a standard oral glucose tolerance test was administered. Rats fed low doses EtOH had a greater (P<0.05) whole body insulin response than control rats fed a diet with no ethanol. Moreover, hepatic Akt-GSKbeta-SREBP-1 signaling was enhanced (P<0.05) and expression of the glucoseoenzymic enzyme PEPCK was suppressed in low dose EtOH vs. control animals (P<0.05). We conclude that chronic intake of a diet containing low dose EtOH can improve insulin sensitivity. Supported in part by NIAAA008645.

**1364**

**TRICLOSAN MODULATES ESTROGEN-DEPENDENT RESPONSES IN THE RAT UTEROTROPHIC ASSAY.**

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Our previous studies in the juvenile rat indicated that the biocide triclosan may alter steroid hormone levels. Here, we hypothesize that triclosan possesses estrogenic activity. First, we evaluated triclosan using the immature rat uterotropic assay. Female Wistar rats (19 days old) were gavaged for 3 days with corn oil, 5 mg/kg ethinyl estradiol (EE) or 1.17 to 300 mg/kg triclosan either as an oral dose or 300 mg/kg co-treated with EE as compared to the EE alone, indicating a potentiation of the estrogen response on uterine weight. In addition, this result was correlated with a dose response increase in degree of columnar differentiation of the luminal epithelium of the uterus in the females receiving co-treatment with EE. In a
second study, an in vitro estrogen receptor (ER) transcriptional activation (TA) assay using the T47D-Blue cell line was used to determine if triclosan altered estrogen receptor activation. Triclosan concentrations of 0.003 to 10μM were evaluated in this assay. Triclosan were completely soluble and cytotoxicity was not observed after 24 hour incubation. Triclosan alone did not have agonistic activity in the ER TA assay. In addition, co-incubations of triclosan and various concentrations of estradiol resulted in a response comparable to estradiol alone, indicating that the parent compound does not antagonize or potentiate ER TA. It is possible that one of the metabolites of triclosan may be responsible for the observed effect. In conclusion, there appears to be a potentiating effect of triclosan on estrogen mediated uterine response in the weaning female rat. Current studies are evaluating other potential modes of action for this effect. This abstract does not reflect EPA policy.

1365 EFFECTS OF A MODEL INDUCER, PHENOBARBITAL, ON THYROID HORMONE GLUCURONIDATION IN RAT HEPATOCYTES.


In vivo, hepatic enzyme inducers such as phenobarbital (PB) have been shown to decrease circulating thyroid hormone (TH) concentrations. This decrease in circulating TH occurs in part through extrathyroidal mechanisms. Specifically, induction of the metabolism of xenobiotic metabolizing enzymes such as uridine-diphosphate-glucuronosyltransferases (UGTs), thyroid hormones are catabolized and cleared through biliary elimination. This study further examines the catabolism and clearance of THs by analyzing the formation of T4-glucuronide (T4G) using rat cryopreserved hepatocytes. Hepatocytes were plated in a monolayer and treated for 72 hours in serum-free media with or without 1μM PB. After removal of media, 0.05, 0.5, 2.5, or 5.0μM T4I125 was added to cells and incubated for up to 24 hours at 37°C. After 24 hours, 3.3, 4.3, 4.5, and 5.7μM T4I125 accumulated within hepatocytes exposed to 0.05, 0.5, 2.5, or 5.0μM T4I125, respectively. In hepatocytes pretreated with 1μM PB, 2.1, 2.8, 3.2, and 4.3μM T4I125 accumulated within hepatocytes to 0.05, 0.5, 2.5, or 5.0μM T4I125, respectively. In controls, Vmax was 0.85μM T4G/min/million cells, while in PB treated hepatocytes, Vmax increased to 1.94μM T4G/min/million cells. The Km for T4G formation following PB pretreatment was 112.4μM whereas the Km without PB was 44.7μM. These data suggest that treatment with PB increased the elimination of T4I125 through an increase in T4 catabolism and clearance. This study also demonstrates that rat hepatocytes are a valuable tool for evaluating the effects of xenobiotics on extrathyroidal mechanisms of TH4 elimination. (This is an abstract of a proposed study.)

1366 ARYL HYDROCARBON RECEPTOR EXPRESSION AND FUNCTION IN NORMAL ENDOMETRIUM AND ENDOMETRIOSIS.

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The aryl hydrocarbon receptor (AhR) has been suspected to play a role in the development of endometriosis. Using human tissue samples and an endometrial carcinoma cell line, we investigated the interactions between AhR and hormonal pathways involved in endometrial physiology. Expression of mRNA for AhR and its target genes cytochromes P4501A1 (CYP1A1) and P4501B1 (CYP1B1) were all significantly higher in endometriosis samples than in normal controls. Because endometriosis is associated with progesterone resistance during the secretory phase of the menstrual cycle, we next measured expression of AhR in normal endometrial samples from different stages of the cycle. Expression of AhR, CYP1A1 and CYP1B1 were typically highest during the proliferative phase and significantly lower by the mid-secretory phase. Hypothesizing that expression of these genes was regulated by hormones important in the control of the menstrual cycle, we treated ECC-1 endometrial adenocarcinoma cells with daunorubicin, epidermal growth factor, or medroxyprogesterone 1-acetate (MPA). Expression of AhR and its target genes was decreased in cells exposed to MPA as compared to vehicle controls. Finally, we examined the effects of AhR ligands on expression of the progesterone receptors (PR) A and B in ECC-1 cells. The AhR agonists beta-naphthoflavone and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) both significantly decreased expression of total progesterone receptors but not PR-B, suggesting that AhR agonists selectively decrease the expression of PR-A. A combination of TCDD and the AhR antagonist CH-223191 modestly increased PR expression versus the control. Taken together, these data suggest that AhR and its target genes are part of a larger program of gene regulation during the menstrual cycle, and that AhR activity may play a role in the progesterone resistance characteristic of endometriosis.

1367 MULTIPOTENT NEURAL STEM CELL TARGETS ARE LICENSED TO TCDD TOXICITY: POTENTIAL ROLES FOR AH RECEPTOR DURING NEUROGENESIS.

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Neurogenesis is a multi-step process that includes proliferation and differentiation of neural stem cells (NSCs). Pluripotent NSCs abnormally express the aryl hydrocarbon receptor (AhR), which mediates dioxin toxicity. Therefore, we hypothesized that dioxin exposure impeded AhR-mediated signaling events during NSC maturation. In this study, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a pervasive environmental toxicant and a potent AhR ligand, was used in this rat model. We previously determined that C17.2 cell line contained abundant AhR levels and exhibited a multipotent NSC-like phenotype, as characterized by the expression of nestin, a NSC marker. Our studies revealed that TCDD exposure suppressed NSC proliferation by interfering with the G1 to S cell cycle transition. Under defined conditions, we determined that C17.2 cells adopted a neuronal fate, as evidenced by reduced nestin expression, elevated IFN-γ levels and absence of glial markers. AhR protein levels declined but remained detectable following NSC differentiation, suggesting a role for this receptor during NSC maturation to a neuronal lineage. When C17.2 cells were cultured in TCDD under non-depopulating conditions, AhR expression was not downregulated, as would be expected. Additionally, AhR remained in the nucleus and the expression patterns of nestin, IFN-γ, and GFAP proteins were consistent with reduced neural differentiation in response to TCDD. When C17.2 cells were exposed to TCDD in depopulating conditions, AhR expression was reduced and NSC differentiation was not altered. Our data suggest that AhR activation by TCDD disrupted early NSC differentiation into neurons but that TCDD had distinct actions during activity-dependent maturation. These observations suggest that the inappropriate or sustained activation of AhR by TCDD during neurogenesis can interfere with multiple signaling pathways during NSC proliferation and differentiation, which could adversely impact brain cytoarchitecture and function.

1368 COMPARING GENE EXPRESSION ALTERATIONS IN MOUSE EMBRYOS UNDERGOING NEURODETERIATION: DOSE AND TIME-DEPENDENT EFFECTS OF CADMIUM AND ARSENIC EXPOSURES.

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Initiated by known and unknown genetic and environmental factors, Neural Tube Defects (NTDs) represent the second most common birth defect in the human population. When exposed during neurulation, the C57 is more sensitive to metals than other inbred mouse strains, displaying a higher incidence of metal-induced NTDs. In limited studies, cadmium (Cd) and arsenic (As) impact biological processes (i.e. cell cycle) associated with increased toxicity and NTD formation. However, these studies do not account for comparative analyses across dose and time which may be important 1) to develop sensitive indicators of toxicity, 2) to characterize common and unique mechanisms of NTD formation between environmental teratogens and 3) to determine potential application for risk assessment. In this study, we examined dose- and time-dependent effects of As and Cd on gene expression using microarray and qRT-PCR in C57 embryos exposed in utero (GD8) across dose (As, 0–10mg/kg; Cd, 0–4mg/kg) and time (0–12h). We identified significantly altered genes due to metal exposure by ANOVA and corresponding enriched Gene Ontology (GO) functional categories using MAPFinder. Using GO-Quant, we calculated the average impact on gene expression (As/Con or Cd/Con) for genes within enriched GO categories. Our results suggest As and Cd induce dose and time-dependent gene expression alterations, representing common (cell cycle) and unique (glycolysis, one-carbon metabolism (As only)) gene expression-linked GO biological processes. These observations correlate with common and differential impacts on specific genes of interest involved in cell cycle regulation (Cdkn1a), ion regulation (MT1), hypoxia signaling (Ndrg1) and methyltransferase (Tmys). In summary, we have identified As and Cd induced gene expression responses in mouse embryos undergoing neurulation and associated quantitative changes within GO categories across dose and time to developmental toxicity and strain sensitivity. NIEHS/2P01ES09601, U10ES11387

1369 LEAD DISRUPTS DEVELOPING HIPPOCAMPAL NEURON SYNAPSES VIA NMDA RECEPTOR INHIBITION.


Lead (Pb) is ubiquitous neurotoxicant that impairs hippocampal-mediated learning. Pb is a potent N-methyl-d-aspartate (NMDAR) antagonist and developmental exposure to Pb alters NMDAR subunit mRNA and protein ex-
pression in the rat brain. Further, Pb2+ exposure alters glutamatergic and GABAergic transmission both in vivo and in vitro. We used a primary hippocampal cell culture system to assess the effects of Pb2+ in both the pre-synaptic active zone (PAZ) and the post-synaptic density (PSD). Previously, we reported that Pb2+ exposure (0.01-1.0 μM) altered NMDAR (NR1 subunit) targeting to the PSD, reduced levels of Synaptophysin (Syn) and increased the expression of the pre-synaptic scaffolding protein Bassoon. We now report that Pb2+ exposure results in decreased levels of Synaptobrevin, a PAZ protein essential for exocytic release. Based on these findings, we hypothesized that Pb2+ was increasing the expression of pre-synaptic elements. To test this hypothesis, we used FM-4-3 dye to determine if vesicular release was impaired in Pb2+-exposed cultures. Live imaging showed a significant decrease in the number of boutons actively releasing dye (p<0.05). The rate constants from individual release sites and the averaged rate of release were significantly different after exposure to Pb2+ (p<0.05), indicating a shift towards slower rate constants. To determine if both pre- and post-synaptic effects of Pb2+ were mediated by NMDAR inhibition, we chronically exposed DIV7 neurons to 100 μM of the NMDAR antagonist APV, 1.0 μM of Pb2+, or a co-exposure. As observed with 1.0 μM Pb2+, exposure to 100 μM APV resulted in decreased Syn expression and increased targeting of NR1 to the PSD (p<0.05). These data suggest that both pre- and post-synaptic effects of chronic Pb2+ exposure are due to NMDAR inhibition and putative disruption of retrograde signaling to alter pre-synaptic elements. This is the first report to show that both pre- and post-synaptic effects of Pb2+ exposure are mediated by NMDAR inhibition [Supported by ES01619 to TRG, APN supported by T32ES07141].

PL 1370 PERSISTING EFFECTS OF DEVELOPMENTAL COPPER EXPOSURE IN WILDLIFE AND METALLOTHIONEIN KNOCKOUT MICE.

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Developmental exposure to heavy and transition metals such as lead and mercury have been shown to cause persistent neurobehavioral toxicity. Metallothioneins are small proteins, which are crucial for the distribution of heavy and transition metals. Previously we found that knockout of MT1 and 2 potentiated the cognitive impairment caused by development mercury exposure. The current study examined the neurocognitive effects of developmental copper exposure. Wildtype and MT1 and 2 knockout mice (MTKO) were given 10 or 50 mg/l of copper during gestation and until weaning. Mice of both genotypes with no added copper served as controls. When the mice were young adults they were trained for 18 sessions on the win-shift 8-arm radial maze test of spatial learning and memory. MTKO mice with no added copper were significantly impaired on radial-arm maze choice accuracy relative to wildtype controls. This MTKO-induced impairment was significantly attenuated by addition of 10 mg/l of Copper to the drinking water during development. Interestingly, Copper had a non-monotonic dose effect function. The addition of 50 mg/l of Copper to the drinking water during development was not effective in reversing the MTKO-induced impairment. Modest but not high doses of Cu supplementation given during development can effectively reverse the cognitive impairment seen in the radial-arm maze test of mice with knockout of MT1 and 2. (Supported by NIH ES01356 and Autism Speaks)

PL 1371 MODERATE MATERNAL IRON INADEQUACY WORSENS NEUROBEHAVIORAL OUTCOMES OF DEVELOPMENTAL ETHANOL EXPOSURE.

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Prevention of alcohol use is difficult, thus therapies that ameliorate alcohol’s damage are desirable. The severity of fetal alcohol spectrum disorders (FASD) increases with parity, implying the depletion of a protective maternal factor. Iron deficiency is the most common nutritional deficiency in women and causes neurodevelopmental deficits in the offspring that parallel FASD. We hypothesized that moderate maternal iron inadequacy (ID) exacerbates alcohol’s effects upon the developing brain and tested this in rats pups reared by dams fed iron-sufficient (IS) or ID diets. We exposed pups to 0, 3.5 or 5 g/kg alcohol during the brain growth spurt period via gastric gavage (postnatal days 4-9; P4-9). Peak blood alcohol levels for 3.5 and 5 g/kg alcohol were 250 and ~400 mg/dl, respectively. Dams of the ID pups showed a Stage I iron inadequacy without anemia, common in women. At P10, ID pups fed 0, 3.5 or 5 g/kg alcohol (ID-0, ID-3.5, ID-5) were slightly smaller and had lower indicators of iron status including moderate anemia. At P33 we evaluated the pups’ associative learning using delay eyelink classical conditioning (RI).

The frequency or amplitude of conditioned responses (CR) in the ID-0, IS-0, IS-3.5, and ID-5 groups did not differ reflecting the test’s simplicity. In contrast, ID-5 pups had a dramatically reduced frequency and strength of CR; values were ~50% and 28%, respectively, of control values. Main effects for pup iron status and alcohol dose and a significant status x alcohol dose interaction were seen for CR frequency, F(2, 47)=6.44, p<0.005, and strength, F(2, 47)=3.23, p<0.05. Analyses of brain morphology and other behavioral outcomes are underway. This is the first study to show that maternal iron inadequacy profoundly exacerbates alcohol’s effects upon learning in the offspring. We propose that maternal iron status is an important modulator of fetal neurobehavioral outcome following alcohol exposure. (Funded by NIH award T32 ES07015, R32 AA11085 & the Palmer fund)

PL 1372 DEVELOPMENTAL NEUROTOXICITY OF SILVER IN ZEBRAFISH AND PC12 CELLS.

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Silver (Ag+) accumulates in soft tissues and impairs CNS function after prolonged periods of high exposure in adults. Furthermore, Ag+ accumulates in fetal tissues to higher levels than those found after birth, raising concerns about developmental neurotoxicity, especially in light of the nearly 10-fold increase in the number of consumer products incorporating silver nanoparticles (AgNPs). Biological systems can accumulate both Ag+ and Ag+ from AgNP exposure. We are therefore exploring the toxicodynamics of Ag+ as a developmental neurotoxicant to serve as a baseline for comparison with AgNPs. We used zebrafish (Danio rerio) as a model organism that allows for evaluation of Ag+ effects in the context of both human health and the environment. Zebrafish embryos were exposed to Ag+ starting at 48h post fertilization (hpf) and then measured mortality and hatch rate through hpf and newly defined developmentally important endpoints (dfp). We found increased mortality and severe morphological defects at concentrations above 3 μM. Concentrations as low as 1 μM decreased the number of embryos hatched by 72 hpf, when hatching is normally completed. We then examined neurodevelopmental effects at a lower exposure that did not impair hatchability or morphology. After embryonic (0.5 dpf) exposure to 0.3 μM Ag+, larvae showed impaired motor function at 5-10 dpf. We went on to determine potential mechanisms of neurotoxicity using neurotypic PC12 cells; Ag+ impaired cell replication and altered neurodifferentiation. These studies thus point to the likelihood that Ag+ is a developmental neurotoxicant. Our future work will evaluate effects of AgNPs in both these in vivo and in vitro models, since AgNPs may be more toxic than Ag+ due to endocytosis uptake of the AgNPs. Support: NIH ES03156, GM007105.

PL 1373 METHYL MERCURY INDUCED GENE EXPRESSION CHANGES IN CEREBELLUM AND CEREBRUM OF PERINATALLY EXPOSED FEMALE WISTAR RATS.

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A developmental toxicity study was performed on the effects of perinatal exposure to methyl mercury (MeHg) (0.0, 0.1, 0.4, 0.7, 1.0, 1.5, 2.0 mg MeHg/kg BW; p.o.; gestation day 6 to lactation day 10). Offspring was studied until post-natal day (PND) 70, with attention to the developing immune, hormonal and neural systems. For selected dose groups, in vivo imaging in vitro long term potential, toxicokinetics and toxicogenomics were performed. For toxicogenomics, rats were sacrificed on PND21 and PND70. Parts of cerebellum and cerebrum were collected. RNA was isolated using Trizol®/NucleoSpin RNA II, labelled and hybridized to Affymetrix GeneChip® Rat Genome 230 2.0 arrays. For selected dose levels, microarrays were performed on RNA obtained from 5 individual animals, yielding a 68 microarray experiment. Quality control and GCRMA (slow) normalization was performed. Preliminary data analysis (principal component analysis) indicated clear differences between the two different brain regions. Gene expression patterns were distinct between PND21 and PND70, suggesting that neural biochemical changes concur with development. The effects of methyl mercury on gene expression in cerebellum were modest: a union of 159 genes changed significantly in any of the dose groups on PND21 and PND70. For most of these genes, the effect of methyl mercury was opposite on PND70 in comparison to PND21. The differentially expressed genes were associated with G0 term and functions that are relevant for brain development, including synaptic transmission, neuron projection and nervous system development. Together, toxicogenomics proved to be a sensitive method to detect, within the context of developmental toxicity studies, persistent chemically-induced mechanistic changes in neurobehavioral target tissues, even long after cessation of the actual exposure.
**PL 1374** INTERFERON-GAMMA CAUSES DENDRITE RETRACTION IN SYMPATHETIC NEURONS IN VIVO.

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It is hypothesized that environmental chemicals modulate neurodevelopment via activation of inflammatory processes. Further, inflammation is implicated in the pathogenesis of a diverse group of neurological diseases. However, the mechanisms by which inflammation causes adverse effects on neural development and function are not well understood. We demonstrate that a peripheral inflammatory event, induced by viral infection and restricted to airways, causes a decrease in dendritic complexity and territory length sympathetic neurons of the superior cervical ganglia (SCG). Systemic exposure to the proinflammatory cytokine IFNγ (IFNγ), also causes dendrite retraction and synapse loss in the SCG. IFNγ-induced loss of re-ceptive surface in the SCG occurs in the absence of reduced neuronal cell viability or decreased trophic interactions between SCG neurons and their target tissues. Decreased dendritic complexity and synapse loss is coincident with impaired ganglionic neurotransmission as evidenced by an attenuation of the baroreflex in animals treated with IFNγ. Based on these observations we propose a novel pathogenic mechanism of neuroinflammatory disease in which IFNγ disrupts neuronal connectivity via selective retraction of dendrites, leading to decreased neuronal excitability.

**PL 1375** BUILDING A DATABASE OF DEVELOPMENTAL NEUROTOXICANTS: EVIDENCE FROM HUMAN AND ANIMAL STUDIES.


EPA's program for the screening and prioritization of chemicals for developmental neurotoxicity (DNT) necessitates the generation of a list of chemicals that are known mammalian developmental neurotoxicants. This chemical list will be used to evaluate the sensitivity, reliability, and predictive power of alternative DNT assays. A literature review was conducted for >400 compounds that have been suggested as developmental neurotoxicants. Compounds were assigned to one of three groups based on the strength of the evidence for DNT: (1) no evidence: no reports meeting our criteria for evidence or reports showing no DNT; (2) minimal evidence: one report or multiple reports from a single laboratory; or (3) substantial evidence: reports from more than one laboratory. Evidence was defined as (a) mammalian (laboratory animal or human) studies published in the peer-reviewed literature and/or (b) studies submitted to the EPA. Studies on chemical mixtures, in vitro studies, reports where ≥5 g/kg of the chemical was administered, and reports with inappropriate statistical analyses were excluded. The definition of neurotoxicity was broad, encompassing gross structural changes (e.g., reduced brain weight, spina bifida, exencephaly) to more subtle indices of DNT (e.g., changes in brain morphology, neurochemical imbalances, and behavioral impairments). Of the chemicals examined, ~50% were assigned to the no evidence of developmental neurotoxicity group; ~25% had minimal evidence; and the remaining 25% had substantial evidence of toxicity to the developing nervous system. The chemicals in the latter group will be especially useful for vetting protocols that have been proposed as screens for developmental neurotoxicity. (This abstract does not necessarily reflect Agency policy)

**PL 1376** HEXABROMOCYCLODODECANE (HBCD) INHIBITS THE DEPOLARIZATION-INDUCED INCREASE IN INTRACELLULAR CALCIUM LEVELS AND NEUROTTRANSMITTER RELEASE IN PC12 CELLS.

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Neurotoxic effects have been described for several brominated flame retardants (BFRs). The BFR hexabromocyclodecane (HBCD) has been shown to cause adverse effects on learning and behavior in mice, as well as on dopamine uptake in rat synaptosomes and synaptic vesicles. As other BFRs have previously been demonstrated to alter the intracellular Ca2+ homeostasis, the aim of this study was to investigate whether the technical HBCD mixture and individual HBCD-stereoisomers (α-, β- and γ-HBCD) affect the intracellular Ca2+ concentration ([Ca2+]i) in a neuroendocrine in vitro model (PC12 cells). ([Ca2+]i) and vesicular catecholamine release were measured using respectively imaging of the fluorescent Ca2+ sensitive dye Fura 2 and fura-2 calciumometry. Exposure to 25 µM HBCD increased the amount of free calcium in PC12 cells, and released dopamine from vesicles. KCl induced an increase in [Ca2+]i that was dose dependent in the presence of 0.5 µM HBCD. ([Ca2+]i) was increased only when HBCD was applied at least 5 min before depolarization, while the maximum effect was observed after 20 min exposure. Additionally, using specific blockers of L- or P/Q-type voltage-gated Ca2+ channels (VGCCs), it was shown that the inhibitory effect of HBCD is VGCC-a-specific. Interestingly, the effects of α- and β-HBCD were similar to that of the technical mixture, whereas the inhibitory effect of γ-HBCD on evoked [Ca2+]i was larger. Summarizing, HBCD inhibits depolarization-evoked [Ca2+]i, and neurotransmitter release. As increasing HBCD levels resulted in a higher response, these findings just additioal efforts to improve (isomer-specific) exposure, hazard and risk assessment.

**PL 1377** THE ROLES OF CHONDROMODULIN I AND NQO1 IN THE INHIBITION OF TUBE FORMATION INDUCED BY THE BENZENE METABOLITE HYDROQUINONE IN HUMAN BONE MARROW ENDOTHELIAL CELLS.

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Bone marrow is one of the major targets of benzene toxicity and NAD(P)H:quione oxidoreductase (NQO1), a protective enzyme against benzene toxicity, is present in human bone marrow endothelial cells which form the hemopoietic stem cell vascular niche. Therefore, we have employed a transformed human bone marrow endothelial cell (TrHBMEC) line to study the adverse effects of the benzene metabolite hydroquinone (HQ) at the level of bone marrow endothelium. In this study, HQ (10µM) inhibited TrHBMEC tube formation at concentrations which were not overly toxic as demonstrated by trypan blue exclusion or MTT analysis. HQ up-regulated chondromodulin I (ChMI), a protein that promotes chondrocyte growth and inhibits endothelial cell growth and tube formation. We also showed that recombinant human ChMI protein could inhibit TrHBMEC tube formation. Anti-ChMI siRNA was used to deplete ChMI mRNA and block the induction of ChMI by HQ. Pretreatment with anti-ChMI siRNA abrogated HQ induced inhibition of tube formation in TrHBMEC. Overexpression of NQO1 in TrHBMEC inhibited the up-regulation of ChMI and abrogated the inhibition of tube formation induced by HQ. Similar up-regulation of ChMI and inhibition of tube formation by HQ treatment was also observed in human umbilical vein endothelial cells (HUVEC) demonstrating that the effects observed are not cell line specific. In summary, HQ treatment up-regulated ChMI and inhibited tube formation in both TrHBMEC and HUVEC. The inhibition of tube formation in TrHBMEC occurred at least in part, through up-regulation of ChMI. The protective enzyme NQO1 inhibited HQ induced up-regulation of ChMI in cells from HQ induced inhibition of tube formation. This study provides a mechanism that may contribute to benzene induced toxicity at the level of bone marrow endothelium. (Supported by NIH grant ES05954)

**PL 1378** GENOTOXICITY AND CELL CYCLE EFFECTS OF A CHICAGO AIRBORNE POLYCHLORINATED BIPHENYL MIXTURE AND AROCLORS IN LUNG FIBROBLASTS.

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Polychlorinated Biphenyls (PCBs) are environmental pollutants that produce various toxicities in humans and animals. Research till now has focused on the commercial mixtures (Aroclors) and individual PCB congeners and no work has been done on the toxicities of airborne PCB mixtures, to which humans are exposed directly. To study the effects of Chicago air PCBs, a similar synthetic “Airborne mixture” was produced. This study is undertaken to determine cytotoxicity, DNA damage and cell cycle effects of this Chicago Airborne PCB mixture and compare it with Aroclor 1242 and Aroclor 1254. Chinese Hamster lung fibroblasts (V79) were exposed for 24 h to the mixtures at concentrations from 1 to 100 µM. Cytotoxicity increased dose-dependently between 10-100 µM concentrations, with Aroclor 1254 >> Airborne PCBs > Aroclor 1242. Micronuclei levels were significantly increased in all groups at 10 and 25 µM concentration, with Aroclor 1254 showing the highest effect and two thirds of the micronuclei being produced by the
loss of a chromosome fragment, not due to whole chromosome loss. Microtubuli could not be counted at 50 and 100 μM due to the high cytotoxicity and damaged nuclei. In the cell cycle phase distribution study, control cells had 32% of cells in G0-G1 phase, 8% in G2-M phase and 60% in S phase. None of the mitudes changed this phase distribution from 1 to 25 μM, but cells started to accumulate in S-phase at 50 μM concentration with the highest percentage in Aroclor 1254 treated samples. In conclusion, Aroclor 1254, which contains higher chlorinated PCB congeners, had the most potent toxic effects suggesting that the airborne PCBs in our inner cities may not be as biologically harmful as feared. However, studies with metabolically competent lung cell lines should be performed to exclude specific organ toxicity due to metabolic activation of the lower chlorinated congeners present in the airborne mixture. (Supported by NIEHS P42ES013661, DOD DAMD17-02-1-0241, and CHEEC)

PL 1379 TOXICOGENOMIC EVALUATION OF PCB153-ELICITED HEPATIC EFFECTS IN C57BL/6 MICE.

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Polychlorinated biphenyls (PCBs) are ubiquitous contaminants found as complex mixtures of coplanar and non-coplanar congeners. Since non-coplanar PCBs do not elicit dioxin-like effects, their toxicity cannot be equated to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The hepatic temporal and dose-dependent effects of the most abundant non-dioxin-like congener, 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB153), were examined in the immature ovariectomized C57BL/6 mice. Animals were gavaged once with 600 mg/kg PCB153 or sesame oil vehicle and sacrificed 2, 4, 8, 12, 18, 24, 72, 120 or 168 h after dosing. In the dose-response study, mice were treated by gavage with 1, 5, 10, 30, 100, 300 and 600 mg/kg PCB153 or sesame oil for 4 h. Significant increases in relative liver weights were noted at 72, 120 and 168 h (600 mg/kg) and at 24 h (300 and 600 mg/kg), accompanied by slight vacuolization. PCB153 altered the hepatic expression of 587 and 177 genes using Agilent 4 x 44 K microarrays (|fold change| ≥ 2, p-value < 0.001) in the time course and dose-response studies, respectively. Prototypical constitutive androstane and pregnane x receptor (CAR/PXR) regulated genes including Cyp2b10, Cyp3a11, Cyp4a14 and Abcc transporters were induced. Functional annotation associated differential gene expression with drug metabolism, DNA damage and growth arrest, transport and lipid metabolism. Computational promoter analysis of the highest induced gene, Cyp2b55 (|fold increase| ≥ 2, p-value < 0.001) identified putative CAR/PXR response elements. Moreover, hepatic PCB153 levels paralleled differential gene expression. Preliminary examination suggests that PCB153 elicits a unique gene expression profile when compared to TCDD that may be mediated by CAR/PXR. Funded by SBRP P42ES049131.

PL 1380 THE PAR4 SUBSTITUTION IS A KEY DETERMINANT OF ACTIVITY OF BROMINATED DIPHENYL ETHERS TOWARD THE TYPE 1 RYANODINE RECEPTOR.

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Polybrominated diphenyl ethers (PBDEs) are widely used flame retardants that bioaccumulate in human and animal tissues. Structurally similar non-coplanar polychlorinated biphenyls (PCBs) sensitize microsomal Ca2+ channels known as ryanoide receptor (RyR). In this study, we investigated the structure activity relationship for select PBDEs and their respective methoxy metabolites towards the RyR type 1 (RyR1) using [3H]Ryanoide ([3H]Ry) binding analysis and measurements of Ca2+ efflux. Para-bromo substitution(s) of were found to critically influence activity toward RyR1. BDE-49 (2,2′,4′-tetrabromo) showed similar activity to BDE-17 (2,2′,4′-tribromo), whereas BDE-42 (2,2′,3′-tetrabromo) showed 3.5 fold higher potency but 2.5 fold lower efficacy. Addition of a methoxy group to the free para-position reduced the potencies and efficacies of the corresponding parent congener. BDE-49 and its metabolite elicite the release of Ca2+ from microsomal vesicles and these effects are completely abolished with the RyR1 blocker ruthenium red. BDE-47 (2,2′,4′-tetrabromo) had a minimal effect on RyR1 activation. Collectively these results indicated that penta-substitution diminishes the activity of PBDEs toward RyRs. Considering RyRs are broadly expressed in excitable and non-excitible cells, these data provide a structure-activity to study how PBDEs influence cellular Ca2+ signaling and Ca2+-dependent processes. Supported by ES11269, ES04699 and ES015171

PL 1381 METABOLISM OF PBDE 47, 99 AND 153 BY HUMAN LIVER MICROSOMES.

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Polybrominated diphenyl ethers (PBDEs) 47 (2,2′,4′-tetrabromo), 99 (2,2′,4′,5′-pentabromo) and 153 (2,2′,4′,5′,3′,4′-hexabromo) are the most abundant congeners that bioaccumulate in humans and biota. The metabolism of PBDEs may contribute to their relative persistence and toxicity, which may in part be due to the formation of metabolites with endocrine-disrupting effects. However, the metabolism of BDEs in humans has not been well documented. This study determined the degree of BDE 47, 99, and 153 metabolism in human liver microsomes and identified specific metabolites formed. Radiolabelled or non-radiolabelled BDE 47, 99, or 153 were incubated for 2 hrs with a pooled specimen of human liver microsomes and specimens from 3 individual donors (A,B,C). After liquid-liquid extraction, radiolabelled samples were analyzed using HPLC/radiochemical detection (RCD) while non-radiolabelled samples were analyzed before and after derivatization using GC/MS. The metabolism of BDE 47 and 99 was found to be linear for up to 2 hrs, while no detectable metabolites were observed for BDE 153. Specimen A had the highest activity, with 51% of BDE 47 and 52% of BDE 99 converted to metabolites over 2 hrs. Lower activity was observed for specimens B and C, with only 9% and 12% of BDE 47 and 12% and 13% of BDE 99 converted to metabolites by the respective specimen. Metabolism of BDE 47 produced two brominated metabolites, one dibrominated compound indicating a cleavage of the ether bond and one dihydroxylated tetra BDE. BDE 99 produced three brominated metabolites, two dibrominated metabolites, an epoxide and monohydroxylated compound, as well as a dihydroxylated penta BDE. This initial study further indicates that there are marked inter-individual differences in the ability of human liver microsomes to metabolize BDE 47 and 99, which could contribute to individual difference in the resulting retention and the potential toxicity of these agents.

PL 1382 TOXICITY OF HIGHLY PURIFIED PCB 180 IN RATS.

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PCB 180 is a non-dioxin-like polychlorinated biphenyl (NDL-PCB) abundantly present in food and the environment. Risk characterisation of NDL-PCBs has been confounded by the presence of highly potent dioxin-like (DL) impurities. We used, therefore, highly purified PCB 180 (DL impurities 2.7 ng TEQ WHO/g PCB 180) for a 28-day toxicity study in young adult rats. Groups of 5 males and 5 females were given total doses of 5, 10, 30, 100, 300, 1000 and 1700 mg/kg PCB 180 by gavage. Total dose was divided into 6 daily loading doses and 3 weekly maintenance doses. No increase in serum ALAT or APHOS activity was found, while increased liver weight and centrilobular hepatocellular hypertrophy were observed at >100 and >300 mg/kg bw in males and females, respectively. A significant increase in liver pentoxysorcinol O-dealkylation (PROD) was found in males at >10 mg/kg bw and in females at >30 mg/kg bw. In both genders, a significant induction of hepatic 7-ethoxycoumarin O-deethylase (EROD) activity was also observed. The CYP induction pattern indicates that PCB 180 acts as an agonist of the constitutive androstane receptor (CAR) and may also have some agonistic activity at the Ah receptor. Plasma T4 and to some extent T3 were dose-dependently decreased in association with thyroid follicle depletion. Red blood cell parameters were dose-dependently decreased in both genders. Thyroid weight was not affected. The results demonstrate a distinct toxicological profile for PCB 180 with some similarities with that of DL compounds, but clearly lower potency. Funded by the European Commission (ATHON, FOOD-CT-2005-022923).
1383 INTESTINAL PERMEABILITY AND DISPOSITION OF CHLORPYRIFOS AND CHLORPYRIFOS-OXON IN THE SINGLE PASS INTESTINAL PERFUSION IN THE RAT.

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Chlorpyrifos (CPF), a common organophosphate pesticide, is metabolized in mammals to the active metabolite chlorpyrifos-oxon (CPF-O), which is primarily responsible for the observed toxicity. As part of our efforts for more accurate exposure modeling, we evaluated the intestinal permeabilities of CPF and CPF-O at environmental levels using the single-pass intestinal perfusion technique with mesenteric veins. Blood samples were collected over the entire perfusion period of 60 min. The perfusate was switched to blank buffer. Outlet perfusion samples and mesenteric vein sampling in rats. Tritiated CPF (25, 250 and 2500 ng/ml) and CPF-O were approximately 10% of the perfusate. After switching the perfusate to the blank buffer system, CPF and CPF-O unexpectedly continued to appear in the blood, which suggests that the intestine was acting as a reservoir for the compound under these experimental conditions. The high luminal and blood permeability values and the apparent sequestering of the two compounds support the use of this technique for exposure modeling and risk assessment.

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1384 EARLY-LIFE ORGANOPHOSPHATE EXPOSURE DISRUPTS LIPID METABOLISM IN ADULTHOOD.


Organophosphate pesticides are developmental neurotoxins but new findings point to lasting effects on metabolism, abnormal weight gain and prediabetes. We gave parathion to rats on postnatal days 1-4, using doses straddling the threshold for early-detectable cholinesterase inhibition and the first signs of systemic toxicity (0.1 or 0.2 mg/kg). In adulthood, we evaluated serum adipokines, lipoprotein and adiponectin, as well as standard lipid markers. Parathion decreased adiponectin and β-hydroxybutyrate in males, while decreasing cholesterol, nonesterified fatty acids and β-hydroxybutyrate in females. When animals were fed a high-fat (HF) diet for 7 weeks, further abnormalities emerged. In controls, there were increases in adiponectin, leptin and all serum lipid markers, verifying the metabolic effect of the HF diet. Males who had received the low neonatal dose of parathion showed an exaggerated increase in adiponectin and leptin on the HF diet, whereas females in the high dose group displayed a decrease in leptin relative to controls fed the HF diet. The sex differences were also apparent in the weight gain elicited by the dietary change: parathion-exposed males gained the same additional weight as controls on the HF diet, whereas females showed enhanced weight gain in the low dose group and suppressed weight gain in the high dose group. These results are consistent with long-lasting perturbations of lipid metabolism even at neonatal parathion exposures that produce minimal signs of exposure. Decreased adiponectin is associated with increased insulin resistance, diabetes, and cardiovascular disease. Further, changes in leptin and adiponectin are likely to play key roles in the weight gain caused by increased dietary fat intake, as these signals are responsible for communicating metabolic status to brain areas that control diet and metabolism. Our findings thus provide further evidence that early-life toxicant exposures may play a contributory role in the explosive increase in the incidence of metabolic dysfunction, obesity, and diabetes. (NIH ESI10356, Leon Golberg Fellowship)

1385 METABOLISM AND CHOLINESTERASE INHIBITION IN LUNGS OF RATS FOLLOWING IN VITRO CHLORPYRIFOS EXPOSURE.

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Chlorpyrifos (CPF) is an organophosphorus insecticide that is routinely applied to crops as a aerosol spray, hence there is potential for workers and bystanders to be exposed via inhalation. Though inhalation represents a primary occupational route of exposure, little is known about lung metabolism or cholinesterase (ChE) response following a CPF exposure. Hence, this study evaluated the in vitro metabolism and ChE response following exposure to CPF or its active metabolite CPF-Oxon in rat lung tissue. Rat microsomes were prepared from naive S-D rat lungs and liver. The comparative metabolism of CPF to the major metabolites trichloropyridinyl (TCPy) was evaluated over a range of CPF concentrations (50 – 500 μM), and the amount of TCPy generated was analyzed by GC/MS. The maximum velocity (Vmax) of lung metabolism was ~44% of the liver (0.72 vs. 1.62 μg/mg/min protein) and the predicted Km for the lung and liver microsomes were 5.9 and 21 μM. For in vivo ChE determination, whole lung from naive S-D rats were homogenized and optimally diluted for the Ellman assay. Substrates for the assay included acetylthiocholine (ATC) and butyrylthiocholine (BTC). The lung homogenates were then incubated with a broad range of CPF-Oxon concentration (1 – 14 nM). Overall, the BTC substrate was ~43% of the ATC response, suggesting that butyrylcholinesterase (BuChE) represents ~40% of the lung ChE activity. The estimated IC50 for CPF-Oxon ChE inhibition in lung for ATC and BTC were 76.5 and 52.7 nM, respectively. These in vivo studies confirm that the rat lung has the capacity to metabolize CPF and CPF-Oxon can readily inhibit lung ChE activity. Future in vivo studies will be conducted to quantify the lung pharmacokinetics and pharmacodynamic dose-response. Finally, these data will then be utilized to further refine the CPF PB/PK/PD model to accommodate inhalation exposure. (Supported by CDC/NIOSH grants R01 OH008173 & OH003629)

1386 SEX-SPECIFIC CIRCADIAN VARIATION IN PHYSIOLOGICAL AND MOLECULAR RESPONSES TO PESTICIDES.

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Circadian clocks synchronize internal biochemical and physiological rhythms to daily light/dark cycles. Many genes, including many involved in xenobiotic metabolism, reveal circadian expression rhythms. Many of the same genes exhibit sex-dependent induction after xenobiotic exposure. Utilizing Drosophila melanogaster as a model organism, we describe crosstalk between the circadian clock, sex, and xenobiotic metabolism. Flies were maintained in 12h:12h light/dark conditions, or moved to constant darkness or light prior to testing. Flies were acutely exposed to a model organ, we describe crosstalk between the circadian clock, sex, and xenobiotic metabolism. Flies were maintained in 12h:12h light/dark conditions, or moved to constant darkness or light prior to testing. Flies were acutely exposed to a dose of propoxur, malathion, deltamethrin, or permethrin by moving them to scintillation vials coated on the interior with chemical for one hour. Exposures or collections were every four hours for 24 hours. Mortality was scored two days later. Collected flies were processed for qRT-PCR, or to assay enzyme activity. Comparing dose responses at different times of day, we have established sex-specific daily susceptibility profiles. Females were generally less sensitive than males, and in the case of propoxur, LC50 was 100 fold that of males at ZT4. Similar rhythms were found in enzyme activities associated with xenobiotic metabolism, including ECOD, esterase, and GST activity. Mortality and enzyme activity rhythms continue in constant darkness and are lost in constant light, as has been observed in other clock-controlled rhythms. Using qRT-PCR, we are currently comparing daily expression profiles of clock genes, nuclear receptors, and xenobiotic metabolizing genes in males and females. Our previous work has indicated that defects in the central clock genes Pdp1 and CLK/CYC lead to increased susceptibility to pyrethroids in male flies. Our future work includes comparing daily susceptibility, expression, and enzyme activity profiles of these flies to wild-type males and females. This work will detail how the circadian clock modules sex differences in xenobiotic metabolism in Drosophila. This will in turn lead to further understanding of how circadian disruption affects health in humans.

1387 STRUCTURAL INSIGHT INTO INHIBITION/AGING OF NEUROPATHY TARGET ESTERASE (NTE) FROM X-RAY CRYSTAL STUDIES OF ITS CATALYTIC DOMAIN HOMOLOGUE, PATATIN-17 (PAT17).

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NTE is a neuronal protein of unknown structure whose inhibition/aging by organophosphorus compounds (OPs) is linked to OP-induced delayed neurotoxicity, OPIDN. We hypothesized that NTE inhibition/aging results in toxic gain of function via conformational change. To test this hypothesis, we solved the crystal structure of the NTE catalytic domain homologue, pat17, inhibited/aged with diisopropylphosphorofluoridate (DFP). We purified histagged pat17 via affinity and size-exclusion chromatography and determined the 20-min 50 of DFP against its phenyl valerate hydrolase activity to be 172±μM. We grew single crystals of DFP-inhibited pat17 within one week via vapor-diffusion at 4°C in 0.1M Na-acetate (pH

Voltage-gated sodium channels are important sites for the neurotoxic actions of pyrethroid insecticides in mammals. The pore-forming α subunits of mammalian sodium channels are encoded by a family of 9 genes, designated Nav1.1 - Nav1.9. Native sodium channels in the adult central nervous system (CNS) are heteromeric complexes of one of these 9 α subunits and two auxiliary (β) subunits. Here we compare the functional properties and pyrethroid sensitivity of the rat and human Nav1.3 isoforms, which are abundantly expressed in the developing CNS and are potential targets for developmental neurotoxic effects of pyrethroids. Coexpression of the rat Nav1.3 and human Nav1.3 α subunits in combination with their respective β1 and β2 subunits in Xenopus laevis oocytes gave channels with markedly different inactivation properties and sensitivities to the pyrethroid insecticide tefluthrin. Rat and human Nav1.3 channels differed in their kinetics of fast inactivation and their voltage dependence of steady-state inactivation. Exposure of rat and human Nav1.3 channels to 100 μM tefluthrin in the resting state produced populations of channels that activated, inactivated and deacti- vated more slowly than unmodified channels. Application of trains of depolarizing prepullses enhanced the extent of tefluthrin modification of both channels approximately twofold; this result implies that tefluthrin may bind to both the resting and open states of the channel. Modification of rat Nav1.3 channels by 100 μM tefluthrin was four-fold greater than that measured in parallel assays with human Nav1.3 channels. Human Nav1.3 channels were also less sensitive to tefluthrin than rat Nav1.2 channels, which are considered to be relatively insensitive to pyrethroids. These data demonstrate that orthologous channels with a high degree of amino acid sequence conservation differ in both their functional properties and their sensitivities to pyrethroid insecticides.

**1389**

**DO PESTICIDES CAUSE PARKINSON'S DISEASE? SYNERGISTIC EFFECTS OF PESTICIDES ON CELL DEATH AND PROTEASOME ACTIVITY IN A DOPAMINERGIC CELL LINE.**


According to epidemiological and laboratory studies, pesticide exposure is a risk factor for Parkinson’s disease (PD). People are usually exposed to mixtures of pesticides rather than to individual chemicals, but the toxicity of complex pesticide mixtures (i.e. more than binary combinations) is not well understood. We hypothesized that commonly used pesticides would cause more than additive effects on PD associated endpoints.

We examined the effects of chlorpyrifos (CPF), 2,4-D, dieldrin, atrazine, trifluralin (TFL), glyphosate, paraquat, mancozeb, maneb and chlorpyrifos on cell viability, proteasome activity, and superoxide (O2−) formation (assessed by XTT, succ-LTVY-AMC, and hydroethidine, respectively) in a dopaminergic rat midbrain cell line (N27). Using an effect addition approach, we tested combinations of the most toxic pesticides (CPF, TFL, 2,4-D and dieldrin). Three of six binary combinations synergistically reduced proteasome activity and caused cell death, but had no effect on O2− formation. Tertiary combinations: CPF + TFL + dieldrin and 2,4-D + TFL + dieldrin were synergistic in all three assays. Finally, the combination of all four pesticides was synergistic in all assays. The PD type cell death was confirmed by immunocytochemistry showing loss of cell and nuclear integrity, and ubiquitinated protein inclusion bodies in exposed cells. Our results show that complex pesticide mixtures have synergistic effects on PD related endpoints in dopaminergic cells in vitro. These results will provide a better foundation for risk assessment, and elucidate the mechanisms responsible for the epidemiological association between pesticide exposure and PD. In future studies we will use a complex pesticide mixture with a range design to determine which pesticides have more than additivity and the magnitude of the interactions. Supported in part by NIH grant R01 ES014675-01 to RFS.

**1390**

**DICHLORVOS- AND METHOMYL-INDUCED RESPIRATORY TOXICITY RESULTS FROM CENTRAL MUSCARINIC EFFECTS.**

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Objective: We previously showed atropine (A) but not methylatropine (MeA) induced the correction of paraoxon-induced respiratory toxicity. The aim of this study was to assess the peripheral or central origin of respiratory toxicity induced by dichlorvos (D), and a carbamate, methomyl (M). Methods: male Sprague-Dawley rats were poisoned using D (5.76 mg.Kg-1; i.e. 45% of the sc MLD) or M (2.3 mg.Kg-1; i.e. 50% of the ip MLD). Poisoned rats were treated with A (base: 10 mg.Kg-1) or equimolar MeA (base: 5.42 mg.Kg-1) by intramuscular injection at the time of maximal respiratory effects, 5 min post injection of D or M. Respiratory function was assessed using whole body plethysmography and central temperature using infra-red telemetry. Results are expressed as mean +/- SEM. Statistical analy- sis used parametric tests with p<0.05. Results: In rats with intraperistaltic telemet- riy, greater dose of D or M than about 50% of the MLD resulted in death. M but not D induced a significant decrease in core temperature. D and M induced a de- crease in respiratory rate resulting from an increase in expiratory time. M but not D increased the tidal volume. The onset of respiratory toxicity occurred 5 min after injection for both D and M. The decrease in respiratory rate induced by M and D lasted 20 and 30 min, respectively. A (10 mg.Kg-1) completely reversed the D- and M-induced respiratory toxicity while an equimolar dose of MeA (5.42 mg.Kg-1) was without significant effects. Discussion: A crosses the blood-brain barrier and induces peripheral and central muscarinic effects. In contrast, MeA does not cross the blood-brain barrier only resulting in peripheral effects. In addition to paraoxon, our study showed that A resulted in the complete correction of D- and M-induced respiratory toxicity. In contrast, MeA did not induce any significant effect. We con- clude the respiratory toxicity induced by anticholinesterase insecticides including paraoxon, dichlorvos, and methomyl at dose about half the MLD results from ef- fects mediated by central muscarinic receptors.
1392 HAPLOTYPING OF THE FULL XPC GENOMIC SEQUENCE REVEALS A CLUSTER OF VARIANTS ASSOCIATED WITH SENSITIVITY TO THE GENOTOXIC EFFECTS OF TOBACCO SMOKE.

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The Xeroderma pigmentosum complementation group C protein, encoded by the XPC gene, plays a key role in the nucleotide excision repair process. XPC is highly polymorphic, but only a few single nucleotide polymorphisms (SNPs) have been studied as modifiers of cancer risk. To date, the phenotypic effects of these SNPs have not been characterized, not has their impact on DNA damage response and DNA repair capacity been determined. In this study, we constructed a comprehensive haplotype map encompassing the common SNPs in the XPC gene, and evaluated their effect on DNA damage associated with smoking, using chromosome aberrations (CA) as a biomarker. We hypothesized that if certain haplotypes have phenotypic effects, there would be a correlation between these haplotypes and CA in smokers. Our results indicate that out of 92 SNPs identified, 35 had a minor allele frequency ≥0.05. A haplotype-tagging (ht) approach was used to identify 12 htSNPs representing these 35 SNPs. We used these htSNPs to genotype a population of smokers matched to non-smokers (n=123), and to construct corresponding haplotypes. Out of 48 haplotypes we identified 20 that existed with a frequency ≥0.05. Phylogenetic analysis revealed that these 20 haplotypes segregated into 5 distinct phylogenetic groups of haplotypes (PGHs A to E). When we evaluated the relationship between these PGHs and CA, we observed that smokers with PGH-E had double the mean CA frequency (mean CA/100 cells ±SEM = 1.25±0.27) compared to smokers with other haplotypes (0.56±0.17). We also observed significant interactions between smoking and PGH-B (p=0.042). Among those negative for PGH-B, CA was 1.35 times higher in smokers compared to non-smokers; whereas among those who were positive for PGH-B, CA was 41% less in smokers compared to non-smokers. Given the strong association between CA and cancer, our data suggest that XPC haplotypes could significantly affect the risk of smoking-related cancers.

1393 GENETIC INSTABILITY IN THE PERIPHERAL LYMPHOCYTES AND BUCCAL CELLS OF HEAD AND NECK CANCER PATIENTS AND THEIR FIRST DEGREE RELATIVES: INFLUENCE OF XRCC1 ARG399GLN POLYMORPHISM?

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It may be important to know the influence of genetic polymorphisms on chromosome damage with respect to the improvement of cytogenetic biomarkers and also to the identification of at-risk groups. We studied the baseline micronucleus (MN) and radiation-induced micronucleus (Ind-MN) frequencies in lymphocytes and the buccal cell MN frequencies and influence of XRCC1 gene polymorphism (Arg399Gln) both for head and neck cancer (HNC) patients (n=59) and their first degree relatives (FDRs) (n=54) as well as controls (n=31). The XRCC1 alleles were detected using the PCR-RFLP technique. For the Ind-MN assay, blood samples were exposed to 2 Gy gamma rays (60Co) at a dose rate of 0.62 Gy/min. The mean(SD)MN and Ind-MN frequencies(%)in lymphocytes of HNC patients, FDRs and controls were 27(19.5), 172.7(44.8),14.1(5.2),169.5(47.1) and 9.0 (6.9), 150.9(45.3), respectively. Significant differences in baseline MN frequencies between studied groups were found (p<0.05), however, there were no differences in Ind-MN frequencies between all groups (p>0.05).Significant differences in buccal MN frequencies between HNC patients and FDRs as well as controls were found (p<0.001). Number of subjects with wild genotype (Arg/Arg), heterozygous subjects (Arg/Gln) and mutant subjects (Gln/Gln) are 23, 12, 12; 21,13, 14 and 8,5,5 for patients, FDRs and controls, respectively. Our data suggest that MN frequencies in lymphocytes and buccal cells may be predictive for cancer risk. It might be possible to identify high risk family members by analyzing background MN frequencies in lymphocytes but not in buccal cells. XRCC1 variant genotypes did not significantly modulate the frequency of MN in studied groups. Further work is needed to resolve the importance of other polymorphisms of XRCC1 including codon 194, as well as other DNA repair systems.

1394 CYP1A1 (Ile462Val) POLYMORPHISM, RESPONSE TO CHEMOTHERAPY AND SURVIVAL IN ADVANCED NON-SMALL CELL LUNG CANCER PATIENTS: IS THERE ANY ASSOCIATION?

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Lung cancer is an increasing worldwide public health problem particularly in men. Recently, the mutations in CYP1A1 gene have been suggested to be responsible for decreasing of the survival rates of patients with non-small cell lung cancer (NSCLC) by forming aggressive tumors by P53 inactivation and thereby altering sensitivity to chemotherapy. However, the limited studies, which focused on the influence of CYP1A1 polymorphism, did not provide data with respect to their relation to response to therapy, had contradictory results. In this study, the association between CYP1A1 (Ile462Val) polymorphism, and response to platinum based chemotherapy and survival rates in 138 (126 men and 12 women) NSCLC patients who are treated with platinum based drugs have been investigated. The polymorphism of CYP1A1 (Ile462Val) did not significantly influence the responses to platinum based chemotherapy. No significant associations were noted between the responses of genotypes to chemotherapy and age, sex, smoking status, chemotherapy treatment status, tumor stage and histology. Significant survival was not observed in patients with CYP1A1 genotypes (median survival of 18 months for wild type (Ile/Ile) genotype and 16 months for variant (Ile/Val or Val/Val) genotype (p<0.523). Multivariate analysis, (after adjustment for age, gender, tumor histology, disease stage, smoking status and response to chemotherapoy) also revealed no significant hazard ratio (HRs) of death associated with CYP1A1 genotypes (HR, 1.46; 95% CI, 0.69-3.08, p=0.318). These results showed that there is no association between CYP1A1 genotype and response to chemotherapy or survival in advanced NSCLC patients (Supported by Research Fund of Ankara University, Grant No: 2007/0830/505/DPI).

1395 ROLE OF POLYMORPHIC HUMAN CYTOCHROME P450 ENZYME IN ESTROGEN METABOLISM AND BREAST CANCER RISK IN THAI WOMEN.

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Estrogen and its metabolites are believed to play important roles in breast cancer and its determinants include both genetic and lifestyle factors. The objective of the study is to investigate association of breast cancer risk in Thailan with genetic polymorphisms in several genes involved in estrogen synthesis and metabolism. Five hundred and seventy patients with histopathologically confirmed breast cancer and 497 controls were included in the present study. Forty single nucleotide polymorphisms (SNPs) in the CYP1A1, CYP1A2, CYP1B1, CYP17, CYP19, CYP2C19, AhR, ER1, PGR, ERRG, COMT, HSD17B2, HSD17B1, EPHX1, and NQO1 genes were genotyped. Association of genotypes with breast cancer risk were evaluated using multivariate logistic regression. Heterozygote carriers of SNPs in CYP2C19 (rs917623), AhR (rs2066855), ERRG (rs1857407) and homozygote carriers of SNPs in CYP1A2 (rs762551), CYP2C19 (rs4917623), ERRG (rs945453) had altered risk of developing breast cancer. In addition, a stratified analysis by menopausal status indicated that the association of the CYP1A2 (rs762551) and CYP17 (rs45572) polymorphisms with breast cancer risk were mainly evident in premenopausal, while those of CYP1B1 (rs62556) and ERRG (rs1857407) significant in postmenopausal women. These findings suggest that CYP1A2, CYP1B1, AhR, ERRG, and CYP1B1 polymorphisms may play an important role in estrogen metabolism and modify individual susceptibility to breast cancer in Thai women.

1396 UNEXPECTED DEATH DUE TO METHADONE OVERDOSE MAY BE ASSOCIATED WITH GENETIC POLYMORPHISMS OF THE CYP3A4 GENE.

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Methadone usage can lead to unexpected death due to inter-individual variability in its pharmacokinetics. CYP3A4 is a key P450 involved in the metabolism of methadone to 2-ethyl-1,5-dimethyl-3,3-diphenylpropionlde (EDDP). The methadone/EDDP ratio was higher in individuals who had overdosed on
methadone compared to those successfully undergoing maintenance treatment in a retrospective study of methadone-only deaths (WV Office of the Chief Medical Examiner). A high methadone/EDDP ratio may be associated with a poor-metabolizer phenotype for methadone due to a genetic defect associated with one or more single nucleotide polymorphisms (SNPs) in the CYP3A4 gene. To assess this possibility, 93 individuals who had died of a methadone-only overdose were genotyped at four different SNP sites on the CYP3A4 gene. Genomic DNA was isolated from blood collected during autopsy, and SNP genotypes were determined by Taqman Allelic Discrimination Analysis. All loci studied were within Hardy-Weinberg Equilibrium. The observed genotype frequencies were not significantly different from those expected based on population statistics for the rs2246709, rs3753541, and rs6646437 SNP loci. The genotype frequency was significantly different than the frequency for the general population (p=0.001) for the rs2246480 SNP. Western blotting for CYP3A4 protein was performed on nine different human liver microsomal samples from overdosed individuals. Three of the samples with the highest methadone/EDDP ratio did not appear to have detectable amounts of CYP3A4 and five samples with the lowest methadone/EDDP ratio had detectable amounts of CYP3A4. These initial findings indicate that a genetic polymorphism on the CYP3A4 gene may contribute to unexpected death from methadone due to decreased function and/or production of CYP3A4.

1397 DETECTION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN CYTOCHROME B5 PROMOTER.

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Cytochrome b5 (CYB5A) and cytochrome b5 reductase (CYB5R) catalyze the detoxification reaction of hydroxylamine metabolites of arylamine drugs, specifically sulfamethoxazole hydroxylamine (SMX-HA) to its amine parent sulfamethoxazole (SMX). In the absence of this reaction, the SMX-HA intermediate metabolite is spontaneously transformed to sulfamethoxazole nitros (SMX-NO). Consequently, SMX-NO is thought to lead to sulfonamide hypersensitivity reactions due to formation of a hapten. The effectiveness in detoxifying the SMX-HA metabolite mainly depends on the variability in gene expression and catalytic activity of CYB5A and CYB5R. The aim of this study was to screen individual human liver samples with outlying high or low activity for SMX-HA reduction or outputting CYB5A protein expression, for single nucleotide polymorphisms (SNPs) in the promoter region of CYB5A, specifically from +1 to +551 bp. Among 55 individuals, we found 5 novel promoter SNPs and 1 known promoter SNP (rs3769396). Four of the SNPs were located in the silencer region (-302 bp to -449 bp). Two of these SNPs may be important in the regulation of CYB5A gene expression at the transcriptional level. First, rs3769396 (S365 UAA) was associated with high protein expression and median activity. Secondly, the rs98 G/A SNP was associated with both low protein expression and activity. Finally, an individual who had high protein expression and moderately high activity was heterozygous for a -458 C/T SNP.

In addition, we also found two cases of linkage disequilibrium. One was the SNP rs1502135 cDNA also linked to SNP at -214 C/T.

1398 GENOTYPE DETERMINES SUSCEPTIBILITY TO MERCURY TOXICITY: STUDIES IN TRANSFORMED CELLS EXPRESSING COPROPORPHYRINOGEN OXIDASE (CPOX) AND ITS GENETIC VARIANT CPOX4.

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CPOX catalyzes the 2-step deacetylation of coproporphyrinogen-III (Cp) to proto-porphyrinogen-IX (Pp) with the intermediate formation of hæmoporphyrinogen (Hp) in the hæm biosynthetic pathway. Previously, we identified a polymorphism (A814C) in exon 4 of the human CPOX gene (CPOX4) and showed that the catalytic activity of cloned CPOX4 was ~50% that of the wild type (WT) enzyme in vitro. We obtained comparable findings in human liver samples from CPOX WT, Het and CPOX4 homozygous (Mut) subjects. We also showed that mercury (Hg2+) specifically inhibits the second step of Cp deacetylation (Hp to Pp) in a dose dependent manner both in vitro and in liver human fractions. Here, we established a CPOX expression cell model using Epstein Bar virus (EBV) transformed lymphocytes from human subjects to further study the consequences of CPOX genotype on cell viability and the effects of Hg2+ on this process. Consistent with findings in vitro and in liver samples, Hg2+ (5 or 10μM for 24 hrs) promoted excess Hp accumulation in WT, Het and Mut cell lines in a concentration-dependent manner. In cell viability studies as determined by AlamarBlue assay, treatment of cells concomitantly with Hg2+ and the hæm synthesis inhibitor succinyl acetone (0.5 mM) for 24 hrs impaired cell viability in a genotype (WT, Het, Mut) and Hg2+ concentration-dependent manner, with CPOX4 (Mut) cells undergoing 40% loss of viability compared with 27 and 21% of Het and WT, respectively, at 5μM Hg2+. These results suggest that CPOX4 increases the susceptibility of cells to Hg2+ toxicity and that this effect is modulated by hæm deficiency. Additionally, these studies demonstrate the utility of EBV transformed cells as an alternative to transgenic animal models for studying the effects of environmental chemicals on chemical-mediated changes in cell functions. Supported by NIH grants ES04696 and ES07033.

1399 GENETIC POLYMORPHISMS ON ACTIVATION AND DEACTIVATION ENZYMES OF ORGANOPHOSPHATE PESTICIDES ARE INVOLVED IN THEIR NEUROTOXICITY.

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Recent studies have suggested that polymorphisms of enzymes involved in the metabolism of environmental contaminants may confer susceptibility to their toxicity. It is the case of some organophosphate pesticides (OP) that require bioactivation by CYP450 and are deactivated by paraaxonase 1 (PON1). CYP1A2*1F polymorphism increases OP activation, while OP deactivation is reduced by PON1-108C/T and PON1-162A/G polymorphisms and is substrate-dependent for PON1 192Q/R polymorphism; therefore, these polymorphisms may be involved in OP toxicity. The aim of this study was to determine the role of CYP1A2*1F and PON1 polymorphisms on the neurological damage in agricultural workers exposed to OP A cross-sectional study was conducted in Yucatan, Mexico in 80 subjects (43 ± 12 years old). Information about lifestyle, health status, pesticide exposure and symptoms of neurological damage was obtained from a questionnaire and a chronic OP exposure index was constructed. Genetic polymorphisms were determined by RT-PCR or RFLP. Sixty-nine percent of participants reported adverse neurological symptoms associated with pesticide exposure. Workers with *1F/1F (73%), -108C/T (86%), -162G/G (74%) or 192Q/R (77%) genotypes and with the genotype combinations *1F/1F-108C/T (63%), *1F/1F192Q/R (54%) and -108C/T192Q/R (64%) showed higher risks of having more than 10 adverse symptoms and/or have presented an intoxication episode associated with OP exposure. This suggests that CYP and PON1 polymorphisms confer elevated risk for neurological damage and carrying the combination of some unfavourable genotypes further increases this risk (Supported by CONACyT, Grant CO-134).

1400 ASSOCIATION OF GENETIC VARIATIONS IN THE ALPHA1-ANTITRYPSIN AND MATRIX METALLOPROTEINASE GENES WITH LUNG FUNCTION DECLINE IN A GROUP OF FIREFIGHTERS.

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The measurement of forced expiratory volume in 1 second (FEV1) and in decline over time are prognostic indicators of early chronic airflow obstruction. Although α1-antitrypsin (AAT) deficiency (Z allele) was shown to be a risk factor for rapid decline in lung function, individuals with the same AAT genotype may differ in their phenotypes suggesting the presence of other genetic modifiers. Matrix metalloproteinases (MMPs) are a group of proteolytic enzymes that play an important role in connective tissue remodeling and repair. The aim of this study was to investigate whether polymorphisms within genes encoding for AAT and MMPs may be associated with OP exposure. A cross-sectional study was conducted in Yucatan, Mexico in 80 subjects (43 ± 12 years old). Information about lifestyle, health status, pesticide exposure and symptoms of neurological damage was obtained from a questionnaire and a chronic OP exposure index was constructed. Genetic polymorphisms were determined by RT-PCR or RFLP. Sixty-nine percent of participants reported adverse neurological symptoms associated with pesticide exposure. Workers with *1F/1F (73%), -108C/T (86%), -162G/G (74%) or 192Q/R (77%) genotypes and with the genotype combinations *1F/1F-108C/T (63%), *1F/1F192Q/R (54%) and -108C/T192Q/R (64%) showed higher risks of having more than 10 adverse symptoms and/or have presented an intoxication episode associated with OP exposure. This suggests that CYP and PON1 polymorphisms confer elevated risk for neurological damage and carrying the combination of some unfavourable genotypes further increases this risk (Supported by CONACyT, Grant CO-134).
real-time PCR assays. Rate of decline in FEV1 was calculated for each subject by running a simple linear regression of FEV1, over age. After adjusting for covariates, the AAT Z and 3’ variants (both p=0.048) and the MMP1-128A/G (p=0.009) polymorphism were associated with a more rapid rate of FEV1 decline. No association was observed between the rate of FEV1 decline and other variations. These data suggest that genetic variations within genes involved in tissue remodeling and repair may influence susceptibility to progressive decline in FEV1.

This work was supported in part by an Interagency Agreement with the Intramural Research Program of the NIHES (Y1-ES-00001).

**1401 GENETIC VARIATION IN ISOGENIC STRAINS OF LABORATORY MICE ALTERS THE DISPOSITION AFTER ACUTE EXPOSURE TO [14C] BENZENE.**

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Many of the variations in human susceptibility to chemical toxins may be linked to genetic diversity in their disposition. Thus, understanding how the genetic diversity affects the disposition of chemicals in various mouse strains may better define the effects of genetic variability on human susceptibility. Initial studies focused on searching for differences in AUC(0-2h), Cmax and Tmax of [14C] benzene equivalents in whole-blood from genetically diverse strain mice (based on degree of single nucleotide polymorphisms) selected from the NTP-Perlegen Mouse Genome Resequencing Project. Mice (5 per time point) were administered a single oral dose of [14C] benzene, 0.1 mg/kg, 75 μg/kg. Blood and bladder urine were obtained at 5-120 min post dose for analysis of total [14C] content. In addition, expression levels of CYP2B1 & UGT1A6 in hepatic microsomes were determined for each of the strains. The AUC(0-2h), Cmax and Tmax of [14C] equivalents in blood ranged from 25 to 75 min·μmol/mL min, 0.2-0.4 μmol-eq/mL and 5-20 min, respectively. Differences were observed in the quantitative profile of benzene metabolites present in bladder urine among the various mouse strains. Additionally, a unique metabolite of benzene appeared to be present in the urine of male BALB/c mice. Preliminary results suggest inter-strain differences in the expression of benzene metabolizing enzymes (CYP2B1 & UGT1A6). Based on these studies, strains showing notable differences in these parameters will be selected for detailed pharmacokinetic/metabolic studies.

This research was supported in part by the NIHES NTP Grant No. N01-ES-45229 and NIHES-sponsored Southwest Environmental Science Center Grant Number P3-ES-06694.

**1402 MODIFYING EFFECT OF GENE POLYMORPHISMS IN THE RELATION BETWEEN LEAD AND BLOOD PRESSURE.**

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Background: Environmental lead exposure has been associated with an increased risk of developing hypertension. Few studies have addressed the role of genetic polymorphisms in influencing the effect of lead exposure on hypertension.

Methods: We examined the genetic effects of five previously characterized single nucleotide polymorphisms (NOS3 rs2070744, VDR rs731236, VDR rs2239185, PON1 n6062, and PON1 n854560) on the relationship between blood lead levels and hypertension using nationally representative data from the Third National Health and Nutrition Examination Survey (NHANES III) DNA bank, 1991-1994 (n=6016).

Results: The prevalence of hypertension was 22.8%. Among persons with and without hypertension, the mean blood lead was 3.72 and 2.78 μg/dL, respectively. Multivariate regression analysis stratified by lead tertile showed that in the highest lead tertile (>3.3 μg/dL) individuals carrying the PON1 rs6062 G allele had a significantly lower prevalence of hypertension compared to those with the AA genotype (OR: 0.41; 95%CI: 0.24-0.68; p <0.01). In addition, predicted diastolic blood pressure was 2.23 mmHg lower for PON1 n6062 G allele carriers in this tertile (p =0.004). There was no effect modification by the other genotypes on the relations of lead dose with blood pressure.

Conclusion: In a nationally representative sample of the U.S. population, the prevalence of hypertension and estimated diastolic blood pressure was significantly lower for carriers of the PON1 rs6062 G allele compared to individuals homozygous to the PON1 AA genotype at the highest tertile of blood lead. These data, also, provide no evidence that the NO33 rs2070744, VDR rs731236, VDR rs2239185, and PON1 n854560 genes modify the association of lead dose with blood pressure.

**1403 ABNORMALLY DECREASED LEVELS OF NON-STEROIDAL ANTI-INFLAMMATORY DRUG-ACTIVATED GENE (NAG)-1 AND H6D POLYMORPHISM IN LUNG CANCER PATIENTS’ SERA.**

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NAG-1 has anti-tumorigenic and anti-inflammatory activities. There are inconsistent reports on expression/secretion of NAG-1 in various human tissues and serum/plasma obtained from cancer patients and controls. This is due to lack of reagents and methodology to measure only cleaved mature forms and/or to distinguish NAG-1 proteins from H6D polymorphism. Antibodies specific for human NAG-1 or H6D polymorphism have been produced using KLH conjugates of a synthetic peptide unique to NAG-1 or H6D protein. Form-specificity of the antibodies was characterized using synthetic NAG-1 and H6D peptides conjugated to BSA and native NAG-1 and H6D proteins in normal human serum samples. NAG-1 polyclonal antibodies recognized the NAG-1 peptide-conjugated BSA but almost no cross-reactivity was detected with the H6D peptide-conjugated BSA. H6D polyclonal antibodies recognized the H6D peptide-conjugated BSA but almost no cross-reactivity was detected with the NAG-1 peptide-conjugated BSA. The results demonstrated that the NAG-1 and H6D antibodies are form-specific.

Western blot analyses using the NAG-1 and H6D antibodies were carried out with 4 human control serum samples and found that the pattern of cleaved ~40 kDa direct (active form) NAG-1 protein levels among the 4 serum samples differed from the pattern of NAG-1 protein levels. Western blot analyses using the NAG-1 and H6D antibodies carried out with 4 serum samples obtained from lung cancer patients revealed that the levels of the ~40 kDa NAG-1 and H6D dimers dramatically decreased compared with the levels of NAG-1 and H6D dimers detected in 4 control serum samples (significantly different with p<0.04 and p<0.06, respectively). The results suggest that decreased serum NAG-1 or H6D levels may serve as a serum biomarker of lung cancer patients. (Supported by NIHES SBIR Phase I Contract HHSN27520070012C).

**1404 INTER AND INTRINDIVIDUAL VARIABILITY OF GLUCURONIDATION OF SELECTED PHTHALATE METABOLITES IN HUMANS.**


Glucuronidation is a major detoxication pathway in humans for all metabolites of phthalate diesters. Genetic polymorphisms and physiological conditions can affect each person’s ability for glucuronidation. Furthermore, the extent of glucuronidation of phthalates varies depending on the solubility of the metabolite in aqueous media. Although the concentrations of total (i.e., free plus glucuronidated) species of phthalate metabolites are typically reported in exposure assessment studies, the free form of the phthalate metabolite is thought to represent the bioactive dose.

We investigated in eight adults with no documented phthalate exposure, the extent of glucuronidation of six phthalate metabolites in urine: monoethyl phthalate, monobutyl phthalate, monopentyl phthalate, mono(2-ethyl-5-enohexyl) phthalate, mono(2-ethyl-5-hydroxyhexyl) phthalate, and mono(2-ethyl-5-carboxypentyl) phthalate. The conjugated species concentrations were determined indirectly from the pattern of the free and the total species in urine. The observed interindividual differences in glucuronidation did not change significantly between days of sample collection or the urine void volume. Furthermore, our findings suggest that intrindivdual variability of glucuronidation also exist. Nevertheless, the significant associations (p<0.01) between total and free phthalate metabolite urinary concentrations suggest that, in large epidemiological studies, total phthalate metabolite concentrations may be used to represent the free bioactive dose.

**1405 BROMINE VAPOR SKIN STUDIES IN WEANLING PIGS.**

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Due to continued public concern for safety from chemical attacks, the Battelle Biomedical Research Center (B Battelle Bio) and the U.S. Army Medical Research Institute of Chemical Defense (USA/MRICD) have aligned research efforts to establish an anesthetized weanling pig model of superficial dermal (2nd degree) and deep dermal (3rd degree) cutaneous injuries induced by bromine vapor. A bromine
vapor exposure system was fabricated and characterized to evaluate female Yorkshire-cross pigs with four lesion sites (each 3 cm in diameter) between the axillary and inguinal areas of the ventral abdomen. Injury was produced by modifying exposure length to various concentrations of bromine vapor and evaluating the sites after 24 and 48 h. Lesion assessment included clinical observations, digital photography, graph measurements, modified Draize scoring, reflectance colorimetry, and evaporation. Infrared thermographic images were also collected. Histopathologic evaluation of skin collected at 48 h-post-exposure included wound severity, wound depth, and percent of the total area involved. Different exposure lengths and vapor concentrations were explored during model development. The injuries induced by controlled bromine vapor concentrations of 85,000 to 175,000 ppm and exposure durations of 5 to 30 minutes were characterized histologically and ranged from superficial dermal to deep dermal in severity. Follow-on efforts will address efficacy testing of therapeutic compounds to improve wound healing by preventing/reducing the erythema, edema and deep tissue damage to the skin caused by exposure to bromine vapor. This work was supported by the U.S. Army Medical Research and Materiel Command under Contract W81XWH-05-D-0001, Task Order 0010.

1408 TRANSCRIPT PROFILING OF PXR AND CAR KNOCK-OUT AND HUMANIZED MOUSE LIVER PROVIDES INSIGHT INTO ENDOGENOUS ROLES OF THE HUMAN PXR/CAR ISOFORMS.

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We have created mouse lines in which the murine pregnane X receptor (PXR) and constitutive androstane receptor (CAR) genes have been removed (PXR KO, CAR KO mice) and/or replaced with human PXR or CAR (huPXR, huCAR mice). To investigate PXR- and CAR-regulated endogenous and species specific functions we performed transcript profiling and pathways analysis on RNA extracted from the livers of these mice compared to C57BL/6j wild type mice (WT) using Agilent whole mouse genome expression microarrays and Ingenuity Pathways Analysis software, respectively. Upregulation (~2-7-fold) of several genes involved in xenobiotic/fatty acid metabolism (24 genes) and cholesterol biosynthesis (7 genes), e.g., Cyp3a11, Ugt1a1, Acox1 and Hmgcr was observed in huPXR KO mice but these changes were reversed in huCAR mice. Thus huPXR may function as a repressor of genes in these pathways. Induction of Cyp isoforms in the PXR KO mouse liver was accompanied by upregulation of genes involved in redox homeostasis (7 genes) and endoplasmic reticulum stress (3 genes), some of which are regulated by Nr2f2. This effect was not observed in huPXR mouse liver, reflecting the lack of Cyp induction in huPXR mice. Fatty acid metabolism and xenobiotic metabolism genes were also overrepresented in the list of altered genes in CAR KO and huCAR mice. However, these genes tended to be altered with similar polarity in the CAR KO and huCAR mice relative to WT mice. The fold changes observed were generally lower in huCAR mice than CAR KO mice, suggesting partial functional substitution of the mouse CAR gene by its human homologue. Repression of CAR-regulated genes in the CAR KO mice and the moderation of this by huCAR is consistent with the constitutive activity of this receptor in rodents and humans. This work was supported by ITI Life Sciences, Scotland.

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infusion with doses administered more frequently than twice weekly was not benefi-
cial; labor and technical demands were comparable to those in traditional tri-weekly infusion studies with rats administered a maintenance solution during non dosing intervals.

**1410 PRELIMINARY TRIAL FOR THE PREDICTION OF MECHANISM-BASED INHIBITION IN HUMAN USING PXB MICE® WITH HIGHLY REPOPULATED HUMANIZED LIVER.**


To predict the extent of mechanism-based inhibition (MBI) in human, a model system which reflects in vivo human is required. The PXB-mouse is a humanized chimeric mouse model where the liver has been repopulated by more than 70 % with human hepatocytes. We therefore anticipated that the PXB-mouse would be a potentially useful model for predicting MBI in human. PXB-mice and control mice were administered 500 mg/kg of erythromycin, mechanism-based inhibitor of CYP3A4, thrice a day for 3 days as an oral gavage dose. To measure the enzyme activity of CYP3A4, a single oral gavage dose of midazolam as a probe compound was administered 3 days before the start of the erythromycin administration at Day -3 and one day after the end of the erythromycin administration at Day 3. The plasma concentration of midazolam was determined by LC-MS/MS, the mRNA and protein expression levels of CYP3A4 were measured using RT-PCR and western blotting, respectively. AUC_{0-4h} of midazolam was 257.0 ng/h/mL at Day -3 and 1369.8 ng/h/mL at Day 3 (1.8-fold) in PXB-mice, and 49.7 ng/h/mL at Day -3 and 89.4 ng/h/mL at Day 3 (1.8-fold) in control mice. The mRNA and protein expression levels of CYP3A4 increased rather than decreased in PXB-mice. These findings strongly suggest that the PXB-mouse is a valid in vivo model system for estimating and predicting mechanism-based inhibition in humans.

**1411 COMPARATIVE MAMMALIAN GASTROINTESTINAL ABSORPTION: THE INFLUENCE OF VARIOUS GASTROINTESTINAL FACTORS.**

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Noblis, Falls Church, VA.

Absorption of ingested materials through the gastrointestinal (GI) system is an important process. The physicochemical properties of drugs and chemicals (e.g., molecular size, aqueous solubility, lipophilicity) are influential, but do not vary during exposure in different animals. By contrast, factors that characterize the barrier between the lumen and the GI tube, such as the absorptive surface areas and secretions that contribute to the luminal milieu, are greatly affected by species-specific GI anatomy and physiology. These differences can result in disparities in the rate and extent of absorption. To determine which animal best models human GI absorption, data from seven mammalian species (dog, rabbit, mouse, rat, pig, monkey, and human) were evaluated. Parameters examined include intestinal transit lengths and surface areas, GI transit times, and pH of GI segments. With the exception of the rabbit and possibly the mouse, the proportions of small to large intestinal lengths were similar to those found in human. The absorptive surface area of the small intestine (luminal surface area compared to total body surface area) is highest in the human and monkey due to the presence of extensive surface modifications (e.g., folds of luminal epithelium [plicae circulares], villi and microvilli) that are notable especially in the cranial portion of the small intestine. Transit times are generally longer in species with larger body sizes, such that humans have the longest transit times followed by the pig and monkey. The stomach pH in rodents (rats and mice) is nearly neutral, allowing the presence of a rich microflora. In the remaining species, most of which are omnivores, stomach pH is quite acidic. The rabbit, (a lagomorph and strict herbivore) exhibits luminal pH in intestinal tract segments that differs greatly from that of other species, including humans. Our analysis finds rabbit to be most different and monkey, followed by pig and dog, to be the closest models of human GI absorption; however, accounting and accounting for interspecies absorptive factors allows the other species to also be useful.

**1412 ESTABLISHMENT OF A LINE OF SPORTS (SPONTANEOUSLY-RUNNING-TOKUSHIMA-SHIKOKU) RATS THAT FORM LEFT ATRIAL THROMBI.**


We report a new model leading to a high incidence of thrombus formation in the left atrium. This new rat model was named SPORTS (Spontaneously-Running-Tokushima-Shikoku) rat, with high wheel-running activity in this male rat. We found that some of the SPORTS rats died after losing body weight after the age of one year. At autopsy, formation of thrombi in the left atrium was recognized in SPORTS rats. The objective of this study was to clarify the autopsy results in the SPORTS rats related to thrombus formation in the left atrium. The incidences of atrial fibrillation and thrombosis were 57% and 38% in male SPORTS rats and 38% in female SPORTS rats. Systolic blood pressures were 134 ± 16 mmHg (control rats 115 ± 11 mmHg) and heart rates were 459 ± 21 beat/min in male SPORTS rats. Atrial fibrillation, such as atrial fibrillation, were not recognized by electrocardiogram in SPORTS rats. In conclusion, we established a line labeled SPORTS rats with spontaneous thrombi in the left atrium. It is thought that the SPORTS rat might contribute to development of new anti-thrombotic approaches for human atrial fibrillation or familial thrombophilia.

**1413 A NON CLINICAL TOXICITY AND BIODISTRIBUTION STUDY IN CYMOLUMUS MONKEY WITH A NEW LIVE ATTENUATED JAPANESE ENCEPHALITIS VACCINE.**

G. Ravel, C. Meier and S. Goulard.

Non Clinical Safety, sanofi pasteur, Marcy l’Étoile, France and R. D. sanofi pasteur, Marcy l’Étoile, France.

Japanese Encephalitis (JE) vaccine (JE-CV) is a new live attenuated vaccine, which has been constructed using the yellow fever 17D vaccine (YF 17D) and recombinant technology. The YF genes for the pre-membrane/membrane and envelope (prM/M and E) proteins were replaced with those of an attenuated JE virus. The remaining part of the construct contains the non-structural and core elements of YF 17D vaccine which provides the replication engine for producing viruses that display the prM/M and E antigens of JE virus on their surface. As part of the safety assessment of the JE vaccine, a combined toxicity and biodistribution study was conducted in the cynomolgus monkey. The study assessed the general and local toxicity, immunogenicity, biodistribution and the persistence of the vaccine in both target and non-target tissues/organs and its shedding from infectious site and body fluids. Cynomolgus monkeys were given a single subcutaneous dose of either the JE-CV or control, and were observed for 3 or 21 days. The study design included standard clinical observations, body temperature measurements, weight, food consumption, clinical pathology, ophthalmology and full examination at necropsy with organ weights and histopathological examination of a full range of tissues. In addition, the distribution and persistence of JE-CV, as well as its shedding, were evaluated by qRT-PCR analysis. One subcutaneous administration of the vaccine was well tolerated in cynomolgus monkeys. There were no vaccine-related adverse signs of general and local toxicity of JE-CV. All vaccinated animals seroconverted. Half the animals developed low viremia 3 days after immunization, and the biodistribution and the shedding of JE-CV were limited. These results, as well as those from previous neurovirology and toxicology studies, confirm the absence of any major safety concerns with JE-CV.

**1414 A NEW IN VITRO METHOD FOR IDENTIFYING SKIN SENSITIZERS AND PREDICTING LLNA EC3 VALUES.**

L. M. McKim, B. D. Jeffy, M. E. Steffen and H. N. Wagner.

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The purpose of this study was to develop a novel in vitro human cell-based model to detect chemicals and finished products that are skin sensitizing agents. In vitro alternatives to animal testing are needed to support REACH and the European Cosmetics Directive initiatives. The present study describes the use of human (HaCaT) cells in culture and a 3D human skin model to identify skin sensitizers and predict LLNA EC3 values by utilizing concentration response, exposure time, and expression of genes controlled by the antioxidant response element (ARE). The model was developed using a set of 14 chemicals previously classified as non-, very weak, weak, moderate-, strong-, and extreme-sensitizers. A validation study with more than 100 compounds is currently underway. The standard HaCaT cell culture model is efficient, but is limited to chemicals with high solubility. In contrast, the human 3D skin models commercially available from SkinEthic and MatTek provide a test system that can be used to test finished products and chemi-
cals with low solubility. Test chemicals were applied at concentrations that ranged from 0.01 μM to 1 mM. Exposures were for 24 hr at 37°C in a 96-well format. Viability was monitored with MTT or histology. Total RNA was isolated with TriZol and changes in the expression of quinone reductase (NQO1), interleukin 8 (IL-8), and aldolase reductase (AKR) were measured by RT-PCR. The strong sensi-
tizer 1-chloro-2,4-dinitrobenzene produced significant increases in IL-8 and AKR at an exposure of 10 μM. In comparison, the non-sensitizer benzoic acid had no ef-
fact at an exposure concentration of 1 mM. An algorithm based on an analysis of the magnitude of gene expression, concentration response, the number of genes re-
sponding, and time provides a single value of response, the in vitro toxicity index (IVTI). Comparison of the IVTI to known LLNA EC3 values by exponential re-
gression analysis resulted in an R value of 0.92 in both cell models. This in vitro system provides a useful tool for identifying sensitizers and predicting LLNA EC3 values without the use of animals.

1415 PROTECTIVE EFFECT OF ASCORBIC ACID SUPPLEMENTATION ON LEAD TOXICITY IN RAT BLOOD.


It is well known that lead is an environmental toxicant that can have long-term adverse health effects on humans. Lead has been evident, used, and consumed in many facets of human life. This study focused on a possible alternative method of using ascorbic acid supplementation in the treatment of lead poisoning. Sprague-Dawley rats, weighing 100-124g, were exposed to 0.1% lead acetate in drinking water for 2 and 4 week periods. They were then allowed to recover for 5 days with 5.5g/ml ascorbic acid supplementation. Rats were sacrificed and blood was collected via cardiac puncture. Blood samples were wet digested using nitric acid and analyzed using optical emission spectrophotometry. Results indicated that the 2 weeks treated animals showed a reduction in blood lead levels of approximately 50%. This study illustrates the potential of ascorbic acid supplementation on lead toxicity.

1416 COMMON NEOPLASTIC LESIONS IN THE WISTAR HANNOVER RCH TAN: WIST RAT.

K. Weber1, 2, T. Thio1, 2, H. Iwata1, 2, A. Piersigilli1, 2 and M. Josten1, 2. Harlan Laboratories, Indianapolis, IN and RCC Ltd, Hingen, Switzerland. Sponsor: L. Corney.

Differing data on strains, depending on their source, feed and housing conditions have been published. Generally, before starting a toxicology program in rodents it should be considered that oncogenicity studies will be performed for a majority of compounds successfully completing development. The incidence, type and time of onset of age-dependent lesions and neoplasms and some special considerations of a rat strain selected may be decisive. Therefore, knowledge of the historical background data is needed. The use of a rat strain should be carefully considered at the beginning of the toxicology program. This paper deals with the occurrence of neoplasms that were recorded in 50 oncogenicity studies performed during 25 years at RCC Ltd Switzerland at incidences ≥ 1.0% (3748 males and 3751 females planned for terminal sacrifice: 104 to 130 weeks) including brain: granular cell tumor; pituitary gland: adenoma; adrenal cortex: adenoma; adrenal medulla: phaeochromocytoma; thyroid: follicular and C-cell neoplasms; parathyroid: adenoma; pancreas: islet cell tumors; liver: hepatocellular adenoma; skin: papilloma, keratoacanthoma, fibroma, lipoma; mammary glands: adenoma, fibroadenoma, adenocarcinoma; testes: Leydig cell tumor; ovaries: granulosa cell tumor, theca-granulosa cell tumor; uterus: endometrial and stromal polyps, adenocarcinoma; vagina: stromal polyp; systemic tumors: lymphoma; mesenteric lymph node: hemangioendothelioma; thymus: thymoma. The onset of tumors in 466 males and 450 females scheduled for sacrifices at study periods between 52 and 78 weeks is rare. At these ages, neoplasms consisted mainly (more than 2 tumors per entity within control data) of pituitary gland adenomas, C-cell and follicular cell tumors of the thyroids, mammary fibroadenoma and adenocarcinoma, stromal polyps of the uterus and vagina, malignant lymphoma, hemangioendothelioma of the mesenteric lymph nodes, and lipoma in body cavities. Over a period of 25 years, the Wistar Hannover RCHHanTM: WIST Rat showed a robust stability in its tumor profile regarding the incidences and tumor entries.

1417 DEVELOPMENT OF A RODENT MODEL OF INFLUENZA A INFECTION IN F344 RATS.

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The purpose of this study was to develop a model of influenza pulmonary infection in rats to facilitate examination of the abilities of environmental agents to induce immune suppression and to assess potential new drugs/vaccines for efficacy against influenza infection. Stocks of non-adapted influenza A, strain HKX-31 (H3N2) were prepared and titrated by plaque assay in MDCK (Madin Darby Canine Kidney) cell monolayers. Groups of 8 male F344 rats were administered 100, 10,000, or 1,000,000 plaque forming units (PFU) by intratracheal instillation. Six rats served as vehicle controls. Half of the rats in each group were euthanized 48 hours (expected time of peak viral growth) and 7 days (period where virus was expected to be cleared from the lung) post dosing. The left lung from each rat was fixed for histological evaluation. The right apical lung lobe was collected for assessment of infectious virus load by plaque assay. The remaining right lobe were collected in RNA later for RT-PCR-based analysis of viral load. Infected animals displayed no clinical signs of disease or toxicity. Overall histology lesion scores (based on severity of parenchymal inflammation, as well as changes in the vasculature and conducting airway epithelium) were increased in a dose dependent manner. However, only high dose group scores at both 48 hr and 7 days post infection were significantly increased compared to the controls. In each dose group, lesion scores at 48 hours post infection were not different from corresponding group scores at 7 days. Infectious virus loads at 48 hr post infection ranging from 1000 PFU/100 mg of tissue (~ 13000 PFU/lung) for the low dose to 100,000 PFU/100mg tissue (1,300,000 PFU/lung) for the medium dose. Virus was not detected in the lung at day 7. Results suggest that F344 rats are permissive for HKX-31 infection and that the model may prove useful for assessing immune competence and therapeutic agent efficacy. Research conducted under NIEHS P01 ES10594.

1418 ACUTE AND SUBCHRONIC STUDIES FOR CHARACTERIZATION OF RISKS IN MINI PIGS: CLINICAL PATHOLOGY AND HISTOPATHOLOGY ASPECTS.

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Scope: For evaluate the possible toxic effect caused by the acute and subchronic oral administration of the Haloacetamido, a new molecule to be used in the combat of Leishmaniose, we use mini-pigs like non-rodent animal model. Experimental procedures: Acute oral toxicity test and chronic studies, 4 and 13 weeks, had been carried through using mini pigs, males and females, between 25 and 32 kg, with about 12 and 14 months of age. The animals had been acclimatized for terminal sacrifice: 104 to 130 weeks) including brain: granular cell tumor; pituitary gland: adenoma; adrenal cortex: adenoma; adrenal medulla: phaeochromocytoma; thyroid: follicular and C-cell neoplasms; pancreas: islet cell tumors; liver: hepatocellular adenoma; skin: papilloma, keratoacanthoma, fibroma, lipoma; mammary glands: adenoma, fibroadenoma, adenocarcinoma; testes: Leydig cell tumor; ovaries: granulosa cell tumor, theca-granulosa cell tumor; uterus: endometrial and stromal polyps, adenocarcinoma; vagina: stromal polyp; systemic tumors: lymphoma; mesenteric lymph node: hemangioendothelioma; thymus: thymoma. The onset of tumors in 466 males and 450 females scheduled for sacrifices at study periods between 52 and 78 weeks is rare. At these ages, neoplasms consisted mainly (more than 2 tumors per entity within control data) of pituitary gland adenomas, C-cell and follicular cell tumors of the thyroids, mammary fibroadenoma and adenocarcinoma, stromal polyps of the uterus and vagina, malignant lymphoma, hemangioendothelioma of the mesenteric lymph nodes, and lipoma in body cavities. Over a period of 25 years, the Wistar Hannover RCHHanTM: WIST Rat showed a robust stability in its tumor profile regarding the incidences and tumor entries.

1419 “ARTHROSIS-ASSOCIATED MYOPATHY”, A CHARACTERISTIC PATHOLOGY OF PRIMATE MODELS.

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We have established a collagen-induced arthritis (CIA) model in cynomolagus monkeys (CM) to assess the effectiveness of new therapeutics in preclinical development (e.g., candidate compounds for anti-rheumatic drugs). Based on similarities in human and CM drug metabolism, primate models have been considered to be predictive of drug effects in clinical applications. In this study, we introduce an additional advantage in the employment of primate CIA models in pre-clinical; demonstrating our finding that “arthrosis-associated myopathy” appears frequently in CIA models of CM. CIA was induced in 22 female CM by two immunizations with bovine type II collagen in the presence of Freund’s complete adjuvant. Symptoms of arthritis were monitored with arthritis score, and food intake, body weight, and serum biochemical parameters (CPK, Creatinine, CRP, serum IL-6 and cortisol) were measured sequentially. The animals were sacrificed in stages, 5 to 6 animals per timepoint (Day 11, 21, 35 and 63 after the first immunization) for histopathology and necropsy of muscle fiber at quadriceps femoris was observed only on Day 11.
The most significant findings such as atrophy, degeneration and/or regeneration of muscle fiber were most frequently observed on Day 35. A marked decrease in body weight was reproducibly observed in this model, which positively correlated with serum creatinine at all timepoints. CKP increased markedly on Day 11, and thereafter CRP and IL-6 rose and peaked on Day 28. These suggested that the procedure of CIA targets skeletal muscle tissues as well as joints independently of systemically-induced inflammatory reaction. In this context, a biphasic increase in serum cortisol was observed on Days 11 and 35, thus indicating that cortisol withdrawal might be associated in the development of "arthritis-associated myopathy".

In conclusion, repeated intra-articular administration of dose volumes up to 0.2 mL/kg is fea`sible in the marmoset model, thus adding another route of administration for this species in toxicity studies. 1. Korte et al. (2004) Toxicologist, 78:S:1-2; 2. Hunnyball (1983) Rheumatol Int. 3:69.

**1420 COOPERATIVE DEVELOPMENT OF AN AUTOMATED SYRINGE INFUSION PUMP FOR PRECLINICAL TOXICOLOGY**

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Historically, preclinical infusion studies have used syringe pumps designed for laboratory or human use; often requiring extensive manual documentation and pump manipulation to meet regulatory requirements for GLP studies. Infusion experts at Covance have co-developed with Solomon Scientific the Orchesta™ syringe infusion pump with wireless communication incorporating input from regulatory and technical stakeholders, to meet the specific needs of preclinical toxicology. The pump features a magnesium splash-proof case and fully enclosed drive mechanism to prevent contamination; these features will extend the life of the pumps over that of those currently available. Pumps may be programmed and operated individually or numerous pumps may be operated in tandem wirelessly (IEEE 802.15.4 WAPN standard) from a single host PC. Tandem operation allows the infusion setup to be entered once on the host PC at the beginning of the study, a significant improvement over the current paradigm of manually programming each pump for each dose. This pump accommodates syringes of 1 to 140 mL, can deliver infusion rates of 0.01 mL to 3 L per hour, and is therefore scalable for small or large animal infusion studies for a variety of study designs. The pump software features robust electronic data collection systems to query for animal ID, body weight data, and dose parameters and to facilitate reporting of infusion-related data and can be upgraded to interface with a variety of data collection systems. The system features robust electronic documentation, facilitating reporting of infusion data and eliminating the need for paper documentation. A unique user code is required for all pump or system interventions and each action is automatically documented by time and user identification. With the Orchesta™, each syringe pump is engineered individually or numerous pumps may be operated in tandem wirelessly, avoiding kinks in the tubing. Following this, the accuracy of the infused volume was checked and measurements were performed as in a routine toxicology study. The infusion pump with wireless communication will improve the accuracy, reproducibility, and regulatory compliance of preclinical toxicology infusion studies.

**1421 FEASIBILITY OF REPEATED INTRA-ARTICULAR ADMINISTRATION IN THE MARMOSET MONKEY (CALLITRIX JACCUS).**

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The common marmoset (Callitris jacchus) provides an alternative species to macaques in preclinical safety testing (1) and has also been used as a model for induced Parkinson's disease, multiple sclerosis and rheumatoid arthritis. For the latter indication, administration of specific testis items such as biopharmaceuticals directly into joints is required in some instances, and for some biopharmaceuticals the marmoset represents the only relevant animal model. This work investigates, therefor, the feasibility of repeated intra-articular dosing in the knee joint in this animal model in the context of toxicity studies. Male and female marmosets (n = 6/group), weighing 278-455 gram and 1-9 years old, were fasted and slightly anesthetized with 0.2 mL/animal ketamine hydrochloride. Knees were shaved and intra-articular administration was done under aseptic conditions using sterile NaCl.

Based upon consideration of the complete synovial fluid volume, maximal injection volume was determined as 0.20 mL/kg. Four bolus doses were injected into the same knee in weekly intervals in every animals. The contralateral knee served as control. Post dosing observations were performed 3 and 5 hours after administration during the first 3 days after dosing. Macroscopic and histopathology evaluation after last dosing did not reveal inflammatory reactions in comparison with the control knee. Post dosing observation did not indicate any procedure related swellings. Activity and movement patterns appeared unaffected in all animals. Body weights and clinical pathology parameters did not indicate any adverse findings. For single injection, intra-articular dosing volumes of 0.35 mL/kg have also been reported (2). In conclusion, repeated intra-articular administration of dose volumes...
moxifloxacin using semi-automated analysis software to evaluate electrocardiography (ECG) data. Methods: Four male and four female cynomolgus monkeys were implanted with telemetry devices and administered moxifloxacin at 0 (control), 10, 50, or 175 mg/kg in separate Latin Square designs. ECG, heart rate, body temperature, and blood pressure data were collected during each dose. ECG data were analyzed using ECG PRO (Data Sciences International). At least 100,000 waveforms were analyzed per animal following each dose. Plasma was collected at 5 and 29 hours postdose and moxifloxacin levels were measured using liquid chromatography/mass spectrometry (LC/MS). Results: Moxifloxacin induced QTc and QTc interval prolongation in male and female monkeys. When males and females were analyzed separately in four animal Latin square designs, significant QTc interval prolongation was detected from 12 to 97 msec for hourly average and 30 msec for an analysis block (defined by day/night). Power analysis demonstrated that QTc interval changes using semi-automated ECG analysis software in these study designs can be detected at 16 (4 animals) and 10 msec (8 animals) with 80% power. Moxifloxacin plasma levels at 5 and 29 hours postdose were 760 and 50 ng/ml (10 mg/kg), 3700 and 290 ng/ml (50 mg/kg), and 6800 and 2250 ng/ml (175 mg/kg). Conclusion: This study demonstrates that semi-automated ECG analysis software can be used to detect QTc prolongation in monkeys with sensitivities of 16 (n=4) or 10 msec (n=8). This approach allows researchers to analyze large sets of ECG data with comparable sensitivity as conventional methods.

1425 VALIDATION OF THE AUDITORY STARTLE REFLEX IN RATS.


The auditory startle test is a required during preand post-natal development studies. The auditory startle reflex is a protective reaction in rodents which consists of a very fast contraction of muscles provoked by a sudden and intense sound stimulus. In order to validate the auditory startle test system, two pharmacological reference drugs, kanamycin and clonidine, known to modify the reflexes were used as positive controls in adult and/or young Sprague Dawley rats. The auditory startle test consists of three minutes of acclimation to the arena followed by two consecutive stimuli performed with a one minute interval. The first stimulus was composed of a sound at 107 dB, 10 kHz, 153 ms and the second stimulus had a higher intensity (119 dB). The test was performed on 5- and 11-week old rats which had been treated 15 minutes previously with clonidine (0.4 mg/kg) or in traperitoneal injection and on 5- and 2-week old rats which had been treated with kanamycin (400 mg/kg) from postnatal days 9 to 16, also by intraperitoneal injection. The data showed that treatment with clonidine or kanamycin decreased significantly the amplitude of the reflex and disturbed the reflex whatever the intensity of the stimulus. In addition, treatment with clonidine increased the latency of the response. Thus, the auditory startle system test is now fully validated and is used in routine preclinical reproductive toxicity studies at CIT.

1426 PUPILLOMETRY OF ALERT AND KETAMINE-SEDATED NON HUMAN PRIMATES UNDER VARIOUS STATES OF ILLUMINATION.

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Pharmacological agents, such as tricyclic antidepressants, which do not have the eye as a target organ, but have side effects on the eye including mydriasis, may elicit adverse effects such as photosophobia and cycloplegia. Therefore, for some drugs, it is important to determine their drug-mediated effects on pupil diameter changes and to this end pupillometry can be a valuable tool. This in vivo non-human primate study evaluated two methods of determining pupil diameter under various illumination conditions in conscious animals, and also evaluated the effect of ketamine. Pupil diameters were determined by manual measurements and by evaluation of video recordings under illumination of (1) room light, (2) darkness, and (3) direct exposure to a pen light (to elicit maximal light-mediated constriction) in conscious and properly restrained animals. An infrared video camera, modified to serve as a photoreflector, allowed the determination of pupil diameter in the dark. Cyclopentolate was used as a positive control for pupil dilation when results were compared with the values obtained in the conscious and restrained state. The results of this study suggest that pupil measurements of animals under the effects of ketamine can be compromised due to ketamine’s pupil-dilating effect. Analysis of pupil diameter for both methods showed a mean measured value compared to manual measurements due to its ease in capturing pupil diameter under a variety of illuminations, and its ability to determine additional parameters such as percent constriction of maximum dilation.

Measurements under sedation with ketamine showed a moderate decrease in pupil dilation when results were compared with the values obtained in the conscious and restrained state. The results of this study suggest that pupil measurements of animals under the effects of ketamine can be compromised due to ketamine’s pupil-dilating effect. Analysis of pupil diameter for both methods showed a mean measured value compared to manual measurements due to its ease in capturing pupil diameter under a variety of illuminations, and its ability to determine additional parameters such as percent constriction of maximum dilation.

1427 HOW TO INTEGRATE SAFETY PHARMACOLOGY END-POINTS DURING TOXICOLOGICAL STUDIES IN NON HUMAN PRIMATES FOR BIOLOGICS?


Existing guidance ICH M-3; S6; S7A; S7B, provide requisites for the conduct of appropriate safety pharmacology and toxicology assessment. However, inherent differences between biologics and small molecules support different approaches in nonclinical safety assessment. As a result, opportunities exist for alternative strategies for the integration of safety pharmacology end-points in general toxicological studies.

Based on our own experience, we describe an approach in non human primates to explore a new biologic that matches not only toxicological criteria, but also meets guideline criteria. Requirements from the ICH S7A core battery were met by the following: Respiratory function is evaluated by the use of a facial mask. Respiratory volumes, flows and bronchoconstriction index are recorded on different occasions. Cardiac vascular functions are assessed by measurements of blood pressure using tail/cuff while the ECGs are recorded on separate occasions by external telemetry. Finally, some aspects of CNS are investigated during the study, including detailed clinical observation, social behavior and body temperature - TK measurements are also made at the most appropriate time-points. The sequence of evaluation of these different parameters is a critical issue in order to avoid interferences between the different measurements. Since ECG is considered as the most critical end-point, it has been decided to perform it on days when no other parameters are to be assessed. Blood pressure, respiratory function as well as body temperature are monitored every other day under light anesthesia and can be associated with TK samples. Such a design while fulfilling most regulatory requirements, presents not only the additional benefit of reducing quantities of compound, time of development and cost but also meets the ethical constraints of reducing the number of animals used.

1428 COMPARISON OF BACKGROUND DATA FROM NORMAL (NON-CANNULATED) VERSUS DATA FROM SURGICALLY-CANNULATED, INTRAVENOUSLY INFUSED, RATS, DOGS, MONKEYS AND PIGS.

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Extended IV infusion, employing surgically implanted catheters, continues to be critical for nonclinical development of many IV pharmaceuticals (e.g. anti-cancer agents, large molecules), or for certain pharmaceuticals that cannot achieve high systemic exposure by other routes. To reliably undertake such non-clinical studies, confidence in the methods and appreciation of procedure-related changes are critical. To that end, establishing background ranges, and understanding how parameters may be temporarily or permanently altered by the procedures, is essential. Having conducted many continuous intravenous studies in all major laboratory species, we have established the data sets required to compare to normal animals. In all rodent and non-rodent laboratory species evaluated, the methodology was the very similar and involved cannulation of the vena cava, via a femoral vein, followed by exteriorization of the catheter via a jacket and tether which permitted connection to an infusion pump. Patency was maintained, during non-treatment periods, by continuously infusing normal saline at a low rate (varied depending on species and catheter size). During the studies the infusion rates were adjusted, at least weekly, based on individual body weight. Despite the jacket and tether, specialized handling procedures facilitated routine clinical examinations, body weight and food intake measurements, blood and urine collections, as well as specialized examinations (e.g. ECG, ophthalmology). At termination, particular attention to gross and tail/leg cuff while the ECGs are recorded on separate occasions by external telemetry. Finally, some aspects of CNS are investigated during the study, including detailed clinical observation, social behavior and body temperature - TK measurements are also made at the most appropriate time-points. The sequence of evaluation of these different parameters is a critical issue in order to avoid interferences between the different measurements. Since ECG is considered as the most critical end-point, it has been decided to perform it on days when no other parameters are to be assessed. Blood pressure, respiratory function as well as body temperature are monitored every other day under light anesthesia and can be associated with TK samples. Such a design while fulfilling most regulatory requirements, presents not only the additional benefit of reducing quantities of compound, time of development and cost but also meets the ethical constraints of reducing the number of animals used.
Investigated. Sampling was performed via an implanted cannula in the cisterna serial sampling of CSF (30 μL/sample) at 4 time points over 24 hours in rats was successfully injected into this space. The feasibility of rect puncture through the exposed dura between the L4/L5 vertebrae above the level. Presence of the catheter in the IT space resulted in expected histopathological formation. At necropsy, catheters were confirmed to be in the IT space, at the lumbar 20 to 22 days and were thereafter euthanized and post-mortem examinations performed. Demonstration of key relationships between biomarkers and verifiable carcinogenesis require substantial verification data before a study design may be considered adequate for risk assessment and serve as a photocarcinogenesis bioassay surrogate. The use of varying UVR exposure regimens and selected biomarkers (thymine dimers, TUNEL, p53, MSH2, DNA synthesis, histopathological changes) was investigated in several studies with known photocarcinogens in hairless albino mice (e.g., retinoids, fluoroquinolone antibiotics), the phototoxic but not photocarcinogenic agonist anthracene, and the human photocarcinogen 8-methoxypsoralen. The data from recent studies has not proven to be predictive of experimental photocarcinogenesis. For example, thymine dimers from two levels of UVR doses that cause differences in time to tumor in mice did not show such difference at the times evaluated; furthmore, anthracene caused the highest change in p53 values, even though it is not a photocarcinogenic in mice. Therefore, the use of these biomarkers in alternative assays is not yet ready to replace the accepted hairless mouse photocarcinogenesis bioassay. Recent studies have, however, provided leads on in the further development of abbreviated tests.


Intrathecal (IT) injection and infusion in rats were validated as routes of cerebrospinal drug delivery. Intracerebroventricular (ICVS) injection or infusions in rats were previously validated and IT injections or infusions in non-rodents are well-established methods in our laboratories. Validation of the IT methods in rats, permits conduct of regulatory compliant efficacy or toxicity assessments of neuroactive compounds. The use of the cerebrospinal routes allows for an effective way of bypassing the blood-brain barrier when administering compounds that would not otherwise reach their desired target. This directed administration also avoids potential toxicity that could arise from systemic exposure to therapeutic concentrations of neurologically active drugs. In addition to dose administration procedures, we evaluated the feasibility of serial sampling of cerebrospinal fluid (CSF) in additional populations of rats. For IT infusion, rats were instrumented with a catheter, entry via the cisterna magna and tracked to the lumbar (L) region with placement of the tip targeted at L2 to L4. The animals were maintained on infusion for up to 20 to 22 days and were thereafter euthanized and post-mortem examinations performed. At necropsy, catheters were confirmed to be in the IT space, at the lumbar level. Presence of the catheter in the IT space resulted in expected histopathological changes consisting primarily of inflammation. IT injections were performed by direct puncture through the exposed dura between the L4/L5 vertebrae above the cauda equina. Intrathecal placement was verified by flick of the tail upon entry of the needle. Up to 50 μL was successfully injected into this space. The feasibility of serial sampling of CSF (30 μL/sample) at 4 time points over 24 hours in rats was investigated. Sampling was performed via an implanted cannula in the cisterna magna. Intrathecal and ICVS infusion or injection in rats together with serial collection of cerebrospinal fluid was concluded to be successfully performed in our laboratories to be used for conduct of efficacy or toxicity testing.

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EPA has proposed a protocol to replace the current multigeneration reproduction study based on an original proposal from ILSI/ACPA. This extended 1-generation study has now been submitted to OECD. The drivers for replacement were to reduce animal numbers on reproduction studies and the testing demands of REACH for reproductive toxicity. Some surveys had indicated that the F2 generation may add little extra information to hazard characterization, although this inference is disputed. The NTP recently modified its default approach for the rat cancer biosay by incorporating a perinatal exposure period (gestation, lactation and puberty) into the 2-year design that starts treatment with animals at 5-6 weeks of age. A cancer biosay would normally require a prior sub-chronic toxicity study with the same exposure paradigm, for basic toxicity information and dose level setting that can stand alone, or be adapted for a reproductive study. This new NTP design focuses evaluations on the F1 animals with continuous exposure during the study. It commences with timed pregnant females exposed from implantation through lactation, to weaning of their offspring. Various offspring cohorts are then selected to include one for standard toxicity assessments with exposure continuing to sexual maturity (2 males and females/litter). A second cohort designated for breeding on reaching maturity (1 male and female/litter) and a third cohort (1 male and female/litter) would be bred, and a teratology exam on pregnant females conducted just prior to term. The second cohort of pregnant females could be terminated in their last month of gestation and were allowed to deliver normally. In the first days after parturition, the piglets were randomized by cross-fostering (litters were mixed and reattributed to the sows). The 20 newborn piglets were then allocated to groups treated with different compounds and submitted for tests in order to collect specific data on juvenile animals for blood chemistry, electrocardiography, neurological evaluation and ophthalmology. All the piglets were sacrificed on day 34 and submitted for macroscopic and microscopic examination. One group of piglets was treated with enalapril (Angiotensin II Converting Enzyme Inhibitor) by oral gavage from day 7 to day 35. Another group was treated with purified water under the same conditions and acted as a control group. Increased uremia and creatininemia associated with increased renal weight and macroscopic and microscopic lesions in the kidneys were observed in the piglets given enalapril. A third group of piglets received sotalol (antiarrhythmic agent) on the day of electrocardiography, inducing decreased heart rate, PQ and QT interval prolongation. This study supports the interest of the Göttingen Minipig model for the assessment of toxicity in the pediatric population.

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In previous studies, we reported ethanol-induced pancreatic injury in hepatic alcohol dehydrogenase-deficient deer mice. In order to elucidate the mechanism of alcoholic chronic pancreatitis (ACP), a do-dependent study was conducted in ADH- and hepatic ADH-normal (ADH+) deer mice fed 1.2 and 3.5% ethanol via liquid diet daily for 2 months. Metabolism of ethanol and pancreatic injury and endoplasmic reticulum (ER)-stress were evaluated. The blood alcohol levels (BAL) and nonoxidative metabolism of ethanol as measured by quantifying fatty acid ethyl esters (nonoxidative metabolites of ethanol) in the plasma and pancreas were several folds higher in ADH+ vs. ADH+ deer mice fed 3.5% ethanol. However, the blood acetaldehyde levels were not significantly altered in both strains. ADH+ deer mice fed 3.5% ethanol also showed significant pancreatic injury (edema and increase in tryptophan activation peptide and cytotoxic cathepsin B). The most prominent ultrastructural change observed in the ADH+ vs. ADH+ deer mice fed 3.5% ethanol was endoplasmic reticulum (ER)-stress. This was further confirmed by assessing the glucose-regulated protein 78 (GRP78, a marker of unfolded protein response) using Western blot analysis and immunohistochemistry. About 9-fold increase in the expression of GRP78 was estimated in the pancreata of ADH+ vs. ADH+ deer mice fed 3.5% ethanol. These results suggest that increased nonoxidative metabolism of ethanol is an important determinant for the activation of ER-stress in the pancreas and that the observed pancreatic injury could be a down stream effect of ER-stress.
mid-gestation and implants assessed, or allowed to litter their offspring and raise them to weaning. This approach would maximize the use of animals produced, maintain a 10 week pre-breed period, to ensure that male germ cell effects could be noted as a functional change, and increase the power to detect potential developmental effects on the reproductive system. The total number of animals used would be considerably less than a multigeneration study and similar to the EPA proposal, but provide more information on developmental outcomes.

PS 1434 TESTING THE UTEROTROPHIC ACTIVITY OF PERFLUOROOCTANOC ACID (PFOA) IN THE IMMATURE CD-1 MOUSE.

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The uterotrophic assay is an in vivo screening tool used to determine the estrogenic or anti-estrogenic potential of an exogenously administered compound. Recent studies reported that PFOA increased activity of estrogen-responsive genes in fish. Thus, we explored the effect of PFOA on the immature mouse uterus using a standardized uterotrophic assay. Timed-pregnant CD-1 dams (N=17) delivered litters that were equalized to 10 female pups. On postnatal day (PND) 18, pups were weighed, sexed and assigned to treatment groups of equal weight. That same day, pups were treated by oral gavage with PFOA (0.01, 0.01, 0.1, or 1 mg/kg body weight (BW)) with or without 17-B estradiol in corn oil (E2, 500 ug/kg, s.c.). All mice not receiving E2 received a corn oil vehicle s.c. injection. The mice were weighed and dosed for 3 consecutive days and killed on PND 21. BW and uterine wet weights (UWW) were recorded, and uteri were fixed in buffered formalin for future histopathology. BW was unchanged by PFOA treatment. There was a significant increase in the UWW and UWW:BW ratio (% BW) at 0.01 mg PFOA/kg (p<0.05) that is most likely due to enlargement of the uterine cervix junction, visibly enlarged in >75% of the PFOA-exposed animals regardless of E2 status. UWW and % BW ratios for 0, 0.01, 0.1, and 1 mg/kg PFOA were 13+/-1, 20+/-1, 16+/-2, and 16+/-2 mg, and 0.11+/-0.01, 0.16+/-0.01, 0.14+/-0.01, and 0.13+/-0.01, respectively. E2 induced a significant increase in UWW in all groups (mean % BW 0.86+/-0.06). No anti-estrogenic effects were found by co-administration of PFOA with E2. PFOA produced a unique effect at the utero-cervical junction that was distinct from the uterine effect of estrogen. A histopathological analysis will be required to determine if these uterine types affected by PFOA exposure. These data do not necessarily reflect EPA policy.

PS 1435 COMPARISON OF THE EFFECTS OF GENISTEIN AND ETHINYL ESTRADIOL (EE2) IN SPRAGUE-DAWLEY (SD) RATS IN MULTIGENERATIONAL REPRODUCTIVE AND CHRONIC TOXICITY STUDIES.

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The complexity of estrogen signaling and/or interaction with non-estrogen receptor targets leads to distinct but overlapping patterns of effects from compounds interacting with estrogen receptors. Genistein, a soy isoflavone, and EE2, which differ in potency and receptor selectivity, were studied in multigenerational reproductive and chronic toxicity studies with different treatment intervals among generations. SD rats were exposed to genistein (0, 5, 100, or 500 ppm) or EE2 (0, 2, 10, or 50 ppm) in a low phytoestrogen diet. Both produced similar effects in female rats exposed continuously, including decreased body weights, accelerated vaginal opening, and altered estrous cycles. Litter size was reduced in genetically directly exposed to genistein. Chronic studies involved treatment from conception to 2 years (F1C), from conception to PND 140 followed by control diet to 2 years (F1-T140), or exposure of the offspring of three exposed generations (F1C, F1-T140, and F1-T140) to refusal of the offspring at 2 years (F1-T21). EE2, but not genistein, significantly increased the incidence of uterine lesions (atypical focal hyperplasia, squamous metaplasia) in F1C and F1-T21, but not F1-T140, animals. High dose genistein, but not EE2, reduced mammary fibroadenomas (F1C) and increased adenomas/adenoacarcinomas (F1C and F1-T21) in females. Both compounds resulted in increasing trends in preputial gland tumors in males only in F1-T21. High dose genistein, but not EE2, induced early onset of aberrant estrous cycles in all exposure intervals, and all dose levels produced early onset of aberrant estrus cycles in F1-T21 animals. Thus, there was apparent increased sensitivity in offspring of prior exposed generations (F1-T21) for several endpoints. Supported by Interagency Agreement 224-07-007 between FDA and NIEHS.

PS 1436 CONTROL DATA OF REPRODUCTIVE TOXICITY STUDIES IN THE WISTAR HANNOVER RCCHTAMT: WIST RAT.

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A Segment I male fertility study allows detection of effects of spermatogenesis and fertility. Stable data were obtained. In the segment III studies, the reproductive success was comparable in the P and the F1 generation. It can be expected that any latent effect in a substance would lower the reproductive success of the F1 generation and would result in high pre- and post-implantation loss. The mean duration of gestation of the P generation was 21-22 days. Post-implantation loss affected up to 6.3% of implantations. No dead pups were found at the first check of the litter. During the first 4 days of lactation, pup loss was up to 5.8% of the pups born. Loss between days 5-21 was up to 11.8% of the pups born. The percentage of males and females was close to 50% in all litters. The pup weights at birth ranged from 66.1-68.3 grams, the male being slightly heavier than the female pups. On day 21 of lactation, pup weights were 59.4-52.0 grams. Developmental landmarks included the unfolding of the pinna on day 3 - 4 of lactation and the opening of the eyes on day 14. In the F1 generation, there was an average of 13.2 corpora lutea per dam and a pre-implantation loss of 5.1% of all corpora lutea. Post-implantation loss was up to 8.3% of all implantation sites. No dead fetuses were noted. In two-generation studies, the loss of pups after birth is low in the Han Wistar rat in both the P and F1 generations and there is little difference between the generations. Organ and body weights are slightly higher in the F1 than the P generation but are comparable. Pathology data revealed no major differences in females compared to animals in other study types at similar ages, except of higher incidences of hepatocellular hyperplasia as well as physiological changes in the cycle after birth. These studies suggest that the Han Wistar rat has a high fecundity and there is little variation in reproductive success between the generations. Thus, it is a strain well suited for reproductive toxicity studies.

PS 1437 COLLABORATIVE WORK ON EVALUATION OF OVARIAN TOXICITY/EFFECTS OF 2- OR 4-WEEK REPEATED DOSE STUDIES AND FERTILITY STUDY IN FEMALE RATS.

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In this study, 2- or 4-week repeated dose studies with ovarian histopathological examinations were conducted. Female fertility study was also conducted to compare the results to those of ovarian histopathological findings. A total of 17 test substances was evaluated and categorized into hormone analogues, primordial follicle damaging agents, metabolic imbalance inducers, and endocrine imbalance inducers. At the end of treatment, the reproductive organs were prepared for future histopathology. A 2-week dosing period may be sufficient to detect ovarian toxicity. The current repeated dose general toxicity study has been considered insufficient in terms of evaluating female reproductive function due to a lack of evidence indicating the appropriateness. Evaluation of ovarian toxicity by precise histopathological examination was performed.
**1438 COLLABORATIVE WORK ON EVALUATION OF OVARIAN TOXICITY: EFFECTS OF INDOMETACIN IN 2- AND 4-WEEK REPEATED DOSE STUDIES AND FERTILITY STUDY IN FEMALE RATS.**


The 2- and 4-week general toxicity studies in rats were performed with indometacin. A female fertility study was also conducted to compare the study results with ovarian histopathological findings obtained in general toxicity studies. Indometacin is a well-known nonselective inhibitor of cyclooxygenase I & 2, and has been reported to inhibit ovulation by acting directly on the ovary. The main purposes of present studies are to assess whether a precise histopathological examination, taking account of morphological changes in the female reproductive organs, in estrus phase, can evaluate ovarian toxicity and to determine an optimal dosing period for detecting ovarian toxicity. Female Sprague-Dawley rats were orally induced at doses of 0.3, 1.3 or 4 mg/kg (10 rats/group) in all studies. In the general toxicity studies, luteinized follicular cyst was observed in 5 and 2 animals at 4 mg/kg in the 2- and 4-week studies respectively, and atretic follicular cyst was noted in 1 animal at 4 mg/kg of the 4-week study. There was no abnormal finding in the uterus, vagina, or pituitary at any dosage group. In the fertility study, effects of indometacin on the female fertility parameters could not be evaluated at the top dose, since 8 rats at 4 mg/kg died or were sacrificed moribund before completion of the mating period, whereas no effect on any fertility indexes was detected at 1.3 or 0.4 mg/kg. In conclusion, ovarian toxicity induced by indometacin could be detected at 4 mg/kg in the 2-week and 4-week general toxicity studies. In the fertility study, although toxic effect on female fertility could not be evaluated at 4mg/kg due to over-dosing because of animal death, no effects were detected at lower doses. These results suggest that the dosing period of 2 weeks is sufficient to detect the ovarian toxicity induced by indometacin.

**1439 COLLABORATIVE WORK ON EVALUATION OF OVARIAN TOXICITY: EFFECTS OF 2- OR 4-WEEK REPEATED DOSE STUDIES AND FERTILITY STUDY OF BROMOCRIPTINE IN FEMALE RATS.**


The main focus of this study is to determine the optimal administration period concerning toxic effects on ovarian morphological changes in a general toxicity study. To assess morphological and functional changes induced in the ovary by bromocriptine, the compound was administered to female rats at doses of 0.08, 0.4 and 2 mg/kg for the 2- or 4-week general toxicity study, and for the female fertility study from 2 weeks prior to mating to day 7 of gestation. In the 2-week general study, increase of ovarian weights was observed at 2 mg/kg. In the 4-week general study, ovarian weights were increased at 0.4 and 2 mg/kg. The number of corpora lutea were increased in the 0.4 and 2 mg/kg groups of the 2- and 4-week general toxicity studies by the histopathological examination of the ovaries. Bromocriptine did not affect estrous cyclicity in the 2- and 4-week repeated dosing. In the female fertility study, although animals in any groups mated successfully, no females in 0.4 and 2 mg/kg groups were pregnant. There were no adverse effects on the reproductive performance in the 0.08 mg/kg group. Based on these findings, the histopathological changes in the ovary are considered important parameters for evaluation of drugs including ovarian damage. We conclude that a 2-week administration period is sufficient to detect the ovarian toxicity of bromocriptine in a general toxicity study.

**1440 DEVELOPMENT AND VALIDATION OF MULTIPLEX HORMONE PANELS TO EVALUATE RAT PITUITARY, STRESS AND THYROID HORMONES.**

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Hormone multiplexing allows the analyses of multiple hormones in one single assay. The method enables the investigator to reduce sample volume by over 90% and still obtain quality data. Furthermore, this methodology realizes two goals of the 3R philosophy: Reduction in sample volume enables reduction in animal use. Refinement of analytical technique means low bleed volumes and hence better animal welfare. In addition, hormone multiplexing allows for faster data turnaround at a lower cost. For example, for analyses of LH, FSH and PRL, radioimmunoassay (RIA) requires 300 ul of sample, takes 7 working days and costs $2500, compared to the hormone multiplex which requires 25 ul, takes 1 day and costs $1125. We have evaluated the Linco Diagnostics (now Millipore) 5 hormone multiplex panel [rat pituitary, rat thyroid and rat stress hormone panels]. All validations were performed side by side with conventional RIA or enzyme linked immunosorbent assay (ELISA) using the same rat serum samples from hypophysectomized, adrenalectomized, castrated or intact rats. Hormone concentration results showed pituitary and thyroid hormone panels had very good correlation (lower than 10% for most of the hormones), had excellent reproducibility (R' > 0.9) and was capable of detecting biologically relevant hormonal changes. The stress hormone panel is still under evaluation.

**1441 VINCLOZOLIN (V) TREATMENT INDUCES REPRODUCTIVE MALFORMATIONS AND INFERTILITY IN F1 MALE RATS WHEN ADMINISTERED DURING SEXUAL BUT NOT GONADAL DIFFERENTIATION. THE EFFECTS ARE NOT TRANSMITTED TO THE SUBSEQUENT GENERATIONS.**


V produces adverse reproductive effects in male rats when administered during sexual differentiation by acting as an androgen receptor-antagonist. It was recently reported that four generations of SD rats, derived from dams dosed via ip injection GD8-15 with 100 mg V/kg/day, displayed prostate, testis, kidney and immune pathologies in infertility. The authors also reported that these abnormalities were transmitted via epigenetic mechanisms. Our study was designed to determine if such effects could be induced by oral treatment with V. Two groups of SD rats were dosed with 100 mg V/kg/d during either gonadal (GD15-17) or sexual (GD13-17) differentiation. Vehicle controls were dosed GD18-17. GD13-17 V treatment de-masculinized F1 male rats, however none of the effects were transmitted to the F2 or F3 generations. In F1 males, GD13-17 V treatment reduced anogenital distance at birth and androgen-dependent tissue weights, and induced female-like nipples, undescended testes, histopathological lesions in the testis, hypospadias and infertility. The effects of V on AGD, nipple and fertility were not transmitted to the F2 generation. In contrast, GD8-15 V treatment did not reduce fertility, induce reproductive tract malformations, or reduce epididymal sperm numbers in the F1 or F2 generations. F3 males will be examined at middle age and the histology of the testes and other endpoints measured in F3 males to determine if V treatment of P0 dams affected any of these measures in this generation. In summary, oral V treatment during gonadal differentiation did not affect reproductive morphology, sperm numbers or the fertility in the F1 or F2 generations. In contrast, clear adverse reproductive effects were seen in the F1 group exposed to V during androgen-dependent sexual differentiation, however these effects were not transmitted to the F2 generation. This abstract does not necessarily reflect US EPA policy.

**1442 A COMPARISON OF THE GLOBAL GENE EXPRESSION IN RAT GRANULOSA CELLS WHEN CHALLENGED BY METHYCHLOR AND ITS METABOLITE 2,2-BIS-(P-HYDROXYPHENYL)-1,1-TRICHLOROETHANE, HPTE, IN VITRO.**

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Previous studies have examined the effects of methychlor [1, 1, 1-tri-chloro-2, 2-bis-(p-hydroxyphenyl) ethane; MXC] within the female reproductive system. The active toxicant for this mechanism of action is thought to be its estrogenic metabolite, 2,2-bis-(p-hydroxyphenyl)-1,1-trichloroethane (HPTE). The role of MXC and HPTE on gene expression changes within the ovary is unclear. In order to further investigate this effect, granulosa cells were cultured in the presence or absence of follicle-stimulating hormone (FSH, 3 ng/ml) or dibutyryl cAMP (cAMP, 1 mM) during this 48 h period. Microarray analysis using Affymetrix Rat Genome 230 2.0 arrays were used to determine changes in gene expression. Estradiol-17β was also measured in cell-conditioned media using radioimmunoassay. HPTE appeared to have a more potent effect on gene expression changes than did MXC. MXC and HPTE significantly (P < 0.05) altered 2943 and 3633 genes, respectively, of which 826 genes were commonly affected. Enrichment analysis suggested that MXC affects genes regulating the ribosome, cell cycle, DNA polymerase, biosynthesis of steroids and PPAR signaling pathways. In contrast, HPTE
affects genes regulating focal adhesion, regulation of actin cytoskeleton, extracellular matrix receptor interaction, adherens junction, and glutathione metabolism. MXC and HPTE each induce differential gene expression activities in rat granulosa cells, and this marked difference in effect provides insight as to the mechanisms by which these environmental endocrine disruptors can impede female reproductive function.

**1443 CYMOMOLUS MONKEY FERTILITY ASSESSMENTS IN A REPEATED DOSE TOXICOLOGY STUDY: TESTOSTERONE LEVELS, SEMEN ANALYSIS AND TESTICULAR VOLUME.**


The Cynomolus monkey (Macaca fascicularis) male reproductive function assessments are included in chronic preclinical studies in lieu of stand-alone fertility studies. For minimum selection criteria, background reference data is provided as a guide. Sexually mature animals (4-10 years and 5.6-10.2 kg) were evaluated. Testicular volume (TV) was measured twice for each animal (total of 76 animals, 7 to 18 days interval). Testicular volume (right plus left side) was calculated using the formula: volume (mL) = (π x (TxLxW)/6). Semen was collected by penile electro-stimulation for 2 or 3 times/animal (total 76 animals, minimum one week interval). Ejaculate volume (EV) was measured. Computer-assisted sperm analyzer (CASA) was used for sperm motility (SM) and concentration (SC). Total sperm count per ejaculate (TSC) was calculated. Testosterone levels were measured using an ELISA assay. The mean TV was 36.6a±5.9 mL at the first measurement (min. -max. = 18.5 - 62.8 mL). At the second measurement, % difference from the first measurement was 10.4a±10.1% (0.6-45.6%) indicating good reproducibility. Semen analysis parameters notably varied inter-animal and between samples. Average SM, SC, EV and TSC of 2 or 3 measurements were 76 ±20.4, 60.3 ±20.6, 62.2 ±10.1% (0.6-45.6%) indicating good reproducibility. Semen analysis parameter varies between samples. Multiple time-points are required to establish each animal’s baseline data.

**1444 ESTRADIOL PROTECTS OVARIAN ANTRAL FOLLICLES FROM PHthalATE ESTER-INDUCED GROWTH INHIBITION AND ATRESIA.**


Phthalate esters (PEs) are synthetic plasticizers used in a wide range of consumer and industrial products. Di-(2-ethylhexyl) phthalate (DEHP) decreases serum estradiol (E2) levels, prolongs estrous cycles, and blocks ovulation in rats. Mono(oxyethyl) phthalate (MEHP), the active metabolite of DEHP decreases aromatase levels in cultured rat granulosa cells. Our previous studies show PEs inhibit growth and reduce the levels of E2 of antral follicles in vitro. This study tested the hypothesis that E2 protects antral follicles from DEHP and MEHP-induced growth inhibition and atresia. Antral follicles from CD-1 mice were mechanically isolated and cultured in α-minimal essential media a DEHP (100μg/mL), MEHP (10μg/mL), or E2 (1 or 10nM) for 96 hr. Dimethyl sulfoxide (DMSO) was used as vehicle control. Follicles without any treatment were also included to monitor the culture conditions. Follicle size, as a measurement of growth, was obtained every 24 hr and presented as percent change compared to that of 0 hr. After culture, follicles were processed for histological evaluation and were rated for atresia on a scale from 1-4 (1-healthy follicle, 2 ≥ 10%, 3 ≥ 10% to 30%, and 4 ≥ 30% pyknotic bodies/follicles). The results indicate that DEHP and MEHP significantly inhibit follicle growth compared to DMSO treated controls, and E2 partially restores growth of these follicles. (DEHP: DMSO=144.41±4.11, DEHP+E=132.5±1.49, MEHP=DMSO=144.41±5.11, MEHP+E=125±4.06). The results indicate that DEHP and MEHP significantly inhibit follicle growth compared to DMSO treated controls, and E2 partially restores growth of these follicles. (DEHP: DMSO=144.41±4.11, DEHP+E=132.5±1.49, MEHP=DMSO=144.41±5.11, MEHP+E=125±4.06). Further, DEHP and MEHP induced atresia in cultured follicles and E2, treatment protected these follicles from PE-induced atresia (DEHP: DMSO=1±0.3, DEHP+E=0±0.1, DEHP&E, InM=2±0.2a; 1±0.2±0.3; MEHP:DMSO=1±0.3, MEHP+E, InM=1±0.1±0.3, n=3; p<0.05). Collectively, these data indicate that E2 partially restores growth in DEHP and MEHP treated follicles and fully protects against DEHP and MEHP-induced atresia. Support: NIH HD46861.
in the context of an intelligent testing strategy for the evaluation of agrochemicals, aimed at addressing the inefficient development of data (Carmichael et al, 2006). The extended one-generation study is the centerpiece of the life stages paradigm, which has three specific purposes: (1) to determine effects on reproduction, (2) to determine the effects on organ systems of developing animals, and (3) to determine the effect of developmental exposure on the young adult (postnatal day 70/90) offspring. The aim of the presented work was to obtain information on the technical feasibility to conduct an F1-extended one-generation study. This is the first of a series of studies using model (positive control) compounds. Vinclozolin as endocrine modulating (antiestrogenic) model compound was administered at doses of 4, 20 and 100 mg/kg bw/day. Effects include reduced anogenital distance and retained areolae at an oral dose of 20 mg/kg and above, hypoplasias, hypoplastic penis, vagina-like orifices, reduced testicular size, aplasia/agenesis or reduced size of male accessory glands at 100 mg/kg. The overall NOAEL is 4 mg/kg bw. The complexity of the study triggered a number of minor modifications of the ACSA-protocol, particularly in terms of the specific timing of samplings/investigations around PND 21. Despite the challenging logistics the F1-extended one-generation study is technically feasible in a well-trained laboratory. The results of this study prove that the F1-extended one-generation study design is sufficiently sensitive to detect endocrine effects on organ systems of developing animals. At least for this endpoint, it is a scientifically robust approach to reduce and refine the studies required for registration of agrochemicals.

1448 INTRAVAGINAL ADMINISTRATION OF 17β ESTRADIOL TO OVARIECTOMIZED RATS AS A POSITIVE CONTROL FOR UTEROTROPHIC ASSAYS.


The uterine assay (UA, OECD Guideline 440) is used to test compounds for potential estrogen receptor agonism or antagonism. 17β-Estradiol (estradiol), the most potent naturally-occurring estrogen, has been used as a positive control for UA in rodents and is typically administered either orally or subcutaneously. Recommended rodent models include untreated young female or ovariectomized young adult female rat. In the case of compounds that are to be administered intravaginally, only ovariectomized young adult female rodents are relevant models due to the age at vaginal patency (31-37 days in the Crl:CD[SD] rat). In order to develop a positive control of uterotropic activity for intravaginal applications, estradiol in sesame seed oil was administered intravaginally to 60-day old rats (ovariectomized at 41 days) at 0, 0.6, 2, 6, 10 and 20 μg/kg/day once daily for 3 consecutive days. Detailed clinical observations, body weights and food consumption were collected throughout the treatment period and, 24 hours after the last dose administration, wet/blotted uterine weights were measured. At 6, 10 and 20 μg/kg/day, estradiol treatment resulted in dose-related increases in wet and blotted uterine weights. Histopathology performed on uterine and vaginal tissues in 10 μg/kg/day-treated rats compared to vehicle-treated rats demonstrated uterine epithelial hypertrophy, mitotic figures, epithelial necrosis, and smooth muscle hypertrophy; vaginal cornification; and cervical cornification and neutrophil infiltrates. The uterine weight increases and histological changes in 10 μg/kg/day-treated rats compared to vehicle-treated rats demonstrated uterine epithelial hypertrophy, mitotic figures, epithelial necrosis, and smooth muscle hypertrophy; vaginal cornification; and cervical cornification and neutrophil infiltrates. The uterus is a well-established model for investigating estrogenicity in rodent reproductive toxicology, and is the most widely used system for evaluating the estrogenic potential of chemicals. However, the use of estradiol as a positive control for intravaginal studies has limitations. Estradiol is a potent estrogen receptor agonist and is known to have anti-androgenic (antiandrogenic) activity. The use of estradiol as a positive control may not accurately reflect the estrogenic potential of compounds that have anti-androgenic activity. Therefore, it is important to use a range of model compounds with varying estrogenic and anti-androgenic activities to properly evaluate the estrogenic potential of intravaginally administered compounds.

1449 EFFECTS OF FENVALERATE ON CELL PROLIFERATION, APOPTOSIS AND COLLAGEN I PRODUCTION IN HUMAN UTERINE LEIOMYOMA CELLS AND UTERINE SMOOTH MUSCLE CELLS.

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Fenvalerate (Fen; CAS # 16360-58-1), widely used for its high insecticidal potency and low mammalian toxicity, is classified as an environmental disruptor chemical. Recently, Fen has received great attention for its adverse effects on human reproductive health. To determine whether Fen could influence the growth of hormonally responsive uterine leiomyoma (UuLM) cells and normal uterine smooth muscle cells (UuSMC), MTS colorimetric and immunofluorescence (BrDU) uptake assays were done. FACS was used to examine the impact of Fen on cell cycle and apoptotic distribution. Also, real-time PCR and western blotting were employed to determine the expression of Collagen I, a major uterine leiomyoma (fibroid) extracellular matrix component. Fen (10 μM) had a stimulatory effect on the growth of both cell lines at 24 hrs compared with controls by MTS (p<0.01) and BrdU (p<0.01) assays. Cell cycle analysis showed that Fen enhanced the escape of cells from the G0-G1 checkpoint and promoted progression from G2 to mitosis phase. Annexin V assay illustrated that Fen had an anti-apoptotic effect on both cell lines. By Real-Time PCR, we found that Collagen I mRNA expression increased (p<0.05) in Fen-treated cells compared to controls, although it was greater in UuSMC tumor cells. Accordingly, Fen increased (p<0.05) Collagen I protein level in both cell lines and was sustained when compared to controls. Our data show that Fen can stimulate the growth of both uterine leiomyomas and smooth muscle cells, which may provide a combination of enhanced cell-cycle progression and apoptosis inhibition. Additionally, we found this compound can increase Collagen I expression, at both mRNA and protein levels. Our results indicate Fen exposure should be considered a novel risk factor for uterine fibroids.

1450 COLLABORATIVE WORK ON EVALUATION OF OVARIAN TOXICITY: (5) EFFECTS OF 2- OR 4-WEEK REPEATED DOSE STUDIES AND FERTILITY STUDY OF BUSULFAN IN FEMALE RATS.


This study aimed to validate the usefulness of histopathology in a 2- or 4-week general toxicity study as a first-tier screening method for risk assessment of female reproduction. Busulfan, an antineoplastic alkylating agent known to target DNA adducts, follicles (primordial, primary follicle), was given to female SD rats orally (0, 0.1, 0.5, and 1.5 mg/kg/day, n=10 in each group) for 2 or 4 weeks, and ovaries were excised, weighed and fixed. Ovarian histopathology and follicle counts were performed in the greatest cross-sectional area of bilateral ovaries to assess optimal administration period concerning toxic effects on ovarian morphology. In addition, a female fertility study was conducted by dosing the same levels of Busulfan from 2 weeks prior to mating to Day 7 of gestation to compare the Non-toxic doses for female reproduction with those in 2- or 4-week general toxicity studies. In the 2-week study, Busulfan treated rats showed normal estrous cyclicity and no toxicological changes in weights and histopathology of ovaries. In the 4-week study, a decrease in small follicles was observed histopathologically in 1 rat in the 0.5 mg/kg group and in 4 rats in the 1.5 mg/kg group. The follicle counts indicated a decrease in small follicle number in the 1.5 mg/kg group immunohistochemically, although there were no changes in ovarian weight. In the female fertility study, reduced body weight gain, increase in dead embryos and post-implantation loss were observed in Busulfan treated groups, thus Non-toxic doses of Busulfan for reproductive functions and early embryonic development were determined as 1.5 and 0.5 mg/kg, respectively. In conclusion, a 4-week administration period and appropriate assessment of follicle number are needed to detect small follicle depletion in general toxicity study. Moreover, histopathological examination is useful to detect ovo toxicity; however, due to developmental toxicity, careful examination is needed to assess the risk of alkylating agents in female reproduction.

1451 AN ASSESSMENT OF THE REPRODUCTIVE AND DEVELOPMENTAL TOXICITY POTENTIAL OF THE ANTIMICROBIAL 7-ETHYL BICYCLOOXAZOLOIDINE.


7-ethyl bicyclooxazolidine (EBCO), a broad-spectrum bactericidal registered for use in consumer and industrial applications, was evaluated in developmental and reproductive toxicity studies. In the rat developmental toxicity study, groups of 25 time-mated Crl:CD BR0 rats were gavaged with 0, 50, 250, or 650 mg/kg/day (mld) EBCO on gestation days (GD) 6-15. Maternal toxicity consisted of significantly decreased body weight gain (GD 6-15) limited to the 650 mld group. Fetal examination revealed treatment-related findings only in the high dose group, with 18 malformed fetuses from four litters with abdominal wall defects and/or cleft palate; however, 15 of these fetuses were clustered in two litters. The no-observed-adverse-effect-level (NOAEL) for maternal and developmental toxicity was 250 mld. In the rabbit study, groups of 26 time-mated NZW rabbits were administered 0, 15, 50, or 150 mld EBCO by gavage on GD 7-27. Maternal toxicity was limited to hyperemia and increased skin edema in general and materno-fetal weight losses at the start of treatment (GD 7-10), decreased feed consumption (GD 7-13), and decreased fetal output. There were no treatment-related developmental effects at any dose level. The maternal and developmental toxicity NOAELs were 50 and 150 mg/kg/day, respectively. The reproduction study evaluated GD 0-15SD rats given 0, 5, 25, and 150 mld EBCO by gavage for two generations. Parental toxicity consisted of decreased lactation body weight gains, increased liver weights, and stomach irritation effects in the majority of animals given 150 mld. Point of contact stomach irritation of lesser severity and incidence were seen at 25 mld. There were no effects on any pa-
riameter of reproductive performance or offspring survival at any dose level. The NOELs for parental toxicity and reproductive toxicity were 5, 0.5 mg/kg bw/day, and 150 mld, respectively. The NOELs from these studies were greater than those used for the risk assessment, which indicated sufficient margins of exposure for all detailed exposure scenarios.

1452 REPRODUCTIVE AND DEVELOPMENTAL TOXICITY SCREENING TEST OF POTASSIUM PERFLUOROHESANOSULFONATE IN SPRAGUE-DAWLEY RATS.

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This study was undertaken to evaluate the potential reproductive and developmental toxicity of perfluorohesanosulfonate (PFHxS), a surfactant found in sear in the U.S. population. In a modified OECD 422 guideline-based design, 15 rats per sex and dose group (control, 0.3, 1, 3, and 10 mg/kg/day) were dosed by gavage with potassium PFHxS or vehicle 14 days prior to cohabitation, during cohabitation, and until the day before sacrifice (21 days of lactation or gestation day 25 if not pregnant) for females and minimum of 42 days of treatment for males. F1 pups were not dosed by gavage but were exposed in utero and during lactation. Evaluations were made for reproductive success, clinical signs, body weight, feed consumption, estrous cycling, neurological and behavioral effects, bone length, organ weights, and histopathology of selected tissues. Serum and liver concentrations for PFHxS were measured at sacrifice and until the day before sacrifice. An additional 3 rats per sex and group were not dosed by gavage but were exposed in utero and during lactation. An additional 3 rats per sex and group were not dosed by gavage but were exposed in utero and during lactation.

PFHxS doses); 2) decreased prothrombin time (0.3, 3, and 10 mg/kg/day); 3) increased BUN, ALP, Ca++, and A/G ratio (10 mg/kg/day). Serum and liver concentrations for PFHxS were measured at sacrifice and until the day before sacrifice. An additional 3 rats per sex and group were not dosed by gavage but were exposed in utero and during lactation. An additional 3 rats per sex and group were not dosed by gavage but were exposed in utero and during lactation. An additional 3 rats per sex and group were not dosed by gavage but were exposed in utero and during lactation. An additional 3 rats per sex and group were not dosed by gavage but were exposed in utero and during lactation.

1453 PERCUTANEOUS UTEROTROPIC ASSAY OF LAVENDER OIL IN IMMATURE FEMALE RATS.

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The estrogenic potential of lavender oil was evaluated in a percutaneous uterotrophic bioassay in immature female rats by measuring uterine weights at sacrifice. From the use of multiple cosmetic products containing lavender oil as a fragrance ingredient, the maximum daily human skin exposure has been calculated to be 0.2 mg/kg for a 60 kg high-end user of these products. Correcting for the 14.3% skin exposure, the maximum daily exposure is 0.03 mg/kg/day. Four groups of ten immature female rats were randomly selected on postpartum day 16. Three groups of immature rats were administered the vehicle control article (corn oil) or lavender oil at 20 or 100 mg/kg/day in a volume of 5 ml/kg on the shaved back during the 3-day treatment period, days 19, 20, and 21. The positive control group was gavaged twice daily with 2.5 mcg/kg/day of 17α-estradiol estradiol on days 19, 20, and 21. All rats were euthanized 24 hours after the third and final treatment, the uteri and ovaries were removed, and the paired ovaries and uteri were weighed and blistered uterine weights were recorded. No unscheduled deaths occurred. No skin reactions were observed. Both dosages of lavender oil significantly reduced body weight gains after the 3rd day of treatment, but terminal body weights and mean uterine weights did not differ significantly from vehicle control values. Positive controls produced significant increases in body weight and mean uterine weights. At the average data, dosages of 20 and 100 mg/kg of lavender oil equivalent to greater than 3000 times the human maximal daily exposure of 0.03 mg/kg/day were not active in the uterotrophic assay and are not considered to be estrogen agonists.

1454 PLEOTROPIC EFFECT OF AKT1 DEFICIENCY ON FEMALE FERTILITY.

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The majority of ovarian follicles including oocytes undergo atresia through a mechanism involving apoptotic cell death. The purpose of this study was to understand the basis of reduced fertility in mice deficient for the Akt1 gene. Akt1-deficient females exhibit a reduced body size and have decreased ovarian and uterine horn weights. Akt1 deficiency led to a delay in both the age at first litter and onset of estrus and a reduction in average litter size relative to Akt1 wild type and Akt1 heterozygous females. Histological analyses revealed fewer healthy mature follicles in Akt1-deficient females with a significant decrease in the number of follicles at the preantral stage. Primary and secondary oocyte diameter measurements revealed larger primary and secondary oocytes in Akt1-deficient females relative to Akt1 wild type females suggesting defects in the communication between granulose cells and the oocyte. In addition, multiple oocytes per follicle were observed in Akt1-deficient ovaries. Hormonal analyses indicated that Akt1-deficiency has a deleterious effect on FSH and LH levels, as Akt1-deficient females had significantly lower FSH serum levels and significantly higher LH serum concentrations relative to Akt1 wild type females. We found a trend for increased expression of Fas mRNA in Akt1-deficient ovaries. These findings indicate an in vivo role for Akt1 in promoting both follicle and oocyte growth and maturation.

1455 THE ARYL HYDROCARBON RECEPTOR MAY REGULATE GENE EXPRESSION IN THE NEONATAL MOUSE OVARY.

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The aryl hydrocarbon receptor (AHR) is a ligand activated transcription factor known mainly for its role in mediating the toxic effects of numerous environmental contaminants. Recent data also indicate that the AHR plays a role in normal ovarian physiology. Specifically, previous studies have shown that Ahr deletion results in slow follicular growth, decreased estradiol synthesis, and reduced fertility in adult female mice. However, the role of the AHR in early neonatal reproductive function is unknown. Normally, just after birth, the mouse ovary contains a finite number of oocytes available for follicular formation. These oocytes are organized in clusters called oocyte nests that will break apart and form primordial follicles. This study tested the hypothesis that on postnatal day (PND) 3, when oocyte nests are break-
sperm morphology and sperm motility were not affected in F1 and F2 generation male offspring. At present, after the investigation of F1 and F2 pups, the study gives no indication of a transgenerational effect of vinclozolin on the integrity and performance of the male reproductive system, in particular spermatogonial capacity and sperm viability. There were no effects noted at 4 µg/kg bw at any of the investigated generations. Thus, this is the second study which fails to confirm the reported transgenerational effects with vinclozolin. This ip study confirms the absence of such effects as reported earlier following oral treatment.

**1457 COLLABORATIVE WORK TO EVALUATE OVARIAN TOXICITY BY REPEATED DOSE AND FEMALE FERTILITY STUDIES IN RATS: MORPHOLOGICAL CHARACTERISTICS OF NORMAL CYCLING OVARY IN RATS AND THEIR VIEWPOINTS FOR OVARIAN TOXICITY DETECTION.**

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Detection of ovarian toxicities is very important for safety assessment of chemicals including drugs. The detection, however, is very hard without underlying normal ovarian morphology. Since the ovary is used for reproductive physiology, this study was conducted to evaluate practical analysis of ovarian morphology in each estrous cycle stage and guides of morphological viewpoints to detect the toxicities. Single ovary sections transversely dissected in maximum area in 143 rats with normal cycling were examined microscopically. The classification of follicles was referred to Pedersen and Peters (1968). The growing follicles are functionally divided into follicular stimulating hormone (FSH)-independent and dependent ones. The former small and medium follicles relevant to primordial/primary and preantral ones, respectively, were constantly distributed in all sections and easy to detect with immunohistochemical staining for proliferating cell nuclear antigen (PCNA). The latter large follicles relevant to antral or Graafian ones, and atresia showed synchronous histological changes depending on the estrous cycle stage. Regarding to corpora lutea (CL), most recently formed CL underwent remarkable changes in their appearance by each cycle, reflecting ovulation and progesterone production. Combined observation of follicle and CL alterations enhanced reliability of the analysis. This results indicate that qualified morphological analysis in the ovary enables us to classify each estrous cycle. Notification of morphological deviation in follicles and CL from normal estrous cycle is the first tier to detect ovarian toxicities. PCNA immunohistochemical staining is useful to detect follicular loss. Vaginal cytology is also informative to predict the ovarian toxicity.

**1458 GENISTEIN AND ETHINYL ESTRADIOL DIETARY EXPOSURE IN MULTIGENERATIONAL AND CHRONIC STUDIES INDUCE SIMILAR PROLIFERATIVE LESIONS IN THE MAMMARY GLAND OF MALE SPRAGUE-DAWLEY RATS.**

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Genistein, a soy isoflavone and phytoestrogen (PE), and ethinyl estradiol (EE2), a potent pharmaceutical estrogen, were examined in multigenerational reproductive toxicology studies (multigen) and chronic toxicity studies with differing exposure windows across generations (F0-F4). Sprague-Dawley rats were exposed to genistein (0, 5, 100, and 500 ppm) or EE2 (0, 2, 10, and 50 ppb) in a low PE diet. In the multigen studies, generations were terminated at postnatal day (PND) 140. In the chronic studies there were three exposure windows: continuous from conception to 2 yr (F1C); conception to PND 140, followed by control diet to termination (F1T140); conception until weaning at PND 21 of the offspring of three exposed generations (F0, F1, and F2) followed by control diet to 2 years (F2T21). In the EE2 and genistein multigen toxicity studies, there was a dose-responsive mammary gland (MG) hyperplasia, evident even at the lowest dose of EE2 (2 ppb). There was no amplification across generations or significant carry over into unexposed F4. The lower incidence of MG hyperplasia in the chronic studies likely resulted from regression, not progression to neoplasia, because there was no proportionate increase in neoplasms. Sexual dimorphism of MG was retained with no observed feminization in F1 or F2 compared to control. For high-dose genistein or EE2. Furthermore, the morphology of MG hyperplasia did not appear appreciably different across generations in the multigen studies, nor was it different from the low background hyperplasia observed in controls. Supported by Interagency Agreement 224-07-007 between FDA and NIHES.

**1459 EFFECTS OF ETHYLENE GLYCOL MONOETHYL AND MONOBUTYL ETHERS ON THE REPRODUCTIVE AND HEMATOGENOUS SYSTEMS IN MICE.**

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Ethylene glycol monobutyl ether (EGEE) can cause damage to the reproductive and hematogenous systems in animals and in occupationally exposed workers. Ethylene glycol monobutyl ether (EGBE), another member in the family of ethylene glycol ethers, is much less toxic compared with EGEE, and therefore it is used in some cases as an alternative solvent. In this study we investigated the effects of the two ethylene glycol ethers on the reproductive and hematogenous systems when administrated separately or in combination. Wild-type and Aldh2 knockout male mice were orally treated with 600 mg/kg/day of EGEE, EGBE or both compounds for 14 consecutive days. In wild type mice, the motility of sperm in the epididymids was significantly damaged in EGEE group, and to the same extent also in the group of co-administration. In addition, the ratio of testes to body weight in the two groups was smaller than controls, indicating the testicular atrophy. However, EGEE had no such effects and exerted little influence on the reproductive effects of EGEE. In Aldh2 knockout mice, the effect on the reproductive system was not observed in any group, implying a critical role of the enzyme. On the other hand, EGBE treatment caused damage in hematogenous system, such as remarkable decrease in red blood cell count, hemoglobin content and hematocrit value. Furthermore, Calculus was found in the bladder of mice receiving EGEE administration, and its occurrence was 75% in wild and 50% in Aldh2 knockout mice. EGEE did not induce the decrease in blood cells and the formation of calculus, and had no interaction with EGBE on these effects. These results showed that although EGEE and EGBE are similar in molecular structure and chemical properties, these two ethylene glycol ethers have different targets of toxic effects in vivo.

**1460 EVALUATION OF ACUTE TOXICITY OF NOVEL SELECTIVE ET₄ receiving antagonists that control preterm birth in a mouse model.**

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Preterm Birth (PTB), for which the single most common cause is intrauterine infection, is defined as any birth occurring before the completion of 37 weeks of gestation and is a leading cause of perinatal morbidity and death (Rush et al, 1976). Endothelin-1 (ET₁) is a potent vasoconstrictor peptide that is both regulated by inflammatory cytokines and also capable of increasing myometrial smooth muscle tone (Yanagisawa, 1988). We have synthesized a novel series of 1-3-6-trisubstituted-2-carboxy-quadrol-4-one’s that act as selective Endothelin A receptor antagonists (ET₄AR) in vitro. Our overarching hypothesis is that these compounds will control inflammation-associated PTB in vivo without having significant toxic effect. Several different analogues of our compound are tested for an effect on PTB. Briefly, C57BL/6 mice at E15.5 are injected intraperitoneally (i.p.) with lipopolysaccharide (LPS) and then treated with the compound being tested. Mice are then injected with LPS and then treated with vehicle. In addition, the LD₅₀ of the compound is determined by the “Acute Toxic Class” method with slight modifications as per OECD 423 guidelines and with Probit analysis. Neurotoxicity is evaluated further with the rotorod test. Our results show that these novel compounds have a significant effect on the timing of PTB and on the number of pups prematurely delivered (p < 0.001). Furthermore, the estimated LD₅₀ range for at least one of the analogues (n-propyl) is 200-1000 mg/kg, which is several-fold higher than the therapeutic dose range. Rotorod results confirm that the n-propyl analogue causes neurotoxicity at doses several-fold higher than therapeutic doses. Finally, blood was assayed and organs were taken from mice receiving the n-propyl analogue for tissue histochemistry in order to assess organ specific toxicity. We conclude that the novel ET₄AR control PTB at doses that do not have significant toxic effect. These results may impact on a very important clinical problem for which there is currently no satisfactory therapy.

**1461 METHYL-PARAOXON, THE ACTIVE METABOLITE OF THE ORGANOPHOSPHATE PESTICIDE METHYL-PARATHION, ALTERS SPERM QUALITY IN MICE.**

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Methyl-parathion (Me-Pa) is an organophosphate pesticide (OP) forbidden in developed countries but still widely used in Mexico. Hepatic and extrahepatic metabolites of Me-Pa generate methyl-paraoxon (Me-Pox), an extremely reactive compound that is responsible for the anticholinesterase activity. Studies in human
the physiological role of CYP2E1 in the organs changed in the course of growth related to the function of male reproductive organs in mice. It was also suggested that tubules did not show increased degeneration or abnormal spermatogenic cells in malacility was significantly higher in the cyp2e1-/- mice. Stage VII seminiferous seminal vesichles were also significantly higher in the cyp2e1-/- mice of the ages, weeks. Body weights and absolute weights of the epididymides and the prostates-potestive organ weights, sperm counts, sperm head morphological abnormality and hypnosis on the male reproductive system during the growing stages. Body and reproductive organ weights, sperm counts, sperm head morphological abnormality and plasma testosterone concentrations were significantly higher in the cyp2e1-/- mice in the reproductive system. This study aimed to clarify the effects of CYP2E1 deficiency on the male reproductive system during the growing stages. Body and reproductive organ weights, sperm counts, sperm head morphological abnormality and plasma testosterone concentrations were significantly higher in the cyp2e1-/- mice at 6, 8, and 12 weeks of age (n=7-8/group). Tissue sections of the testes of the 8-week-old mice were stained with periodic acid Schiff’s reagent and hematoxylin. Plasma testosterone concentrations were significantly higher in the cyp2e1-/- mice than in the cyp2e1+/+ mice at 8 and 12 weeks of age but not at 6 weeks. Body weights and absolute weights of the epididymides and the prostates- semenal vesichles were also significantly higher in the cyp2e1-/- mice of the ages, but testicular absolute weights were not changed. Sperm counts were also not significantly different between the genotypes in all ages, whereas sperm head abnor- mality was significantly higher in the cyp2e1-/- mice. Stage VII seminiferous tubules did not show increased degeneration or abnormal spermatogenic cells in the cyp2e1-/- mice. The present study revealed that CYP2E1 expression was related to the function of male reproductive organs in mice. It was also suggested that the physiological role of CYP2E1 in the organs changed in the course of growth since the significant differences in the testosterone concentrations were observed at 8 and 12 weeks of age but not at 6 weeks.

**1463 REPRODUCTIVE AND DEVELOPMENTAL EFFECTS OF MOTORCYCLE EXHAUST IN RATS.**

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Fossil fuel combustion products are potential health hazard and environmental hazards. Engine emissions from two-stroke and four-stroke motorcycles create a major environmental burden in urban areas where motorcycles are a popular means of transportation. Motorcycle exhaust (ME) contains higher levels of benzene and bu- tadiene than diesel and gasoline exhaust from passenger cars. Carcinogenic aromatic hydrocarbons such as benz(a)pyrene and benz(a)anthracene are present in organic extracts of ME particulates. The major purpose of the present study was to determine the reproductive and developmental effects using male rats exposed to 1:10 diluted ME, 2 hr daily and 5 days a week for 7 weeks. ME exposure markedly decreased testicular and epididymal sperm counts and increased testicular lipid per- oxidation and interleukin-1β mRNA expression. The ME-exposed male rats were mated with unexposed female rats and their offspring were used to determine the effects of ME on the parameters of reproductive system development. The ME exposure caused decreases of anogenital distance in male offspring on postnatal day (PND) 1 and anovaginal distance in female offspring on PND 14. The exposure re- sulted in an earlier vaginal opening in the female offspring. The ME exposure increased organ weights of prostate, levator ani bulbocavernosus, and bulbourethral glands in the male offspring on PND 56. The exposure also decreased cytochrome P450 content and 7-ethoxycoumarin O-deethylation activity of male rat liver mi- crosomes on PND 56. These present results suggest that ME inhalation exposure of male rats has the ability to produce reproductive toxicity and to cause effects on the parameters of reproductive development in their male and female offspring.

**1464 EVALUATION OF THE EFFECTS OF MDMA (“EXTASY”) ON THE FERTILITY AND REPRODUCTION OF THE MALE RAT.**

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3,4-Methylenedioxymethamphetamine (MDMA, “ecstasy”) is an illicit drug that has become increasingly popular in Europe and North America over the past 20 years. As consumption mainly occurs among young population, it is relevant to take into consideration the effects of MDMA on the reproductive system. The im- pact of MDMA on the health has been repeatedly documented, but its effects on reproduction have not yet been deeply studied. MDMA consumption af- fects several neurotransmitter systems such as serotonergic, dopaminergic and norepinephrinergic which are involved in the neuroendocrine control of reproduc- tive functions. In this study the influence of MDMA on the fertility and reproduc- tion of the male rat was assessed. MDMA was administered (sc) to Sprague-Dawley male rats once a day, three consecutive days a week, simulating human week-end associated consumption. MDMA doses were calculated as 5mg/kg, 5mg/kg or saline (control) during 12 weeks, (from 5 to 17 PNW) covering the puberty onset and the sexual maturity achieve- ment. The highest dose was calculated as 1/5 LD50 and the lowest was in the same order of magnitude of human exposure. Hormonal (LH, FSH and testosterone), haematological, biochemical, histological, genotoxicological ( Comet test in sperm) and, testicular and sperm parameters (mobility, morphology and quantification of spermatozooids and spermatids, epididymis and teste weigh), were evaluated in half of the animals. The remaining animals were mated with untreated sexually recep- tive females to evaluate the mating and pregnancy rates and sexual behaviour No biologically significative differences were found in the studied parameters ex- cept in genotoxicity, food and water consumption in all treated groups, and total weight in the high dose group. In conclusion, these results show the lack of impor- tant effects in male rat fertility after MDMA subchronic exposure.

**1465 ASSESSING THE CUMULATIVE IMPACTS OF CHEMICAL EMISSIONS AND DISCHARGES.**

G. V. Alexeff1, J. Fusi1, A. Kyle2, R. Morello-Frosa1, M. Jerrett2 and L. Zee2. CHEMICAL EMISSIONS AND DISCHARGES.

California legislation to address environmental justice has required the transformation of the risk assessment process to address cumulative impacts in communities. The state’s definition of cumulative impact requires consideration of the combined emissions and discharges from all sources in a geographic area, on public and environ- mental health, taking into account sensitive populations and socio-economic factors. In a collaborative project involving CalEPA and academic partners, we are developing better analytical tools to identify cumulative impact, reviewing existing statutory authorities and regulatory requirements, developing case studies and con- cept papers and creating a framework for analysis to support decision making. While U.S. EPA adopted a very specific interpretation of cumulative risk which re- quires consideration of chemicals that act by a “common mechanism of toxicity,” our focus has been to identify how community exposure disparities contribute to health disparities for environmentally mediated diseases. Further, in addition to ex- posure, toxicity, and health status the process considers increased vulnerabilities that result from non-intrinsic factors such as race, ethnicity and SES. We found that existing exposure disparities contribute to community health disparities. We considered the California Air Toxics Hot Spots program as a case study to examine these concepts in a specific decision context. While the current hot spots program focuses on the cumulative risk of chemical emissions from individual facilities, it does not take into account emissions from other facilities located in the community or incorporate community health status or vulnerabilities. We conclude that a mix- ture of these methods, including those drawn from toxicology and other disci- plines, are needed to address cumulative impacts.
Health risk assessment of chemical mixtures is frequently conducted on the basis of exposure and toxicity information for individual components. Routine application of physiologically based toxicokinetic (PBTK) models in mixture risk assessment is limited by the number of parameters that need to be specified for the full model for mixtures. Since human exposures to environmental chemicals frequently lead to steady-state conditions, it is pragmatic to develop a tool that provides the steady-state solutions of full PBTK models of mixtures by accounting for the nature and magnitude of interactions. A steady-state solution was developed for computing dose metrics (i.e., blood concentration and metabolism rate) during mixture exposures and applied for computing interaction-based hazard index for mixtures. The algorithm is based on critical determinants of the internal dose during chronic exposure to VOCs (i.e., alveolar ventilation rate, blood flow rate to liver, blood:air partition coefficient, maximal velocity of metabolism, Michaelis constant, inhibition constants, and free concentration of chemical at the site of interaction and metabolism). This algorithm, developed as a spreadsheet program, is an iterative procedure for estimating an intermediate term representing sum of ratios of liver concentrations of individual components to the Michaelis parameter (or inhibition constant). Case studies demonstrating the application of this tool were conducted for human inhalation exposure to toluene, xylene, ethylbenzene, carbon tetrachloride and tetrachloromethylene. The results demonstrate that the algorithm appropriately accounts for the saturable metabolism and competitive inhibition among the chemicals in mixtures, as a function of exposure concentration. This risk assessment tool should be of use in establishing interaction thresholds for environmental contaminants, as well as for conducting interaction-based risk assessment for populations exposed to chemical mixtures.

The California Air Resource Board (CARB) collects emissions data from various sources. These emissions data are used to evaluate the ability of environmental pollutants, socio-economic factors, and climate and median age to explain variations in county-specific mortality rates for heart disease, cancer, stroke, chronic obstructive pulmonary disease (COPD) and all causes combined. Generally, the estimations of the models of all diseases for all regions were considered reasonable. This study suggests the need for a better understanding of the pathways through which these factors and mortality are related.
Human health risk assessment consists of bringing to bear a large body of in vitro, animal, and epidemiologic studies on the question of whether environmental exposures to a substance are a potential risk to humans. However, the body of scientific information is typically unclear, often contains apparent contradictions; and therefore, often only possible conclusions about potential human risks may be surmised from the data and may vary from very strong to tenuous. The task, therefore, is to also effectively communicate the uncertainties within the data, giving proper consideration to contrary data and alternative scientifically plausible interpretations.

We propose an approach, HBWoE, to organize, evaluate, and communicate all of the available data on a given chemical exposure, using naphthalene as an example. The National Toxicology Program (NTP) inhalation bioassay data for naphthalene showed an increased risk for nasal tumors in rats and lung tumors in mice, and although cytotoxicity and hyperplasia were observed in mouse nasal and to some extent in rat lung tissue, tumors were not observed in these tissues. CYP2F metabolism of naphthalene to its epoxide and cytotoxicity has been invoked as a potential carcinogenic mode of action (MOA) for naphthalene, and there is CYP2F activity in both tissues and species. We evaluate the available data for naphthalene, through the HBWoE approach, considering potential key events (end-points) in the MOA in each species and tissue: metabolism by CYP2F, cytotoxicity, chronic inflammation, potential genotoxicity, regenerative hyperplasia, and tumor formation. We evaluate the weight of evidence in support of a cytotoxic MOA, in addition to alternative MOAs that have also been offered in the scientific community, noting the explanatory power of the proposed hypotheses, considering consistencies, inconsistencies, and contradictions within the data set, and how compelling each end-point is as evidence of a similar MOA in humans from low levels of naphthalene exposure.

Risk analysts typically make a number of decisions during the conduct of a quantitative risk assessment, and these decisions may have significant impact on the final risk estimate. In this analysis we explore the sensitivity of risk estimates for titanium dioxide (TiO2) to pooling of data, dose-response model selection, animal-to-human extrapolation method, and the choice of a human dosimetry model. The goal was to estimate the occupational exposure concentration of TiO2, associated with a 0.1% lifetime excess risk of lung tumors, based on long-term bioassay data for both fine (pigment-grade) and ultratine (UF) TiO2. The method used was benchmark dose (BMD) modeling, estimating either the model-based 0.1% exposure concentration in rats, or a 10% ‘point of departure’ coupled with linear extrapolation. Potential modeling decisions included use of the best-fitting model, the most health-protective model, or a model average, and whether to use the central BMD estimate or a 95% lower-bound (BMDL). Extrapolation issues include the choice of dose metric – either particle surface area/g lung or particle surface area/lung surface area – and the choice of a human lung particle dosimetry model. The BMD estimates vary by a factor of 29 between the most health-protective dose-response model and the best-fitting, while the BMDLs differ by 2.7. The BMD and BMDL differ by factors of 1.3-1.4, depending on the choice of dose-response model. Pooling fine and UF TiO2 data has little impact on the estimated risk estimates for fine, but does affect the risk estimates for UF. The choice of dose metric for rat-to-human extrapolation impacts the risk estimate by roughly 3-fold, and the choice of dosimetry model alters it by approximately 2-fold. Overall, the estimated occupational exposure associated with a 0.1% excess risk of lung tumors may vary more than 100-fold, depending on the specific assumptions which are made.

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

### 1472 AN ASSESSMENT OF THE IMPACT OF EXPOSURE ROUTE ON THE INTERINDIVIDUAL VARIABILITY FACTOR (IVF) FOR DRINKING WATER CONTAMINANTS (DWCs).

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An IVF of 10 or two multiplicative factors of 3.16 representing pharmacokinetic (PK) and pharmacodynamic (PD) variability are frequently used in risk assessment, independently of the exposure route and sensitive subpopulation. The objective of this study was to evaluate the impact of exposure route on the magnitude of IVF-PK for two frequently identified/presumed sensitive subpopulations (pregnant women and infants (age < 3 years)). A multidose PBPK model framework was obtained from the literature and used for computing the dose metrics (DM, i.e., area under the blood concentration vs time curve (AUC) and the amount metabolized/L liver/24 hr (MET)) in adults, infants and pregnant women following four exposure scenarios to three DWCs – chloroform (CHL) trichloroethylene (TCE) and bromoform (BRO). The exposure scenarios were: (i) ingestion of drinking water, (ii) dermal contact (15 min/day), (iii) inhalation for 24 hr, and (iv) an oral dose (μg/kg/day). Distributions for body weight (BW), height (H) and hepatic CYP2E1 content were obtained from the literature or from P3M software whereas body surface area, blood flows and tissue volumes were calculated from BW and H. The magnitude of IVF-PK was calculated as the ratio of the 95th percentile value of DM in sensitive subpopulation to the 50th percentile value in adults, obtained by Monte Carlo simulations. In neonates, the magnitude of IVF-PK for CHL-AUC was 2.59, 1.25, 1.42 and 1.43 for drinking water ingestion, inhalation, dermal and oral dose, respectively. For CHL-MET, the values were 1.36, 1.14, 1.34 and 0.76. In pregnant women, the corresponding values were 0.41, 2.12, 1.61 and 0.39 for CHL-AUC; and 0.87, 0.47, 0.42, and 0.83 for CHL-MET. Comparable values were obtained for TCE and BRO. Overall, these results indicate that the chemical-specific IVF-PK is route-dependent for the DWCs investigated and the PBPK model-derived values for all three routes were below the default value of 3.16.

### 1473 YOUR RESULTS MAY VARY: EXPLORING THE SENSITIVITY OF TITANIUM DIOXIDE RISK ESTIMATES TO DIFFERENT MODELING ASSUMPTIONS.

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Assessment of the potential for subsurface volatile organic contamination on indoor air quality is widely relied on the Johnson-Ettinger Model (J&EE). However, depending on the availability and types of site-specific data, using the J&EE to estimate subsurface vapor intrusion into buildings generally is a very conservative approach that tends to overestimate actual risks. Therefore, it is essential to fully understand the behavior of each J&E with different medium data sets and the response of the model to various input parameters. The study site contains a large industrial building in an urban area. The contaminants include chlorinated organics such as 1,1,1-trichloroethane, tetrachloroethene, methylene chloride, and trichloroethene which were released into shallow soil and groundwater from past operations. These volatile organics pose potential concern to indoor air quality of the overlying building. Four suites of data including soil, groundwater, soil gas, and sub-slab soil gas were collected from the site. Potential risks from subsurface vapor intrusion are evaluated using the J&EE soil, groundwater, and soil gas models. Results of the J&EE risk evaluations were verified by indoor air samples. This verification analysis provides a guide for selecting data sets when using the J&EE and the ranking of the data sets to be used. Sensitivity analysis will be conducted on each J&E to evaluate uncertainties generated by individual parameters. Fully understanding the behavior of each J&E and the response of the model to individual parameters will provide a more representative estimate of risks.

### 1474 VERIFICATION AND SENSITIVITY ANALYSIS OF THE JOHNSON-ETTINGER MODELS.


In smelter communities, one of the most important pathways to consider in Human Health Risk Assessments (HHRA) is potential exposures to relevant chemicals (i.e., metals) in indoor dust. Wet and dry deposition causes atmospheric emissions from smelters to settle onto local soils and other surfaces. Both the settled material and the airborne chemicals may be transferred into residential homes via human and local meteorological activity. Outdoor yard soil can be transported indoors by wind, household pets, or on clothing or shoes of humans. These outdoor sources, combined with numerous indoor, anthropogenic sources, form typical household dust. Studies have reported that between 20 and 30% of indoor contamination comes from outdoor soil sources. This is an important pathway of exposure in human health risk assessments, especially for sensitive individuals.
The bioaccessibility of a contaminant is the portion that is soluble in the gastro-intestinal environment, and is available for uptake into the blood stream. This soluble fraction can be measured in an in vitro laboratory setting, and can be used as a surrogate for bioavailability. The use of bioaccessibility in risk assessment is considered to be valid and applicable, provided that some basic data requirements are fulfilled to permit regulatory groups (e.g., U.S. EPA, Health Canada) to ascertain the validity and defensibility of such measurements. While in vitro assays are commonly used to evaluate soil, evaluation of bioaccessibility of metals in dust is not routinely undertaken. In a recent community-based HHRA, consideration was given to the bioaccessibility of metals present in indoor dust collected from within homes in the community. Comparison of the dust bioaccessibility to soil for the same community provides some insight to its utility and necessity of collecting this type of data at other locations. Not surprisingly given its ultrafine characteristics, bioaccessibility for dust was slightly higher than for soil. The soil and dust bioaccessibility data for six smelter-related metals will be discussed and compared.

1476 WHEN BACKGROUND RISKS ARE IMPORTANT: METALS IN SOIL.
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Naturally occurring metals in soils can be significant in risk assessment and risk management. This paper has recommended including naturally occurring metals in risk assessments. Background issues are to be addressed in risk characterization, rather than in selecting chemicals of potential concern. This new approach presents a more thorough picture of risks and encourages transparency. However, it may generate confusion by reporting risks which will generally not be addressed in remediation because they stem from background. For some sites it is important to portray total risks from both anthropogenic and natural sources. Sites A, B, C, and D illustrate areas affected by mineralized soils, dredged material used to create land, drainage from mine tailings, or agricultural drainage. Site A was created by filling wetlands with sediments dredged from a bay. The sediments were impacted by drainage from mine tailings carried through a river system. Metal concentrations in original soils are generally much less than those detected in artificial fill soils. Arsenic (As) concentrations in original soils ranged from 2 to 21 mg/kg compared to 0.7 to 49 mg/kg in artificial fill. Manganese (Mn) ranged from 156 to 628 mg/kg in original soils and 27 to 15,559 mg/kg in artificial fill. Potential risks to humans and ecological receptors from exposure to these fill soils can be significant. The cancer risk and hazard quotient (HQ) for future residents are 6E-4 and 1.6 based on the 95th percentile As concentration, and 8E-4 and 2.2 assuming the maximum concentration. The Mn HQ for future residents is 0.9 based on the 95th percentile and 7.5 based on the maximum. Ecological receptors may suffer harm from exposure to local background concentrations that are toxic. For example transport of fill material containing serpentine to areas where ecological receptors are adapted to lower metal concentrations can be deleterious. Addressing high risk estimates can be problematic through traditional remediation because of large areas. Awareness of the potential risk can suggest alternate mitigation measures such as land use restrictions.

1477 LEAD TESTING WIPES CONTAIN MEASURABLE BACKGROUND LEVELS OF LEAD.
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Lead is regulated under the California Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65) as both a carcinogen and reproductive hazard. As part of the process to assess whether consumer products satisfy Proposition 65 with respect to lead, various wipe sampling strategies have been utilized coupled with analytical methods with very low limits of detection (LOD). A method developed by the Consumer Products Safety Commission recommends using Ghost™ Wipes, a commercially available wipe designed for surface lead testing. In this study, four commonly used wipe materials (cotton gauze, cotton balls, absorbent filter paper, and Ghost Wipes) were tested for background lead. A total of 35 samples were collected. Each sample included three units of wipe material that were removed from their packaging, digested together, and analyzed using inductively coupled plasma mass spectrometry (ICP-MS) (LOD = 0.1 μg/sample). Lead was not found in cotton ball (n=3) or filter paper (n=3) samples. One of three cotton gauze samples was found to have 0.02 μg of lead. Every Ghost Wipe sample (n=24) contained measurable amounts of lead. On average, Ghost Wipe material was found to have 0.43 ± 0.11 μg lead/sample (0.14 μg/wipe). Therefore, wipe testing for lead using Ghost Wipes and analyzing them with ICP-MS may result in measurable concentrations of lead regardless of whether or not the consumer product has leachable lead. These results may falsely lead one to conclude that the product causes lead exposure in excess of the Proposition 65 Safe Harbor Levels. Therefore, special attention needs to be paid to the choice of wipe material and the importance of field blank controls.

1478 USING THERAPEUTIC DATA TO ASSESS POTENTIAL HUMAN HEALTH RISKS FROM EXPOSURE TO ORGANIC NITRATES.
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Pentaerythritol tetranitrate (PETN) and nitroglycerine are organic nitrates that have been found as contaminants at several Superfund sites. These organic nitrates are explosives with military and commercial applications. However, they are also used therapeutically as vasodilators to treat angina pectoris by reducing venous pressure, cardiac preload and cardiac output. Because of this therapeutic application, a rich database is available in the pharmaceutical literature. Additionally, these organic nitrate vasodilators tend to reduce arterial pressure, myocardial oxygen demand, and tissue edema. However, several of these therapeutic effects can also cause undesirable consequences in some people. For example, the maximum therapeutic doses of 58 mg/day for nitroglycerine and 160 mg/day for PETN have been reported to cause headache and postural hypotension due to cerebral vasodilation. More severe consequences might result if already hypotensive people are exposed to these organic nitrates. This presentation discusses whether effects from lower doses that are therapeutic in people with angina pectoris should be considered as critical effects for assessing health risks to the general population.

1479 STUDY OF THE EFFECT OF CONCOMITANT INGESTION AND INHALATION EXPOSURE TO THE INTERNAL DOSE OF N-HExANE (HEX) OR CYCLOHEXANE (CYCLO) IN RATS.
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The potential contribution of dermal and inhalation routes of exposure to volatile organic chemicals (VOCs) in drinking water is increasingly drawing attention. The objective of this study was to evaluate, in an animal model (rat), the contribution of multiroute exposures to the total internal dose of HEX and CYCLO in drinking water. Initial experiments showed that the contribution of the dermal route was insignificant for the two compounds. Subsequently, groups of 5 male Sprague-Dawley rats were either given a single dose of HEX (5.5 or 22.3 mg/kg) by oral ingestion (ING) or exposed to 50 or 200 ppm (2 hr) by inhalation (INH). For CYCLO, ING doses were 27.9 and 108.9 mg/kg whereas INH concentrations were 300 and 1200 ppm (2 hr). Additional groups were exposed by the two routes simultaneously (INH and ING) at the high doses for HEX, and both at the high and low doses for CYCLO. Blood samples (15 μl - 200 μl) were collected for up to 6 hr after treatments and HEX and CYCLO concentrations were measured. Overall, the blood concentrations measured after multiroute exposure to the high dose of HEX were higher (~1.45 - 1.70 - fold) than the sum of the blood levels obtained after administration of HEX by each route. In contrast, results for CYCLO after multiroute exposure were similar to the sum of the blood levels obtained for single route exposures to CYCLO (~1.0 - 1.2 - fold), suggesting additivity of the internal dose associated with individual exposure routes. These results suggest that the additivity of internal dose arising from multiple routes is not only determined by the route-specific kinetic characteristics but also by the degree of saturation during aggregate exposures. Supported jointly by ExxonMobil and NSERC, Canada.
nephropathy in males. No treatment-related increase in nephropathy, of minimal to mild severity, was observed in males. No treatment-related increase in protein is due to a concentration effect. The relative weights of kidneys and testes were significantly increased in males at 12 mo of exposure. A treatment-related increase in protein is due to a concentration effect. The relative weights of kidneys and testes were significantly increased in males at 12 mo of exposure.

25 and 52. A complete necropsy was performed at 6 mo (males) and 12 mo (males and females) of exposure. Animals were euthanized and selected tissues examined for histopathology. Lesions were observed. Male rats exposed to jet fuel exhibited an increased accumulation of naphthalene particles were not present. Degeneration of the olfactory epithelium was observed in both F344 and SD strains, both sexes, with increasing incidence and severity that correlated with naphthalene exposure concentration. Lesions were minimal to moderate in severity. At 0.1 ppm, minimal olfactory lesions were observed in female rat only. Fourteen-day recovery animals exposed to 10 ppm also had olfactory epithelium lesions, though at a lower severity, showing evidence of recovery. Minimal to mild nasopharyngeal goblet cell hyperplasia/hypertrophy was related to exposure to 10 ppm naphthalene in all groups of rats.

1481 METHYL TERTIARY BUTYL ETHER (MTBE): ONE-YEAR TOXICITY DRINKING WATER STUDY IN WISTAR RATS.
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The environmental presence of MTBE, particularly in groundwater, underscores the need for information on the toxicology of this compound when administered in drinking water (DW). The current study is part of a 2-yr study designed to examine the toxicity and carcinogenicity of MTBE administered in DW. Male Wistar rats were exposed to 0, 0.5, 3, 7.5 and female to 0, 0.5, 3, and 15 mg/ml MTBE in DW for 12 mo, based on the results of a 90-d pre-chronic study. Body weights, clinical signs, and food and water consumption were monitored. Urinalysis was performed on males at study weeks 25, 38, and 52 and on females at study weeks 25 and 52. A complete necropsy was performed at 6 mo (males) and 12 mo (males and females), including the collection of blood for clinical pathology and hematology. Blood levels of tert-buty1 alcohol (TBA), the major metabolite of MTBE, were determined for males and females at 12-mo. Body weight and food consumption were the same as control and water consumption was less than control (p<0.01), for MTBE-treated males and females. There were no significant changes in hematology or serum chemistry parameters for males or females. Blood levels of TBA increased with dose in males and females. Urine osmolality (OSM) and specific gravity (SG) were not statistically different in body weights related to naphthalene exposure throughout the study. At 0.1 ppm, minimal olfactory lesions were observed in female rat only. Fourteen-day recovery animals exposed to 10 ppm also had olfactory epithelium lesions, though at a lower severity, showing evidence of recovery. Minimal to mild nasopharyngeal goblet cell hyperplasia/hypertrophy was related to exposure to 10 ppm naphthalene in all groups of rats.

1482 PATHOLOGY AND GENOTOXICITY IN F344 RATS IN A TWO WEEK INHALATION EXPOSURE EXPOSED TO AEROSOLIZED JET FUEL.
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Fischer-Tropsch (FT) jet fuel is produced by a synthetic process from natural gas and may be used to augment or replace petroleum-derived JP-8 jet fuel for the US military. During operations and maintenance, personnel may be exposed to jet fuel vapors and aerosols. An inhalation study was conducted in which F-344 rats were exposed to 0, 500, 1000, and 2000 mg/m3 of an aerosol/vapor mixture of jet fuel. Animals were exposed for 6 hours per day, 5 d/wk for two weeks. After the last exposure, animals were euthanized and selected tissues examined for histopathology. Bone marrow smears and aerosol testing of the femur was taken, stained, and examined for micronucleus formation to assess the genotoxic potential of inhaled FT jet fuel. In the lungs of males and females at the highest concentration, we observed multifocal areas of inflammatory cell infiltration. In the nasal airways of males and females exposed to the intermediate and high concentrations, olfactory epithelial degenerative changes were observed. Male rats exposed to jet fuel exhibited an increased accumulation of hyaline droplets in the proximate convoluted tubular cells, characteristic of alpha-2u globulin induction. There was no statistically significant induction of micronuclei observed in the exposed groups.

1483 INTRAVENOUS- AND INHALATION-ROUTE PHARMACOKINETICS OF PROPANOIIC ACID AND ITS METABOLITE, PROPIONIC ACID.
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Propionic acid (PCA) is an intermediate in the metabolism of propionyl-CoA, a metabolic precursor of the branched chain amino acids. PCA is widely used in many industrial applications, potentially leading to inhalation exposures in the workplace. Extrapolation between observed effects in animals and potential human effects requires detailed route-specific pharmacokinetic analyses. Inhalation and intravenous (iv) exposure studies were conducted in rats to determine the blood pharmacokinetics of propanoic acid and its major metabolite, propionic acid. Inhalation-route studies included constant exposures to 500 or 3500 ppm 13C-propionic acid with concurrent measurement of respiratory rates using plethysmography and measurement of 13C-propionic acid in blood. Radiolabeled material was used to isolate the metabolite from endogenous propionic acid. Respiratory rates were steady for the 2 hr inhalation exposures. The average minute volume for 4 rats exposed to 3500 ppm 13C-propionic acid was 116 ± 22 ml/hr. Blood propionic acid and propionic acid peaked within the 2 hr exposure and dropped immediately post exposure. For 3500 ppm exposures, peak propionic acid was 360 ± 67 nmol/ml, several fold higher than observed after a 25 mg/kg iv dose. Propionic acid however was proportionally less than after the iv dose, most likely indicating saturable metabolism of propionic acid. The iv exposures included a 30 minute infusion to achieve steady state with analysis for 13C-propionic acid and 13C-propionic acid in blood and urine. Rats were also dosed iv with 13C-propionic acid to determine the acid kinetics independent of propionic acid metabolism. The Cmax and AUC of 13C-propionic acid were ~6x higher following a propanoic acid dose than following an equimolar dose of propanol. The rate of elimination of propionic acid and AUC of 13C-propionic acid were ~6x higher following a propanoic acid dose than following an equimolar dose of propanol.

1484 INHALATION KINETICS OF PENTAFLUOROPROPANE IN HUMANS.
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1,1,1,3,3-Pentafluoropropane (HFC-245fa) is one of the hydrofluorocarbon (HFC) replacements of the ozone-depleting chlorofluoro- and hydrochlorofluorocarbons. Human toxicologic data on HFCs, and in particular on HFC-245fa, are sparse. Five healthy volunteers of each sex were exposed in random order to 0, 100 or 300 ppm HFC245fa for 2 h during light physical exercise in an exposure chamber. Capillary blood, urine and exhaled air were sampled before, during and after exposure and analyzed for HFC245fa by head space gas chromatography. Urinary fluoride and triluoroacetonic and triluoroacetic acid were analyzed by ion selective electrode and GC/MS, respectively. Symptoms of irritation and central nervous system effects were rated in Visual Analogue Scales (VAS). Biochemical (ASAT, ALAT, ALP, GGT, lactate, creatine kinase (CK), CK-MB) and immunoassays (SAA protein, fibrinogen, D-dimer, C-reactive protein, and IL-6) in plasma/serum were analyzed, based on sporadic findings after exposure to other HFCs. The initial increase in blood was fast and average levels of 1.9 and 5.7 μmol/l were reached within a few minutes of exposure to 100 and 300 ppm HFC245fa, respectively. The biphasic post-exposure decrease in blood was fast as well and parallel to that in exhaled air. The respiratory uptake could not be determined, as inhaled and exhaled air had similar concentrations of HFC245fa. On average, about 0.7% and 0.001% of the total amount of inhaled HFC-245fa was excreted in urine and urine, respectively, after exposure. triluoroacetonic acid was not detected in urine (LOD 0.1 μmol/l) and no increase in trifluoroacetic acid or fluoride above background was seen, suggesting minimal metabolism. The VAS ratings revealed no HFC-245fa-related effects, except a slight increase in subjective symptoms in the low exposure group. The study was financed by Honeywell International and approved by the regional ethical review board.

1485 ASSESSMENT OF THE IRITATION POTENTIAL OF SWEDISH SNU WITH POACHED SNUS INGREDIENTS USING THE EIPOTRALTM TISSUE MODEL.
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Our risk assessment for ingredients used in Swedish style pouched snus differs significantly from that used for traditional combusted tobacco products. Prolonged contact of the snus pouch with the oral mucosa may allow certain ingredients to
exert an irritant potential on mucosal tissue. As part of our risk assessment, we have utilized an in vitro screen for irritancy in order to define non-irritant levels of ingredients for use in snus products. The EpiOral™ tissue model consists of cultured human epidermal keratinocytes in a differentiated multilayer with a buccal phenotype. The irritant effects of topically applied chemicals are measured using the MIT cell viability assay. Ingredients for use in snus were tested in duplicate up to a maximum concentration of 5000 μg/ml using exposure periods of both 1h and 20h. Triton X-100 and SDS were included as positive controls and DMSO as a negative control. None of the ingredients tested at concentrations up to 5000 μg/ml affected the tissue viability at either time point. Actual tissue viabilities relative to controls were: benzyl carbinol (118.6% & 114.5%), cinnamaldehyde (99.1% & 93.3%), citronellol (124.2% & 118.4%) and menthol (121.3% & 122.7%) for 1h and 20h exposure respectively. In contrast, exposure for 1h to 1% Triton X-100 or 1% SDS gave tissue viabilities relative to controls of 40.3±11.4 and 44.4±9.2% respectively, reducing to 7.1±1.8% and 7.6±1.3% respectively after 20h exposure. A reduction of ≥25% tissue viability relative to the negative controls was used to define irritancy within our model. These data demonstrate the utility of the EpiOral™ model for assessing irritation potential to the oral mucosa. In our hands, ingredients for use in snus that may possess an irritation potential are screened using this model at an early stage in our risk assessment paradigm. This assists in defining a level of ingredient use in pouched snus that is without irritant effect.

**1486 HOW DO ANIMAL STRAINS AND VEHICLES IMPACT ON LLNA RESULTS.**

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The murine local lymph node assay (LLNA) is a stand-alone method for objective and quantitative determination of skin sensitizing potential of chemicals. However, various factors, e.g. animal strains and vehicle-effects, may have an influence on the LLNA in different ways and a variety of mechanisms and sometimes even elicit unreliable results. To quantitatively evaluate such influences we made various measurements: (1) the total disintegrations per minute per node (dpm/LN) of 3H-methyl thymidines incorporated in draining lymphocytes, using CBA/CaOlaHsd, CBA/CaHsdRec(SPF), CBA/CaCr(cru)/RI, CBA/Jlbn(SF), CBA/Nglc, Balb/c and NMRI mice. The methods were identical with those described in the OECD Guideline 429. The results obtained demonstrated that the background dpm/LN in strain differences of mice were different. CBA/CaOlaHsd and NMRI mice of those tested presented the lowest and the highest dpm/LN (130 to 554), respectively; (2) dpm/LN derived from CBA/CaOlaHsd and CBA/CaHsdRec(SPF) mice, following topical exposure to dimethyl sulphoxide (DMSO). It was found that CBA/CaOlaHsdRec(SPF) mice presented a significantly higher response to DMSO than CBA/CaOlaHsd mice; (3) dpm/LN derived from CBA/CaHsdRec(SPF) mice following topical exposure to acetone/olive oil (4/1, v/v) (AO), water/acetone (7/3, v/v) (EO) and benzyl alcohol (100%, v/v) (BA). The results demonstrated that the magnitude of SI of HCA with DMSO was clearly decreased due to a high dpm/LN level in the vehicle-control when using CBA/CaOlaHsd mice. The EC50 values were: 1.76 (BN), 5.71 (DMF), 6.36 (EtOH70%), 7.35 (PG), 11.72 (AOO) and 16.21 (DMSO). In conclusion, both animal strain and the choice of vehicle can affect the irritancy within our model. These data demonstrate the utility of the EpiOral™ model for assessing irritation potential to the oral mucosa. In our hands, ingredients for use in snus that may possess an irritation potential are screened using this model at an early stage in our risk assessment paradigm. This assists in defining a level of ingredient use in pouched snus that is without irritant effect.

**1487 TSCA 8(e)/FYI INITIAL SCREEN DATABASE, A PRIORITIZATION TOOL.**

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Under TSCA section 8(e), persons who manufacture/import a chemical must inform EPA of any new information regarding substantial risk of injury to health or the environment. Data received under section 8(e) generally report findings of toxic effects in laboratory animals, accidents of environmental release, toxic effects in aquatic species, or reports of human exposure. FYI (for your information) submissions are voluntarily submitted and generally consist of studies with negative results. EPA's Risk Assessment Division (RAD) screens these 8(e)/FYI submissions to identify chemicals to determine priority for follow-up activities. In April 2006, SRC began to assist EPA in the development of the TSCA 8(e)/FYI Initial Screen Database to store the screening information derived from 8(e) and FYI submissions. Each database record contains a link to an electronic version of the corresponding 8(e) or FYI submission. The database is searchable by chemical name, CAS number, submitter name, date range, key words within text, document number, and submission description. Other fields in the database indicate whether or not the document contains confidential business information (CBI), study information/description, and priority level for follow up. The current prototype of the TSCA 8(e) Initial Screen Database contains data from 2000 to present and supplements the data within the original TSCATS database, which includes the evaluation of chemicals up to the year 2000. A parallel version of this database has been created which contains CBI information in addition to all the information that is present in the non-CBI version.

**1488 HORMESIS KNOWLEDGE AND OPINION SURVEY RESULTS.**

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Studies and review articles about hormesis are being published at an increasing rate by researchers from a variety of disciplines. Due to increased publication interest, we conducted an opinion survey to assess knowledge and attitudes about the hormesis dose response.

Research objectives were to:
1) assess the respondent’s general knowledge about dose response models, including hormesis,
2) determine characteristics of people who would, if allowed by regulation, take potential for hormesis into account when designing or interpreting risk assessments, and
3) compare the characteristics of those who would consider hormesis to those who would not by identifying variables affecting acceptance/rejection.

Methodology: The survey was administered in August and September of 2008. It consisted of 44 questions covering basic demographics, knowledge and attitudes about dose response, knowledge and attitudes hormesis, and knowledge and attitudes about risk assessment principles and practice. The survey was pre-tested and pilot tested by 25 toxicologists and risk assessors representing diverse backgrounds and employers.

The survey was distributed via email to 9,500 potential respondents, all of whom were members of either the Society of Toxicology or the Society for Risk Analysis. Respondents were offered an opportunity to decline participation and to ask questions of the researchers. Survey responses were analyzed in the aggregate using SAS statistical software. QA/QC techniques were used to assure duplicate responses were eliminated from analysis and to evaluate survey reliability and validity.

Conclusion: The survey had an overall response rate of 20% and a completion rate of 73%. The poster presents results of the detailed analysis and identifies the characteristics of those who would employ hormesis in a risk assessment and those who would not. In general the survey indicated 65% of respondents felt hormesis justified a change in hazard assessment protocols and that 87% of respondents felt risk assessments should accommodate the hormesis dose response.

**1489 A PROBABILISTIC CANCER RISK ASSESSMENT MODEL OF KEY SMOKELESS TOBACCO CONSTITUENTS.**

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Smokeless tobacco (ST) use is common worldwide, with a potential increase in consumption in coming years. This report describes a probabilistic cancer risk assessment model of key ST constituents. The model includes a wide range of ST products, evidence of being known or suspected carcinogens, and availability of oral cancer potency data. Such compounds fell into a range of chemical classes including polycyclic aromatic hydrocarbons (PAHs), N-nitrosamines, heavy metals, carbonyls and other organic chemicals. Distributions incorporated in the probabilistic model included: analyte concentration (built from quantitative chemical analysis data collected in triplicate for each product), daily ST consumption (based on market surveys and the peer-reviewed literature), frequency of use and body weight (based on data from the 1999-2006 National Health and Nutrition Examination Surveys) and duration of use (based on U.S. Census Bureau life expectancy data). Cancer slope factors were included as point estimates and potency equivalency factors were used to estimate potency of individual PAHs where measured slopes did not exist. In summary, probabilistic models provide significant improvements over deterministic models in health risk assessments with highly variable and uncertain input parameters such as those associated with ST use. To our knowledge, the present work is the first application of probabilistic approaches in estimating cancer risk linked to ST use.
1492 ESTABLISHING A MUTAGENIC MODE OF ACTION: CASE STUDIES.


Current U.S. EPA guidelines for cancer assessment place emphasis on establishing mode of action (MOA) as a key step in the decision process. For chemicals for which there is some evidence of a mutagenic mode of action (MOMA), the pre-existing, tumor-associated mutagenic expression for POLYCYSTIC TUMORS in rats by xenobiotics.

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The relevance to human cancer risk assessment of treatment-associated tumicaria vagnalis esmolothemias (TVM) in rat cancer bioassays has been questioned. Hormone imbalance brought about by perturbations of the endocrine system has been proposed as a key factor leading to both spontaneous and treatment-associated TVM in rats. Tunicaria vaginalis esmolothemias commonly occur in F344 male rats and are causally associated with an altered hormonal milieu attributable to the high background incidence of Leydig cell tumors of the testes of these animals. Twenty-one chemicals were found that produced a treatment-associated TVM response primarily in F344 rats; of these, 11 had a robust TVM response at various doses. These chemicals were identified as candidates for studies of the role of tumors in the establishment of a mutagenic mode of action as defined by the U.S. EPA. The mutagenic mode of action for each of these chemicals is proposed based on a review of the literature and application of U.S. EPA's Framework for Determining a Mutagenic Mode of Action for Carcinogenicity. For both chemicals, the proposed MOA entails metabolism to DNA-reactive metabolites, covalent binding with DNA, muta- tion, and proliferation of mutated cells. For 2NA, key evidence includes: observa- tion of mutagenicity and genotoxicity following metabolic activation in a variety of organisms and tests; identification of metabolites that form DNA adducts in the bladder in humans and animals and cause mutations in bacteria; formation of tu- mors following less than one year of exposure; and similarity to benzidine, which is recognized by EPA to have a mutagenic MOA. Additional support comes from findings that 1-naphylamine, a 2NA analog, is neither mutagenic nor extensively transformed to a DNA reactive metabolite and does not cause bladder cancer in an- imals or humans. Evidence in support of a mutagenic MOA for 33DMDB includes: mutagenicity and genotoxicity in a variety of organisms and tests; mutations in oncogenes found in rats induced by 33DMDB; demonstration of specific acti- vated oncogenes in 81% of benign and malignant tumors in rats treated with 33DMDB, compared with only 2.5% of spontaneous tumors in controls; and simi- larities to benzidine. Key determinants for a mutagenic MOA for these chemicals included similarities to another mutagenic agent in structure and response, and strong associations between tumor occurrence and occurrence of specific DNA adducts or mutations within target tissues in exposed humans or animals.

1493 FACTORING PRE-EXISTING, TUMOR-ASSOCIATED MUTATIONS AND POLYCYSTIC TUMOR DEVELOPMENT INTO THE REGULATORY RISK ASSESSMENT PARADIGM.

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In quantitative cancer risk assessment, genotoxic and non-genotoxic carcinogens may be regulated differently. It is generally assumed there is no safe dose of a geno- toxic carcinogen, based on the idea that the induction of a single mutation is po- tentially carcinogenic. If a chemical has a mode of action that is not directly geno- toxic, some regulatory groups will use a nonlinear approach to quantitative cancer risk assessment. This is based on the idea that non-genotoxic carcinogens may op- erate through a mechanism that has a dose-related threshold. The realizations that many or most tumors are polyclonal in origin [B.L. Parsons (2008) Mutation Research/Reviews in Mutation Research 659: 232-247] and tumors are polyclonal in origin (B.L. Parsons (2008) Mutation Research/Reviews in Mutation Research 659: 232-247) and tumor-associated muta- tions pre-exist in normal tissues have not been factored into the development of this risk assessment paradigm. A review of data from several studies, obtained using Allele-specific Competitive Blocker-PCR (ACB-PCR), indicates tumor-associated mutations (rat and mice) are measureable in tissues (colon, liver, lung, nasal mucosa, and skin) of control animals (rats and mice), as well as in human colonic mucosa. The observed spontaneous mutant fractions (MFs) range from below the level of accurate ACB-PCR quantitation (10^-5) up to 10^-3, with the majority of sponta- neous MFs above 10^-3. Further, a literature review identified studies which support the idea that pre-existing mutations within a tissue are involved in initiating car- cinogenesis. The hypothesis that tumor initiation involves the spatial interaction between cooperating clones of cells with complementing genetic and/or epigenetic lesions implies that polyclonal tumors may be initiated through mixed modes of ac- tion. Together, this information raises the question of whether the mutagenic ef- fects of chemicals seen at high doses in rodents accurately model mode of action rel- evant to human exposures and whether chemically-induced epigenetic effects have a larger role in human cancer development than previously realized.
1495 ASSESSMENT OF THE RODENT CARCINOGENICITY TESTING REQUIREMENTS FOR PESTICIDES.

The Office of Pesticide Programs (OPP), United States Environmental Protection Agency (US EPA), requires a two sex, two species rodent carcinogenicity bioassay for the registration of all food use pesticides. To date, the results of approximately 500 such studies have been reviewed by OPP. The common rat and mouse strains used in carcinogenicity studies for pesticides were Fischer, Sprague-Dawley, and Wistar rats and CD-1 or Swiss and B6C3F1 mice. The most common tumor types observed in studies with male or female rats were liver, thyroid and testes; in studies with male and female mice, evidence of increased incidences of liver tumors was by far the most common tumor type. The positive carcinogens generally induce tumor responses only in male and/or female mice. Most of these mouse only tumor responses were detected using a Swiss strain of mouse and a few were detected using B6C3F1 mice. The majority of the mouse only carcinogenic tumors induced in both sexes of mice. The most common tumor type reported for the mouse specific carcinogens was liver tumors. Those mouse specific carcinogenic pesticides generally did not appear to cause DNA reactive. Some mode of action data are available on these mouse specific carcinogens that indicate they generally induce tumors either by promoting the clonal expansion of preneoplastic cells by stimulating cell proliferation or by persistent cell killing which in turn may result in a persistent regenerative proliferative response in the damaged tissue. This assessment includes a summary of the tumor profile data from the standard rodent carcinogenicity bioassays for pesticides which may provide insights into the value of these studies. An analysis will be performed on each rodent study and its overall impact on human health risk assessment. The views expressed in this abstract are those of the authors and do not necessarily reflect those of the U.S. EPA.

1496 OXYFLUORFEN: EVALUATION OF THE MODE OF ACTION FOR LIVER TUMORS IN MALE MICE.
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Oxyfluorfen (2-chloro-1-(3-ethyl-4-nitrophenyl)-4-( trifluoromethyl) benzene) is a diphenyl ether herbicide. A carcinogenicity study with oxyfluorfen resulted in a minimal increase in the incidence of combined hepatocellular adenomas and carcinomas in male CD-1 mice following chronic exposure at the highest dose tested, 200 ppm (33 mg/kg/day). The increase in liver tumors was within the range of appropriate historical controls and only statistically significant for one of the two concentrations. No increases in liver tumors in female mice or male or female rats were seen, nor were there any increases in any other types of tumors in either species. A proliferative response in the liver. Peroxisomal acyl-CoA oxidase was increased in the oxyfluorfen treated animals, but electron microscopy provided no evidence of peroxisome proliferation. A PPARα transactivation assay was negative for oxyfluorfen. Additional mechanistic studies include gene expression in male mice fed increasing doses of oxyfluorfen and measurements of liver enzymes and liver porphyrin content. High doses of oxyfluorfen in male mice for 10 days (2500 ppm) or 3 months (2000 ppm) are known to cause hepatic porphyrin accumulation and increased liver weights (Krijt et al., 1993, 1994). Overall, these mechanistic data provide further definition of the MOA in the livers of male mice fed oxyfluorfen.

1497 OPPORTUNITIES FOR PROGRESS IN USING MECHANISTIC INFORMATION IN RISK ASSESSMENT: THE PPAR-α MODE OF ACTION HYPOTHESIS REVISITED.

Data on a chemical’s mode of action (MOA) can be critical to decisions in evaluating human health risks. However, there are several challenges in evaluating and applying MOA data for carcinogenic risk assessment purposes. These challenges include determining whether a MOA causes cancer, and estimating the relative human sensitivity to a MOA. These issues are elaborated in our present review of the PPAR-α MOA for hepatocarcinogenesis. PPAR-α receptor agonism has been posted as a sole, causative factor for hepatocarcinogenesis. In addition, based on a hypothesized reduction in human sensitivity to PPAR-α activation in hepatocytes does not alone evoke liver tumors. We conclude that 1) the hypothesized PPAR-α MOA is no longer tenable as a sole explanation for rodent liver tumors; 2) for most PPAR-α agonists, chemical-specific data are insufficient to characterize the relative human sensitivity to the rodent MOA or to hepatocarcinogenesis; and 3) the available epidemiologic data for PPAR-α agonists are inadequate to provide sufficient information to inform conclusions of human relevance. These findings have broad implications for the assessment of the human health risks of the diverse rodent hepatocarcinogens that are PPAR-α agonists, including many environmental contaminants. They also suggest opportunities for progress in how MOA hypotheses are developed, tested, and applied in human health risk assessment. Disclaimers: These views are those of the authors and do not necessarily reflect US EPA policy or opinion.

1498 USE OF TOXICOGENOMIC DATA AT THE U.S. EPA CANCER ASSESSMENT REVIEW COMMITTEE.

The Office of Pesticide Programs’ (OPP) Cancer Assessment Review Committee (CARC) routinely utilizes mode of action (MOA) data when available for pesticides whose cancer risk assessment is being considered. Typically, a MOA analysis incorporates data from required toxicology studies and supplemental mechanistic data. The CARC has considered genomic data in the weight-of-the-evidence (WOE) for support of an MOA. As part of this effort, standard approaches are being developed for the inclusion of toxicogenomic data, and other new technologies, into the risk assessment process. The Office of Research and Development has developed toxogenomic datasets for several regulated conazoles. Members of this class of fungicides induce mouse liver tumors via an undetermined MOA. Toxicogenomic data, supplemental tissue response information, molecular and biochemical studies, and traditional registration studies were used to determine the value of applying genomic data to the MOA analysis. Postulated key events based on genomic and experimental studies are: nuclear receptor activation, CYP induction, cholesterol inhibition, oxidative stress, effects on retinoic acid and mevalonic acid levels, and in vivo mutagenicity. These key events are being synthesized into MOA assessments that help to explain the toxicological effects of conazoles. EPA Cancer Risk Assessment Guidance approaches will be used to develop a process whereby genomic data can be integrated into our risk assessments. The CARC sees this as an opportunity to bring new approaches that inform our understanding of cancer and increase the efficiency and accuracy of our risk assessment process. The views expressed in this abstract do not necessarily reflect those of the U.S. EPA.

1499 INTERPRETATION OF DERMAL ABSORPTION FOR PESTICIDES AND BIOCIDES IN THE EU: A LIMITED CRITICAL EU GUIDANCE.

Risk assessments for agrochemicals and biocides within the EU require an assessment of dermal absorption: a statement of “percentage dermal absorption” is a required regulatory endpoint. Harmonisation and consistency of data evaluation by the various Member States of the EU has been assisted by the “Guidance Document on Dermal Absorption”, a working document of the European Commission. The “Guidance Document” was established with a primary aim of ex-
tablishing default assumptions in cases where no data, or very little data, are available for a chemical. The document also describes how data from in-vitro or in-vivo dermal absorption studies may be interpreted in order to derive the “percent dermal absorption” value. Use of this guidance document since 2004 has however indicated that further work in the context of additional data can refine the procedures described in this guidance. Circumstances in which additional data could be incorporated in dermal penetration assessments, while remaining appropriately precautionary, are discussed for the following three areas: - Interpretation of in-vitro and in-vivo studies, considering only penetration of radiolabel, without assessment of any toxic, corrosive, or asphyxiant substance that, via inhalation exposure, poses an immediate threat to life or would cause immediate or delayed irreversible adverse health effects or would interfere with an individual’s ability to escape from a dangerous atmosphere in the event of a respirator failure. We developed a process to prioritize IDLH development for high priority chemicals of specific interest to emergency response personnel (i.e., chemical terrorism agents or industrial chemicals subject to emergency or uncontrolled releases). The prioritization process included weighted scores to account for metrics of exposure potential, toxicity, and a variety of secondary considerations (such as toxicity data availability and existence of other acute exposure guidance values). We evaluated the impact of a refined weight of evidence approach described in our prior work on methods for developing IDLH values. The refined approach was applied to 20 case study chemicals, particularly where use of additional data is possible. The resulting preliminary IDLH values and the rationale for their derivation is presented and contrasted to the IDLH values that would have been developed using a default IDLH coloration approach. Lessons learned from these case studies are used to inform further refinements in the IDLH derivation methods as they apply to chemicals of interest to homeland security applications and emergency preparedness.

1500 PROTECTING EMERGENCY RESPONSE PERSONNEL FROM CHEMICALS OF HIGH PRIORITY BY DERIVING IMMEDIATELY DANGEROUS TO LIFE OR HEALTH (IDLH) VALUES USING REFINED METHODOLOGY.

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The National Institute for Occupational Safety and Health (NIOSH) has been investigating methods to improve the derivation of Immediately Dangerous to Life or Health (IDLH) values. IDLH values are 30-minute atmospheric concentrations of any toxic, corrosive, or asphyxiant substance that, via inhalation exposure, poses an immediate threat to life or would cause immediate or delayed irreversible adverse health effects or would interfere with an individual’s ability to escape from a dangerous atmosphere in the event of a respirator failure. We developed a process to prioritize IDLH development for high priority chemicals of specific interest to emergency response personnel (i.e., chemical terrorism agents or industrial chemicals subject to emergency or uncontrolled releases). The prioritization process included weighted scores to account for metrics of exposure potential, toxicity, and a variety of secondary considerations (such as toxicity data availability and existence of other acute exposure guidance values). We evaluated the impact of a refined weight of evidence approach described in our prior work on methods for developing IDLH values. The refined approach was applied to 20 case study chemicals from the list of agents identified by the prioritization process. The resulting preliminary IDLH values and the rationale for their derivation is presented and contrasted to the IDLH values that would have been developed using a default IDLH coloration approach. Lessons learned from these case studies are used to inform further refinements in the IDLH derivation methods as they apply to chemicals of interest to homeland security applications and emergency preparedness.

1501 DRAFT REVISIONS TO THE GUIDELINES FOR AUTHORS OF DRINKING WATER HEALTH ADVISORIES.

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The EPA’s Office of Water is updating the Guidelines for Agency authors for writing Drinking Water Health Advisories (HA). EPA’s Office of Water develops HAs to identify non-regulatory concentrations of drinking water contaminants at which adverse health effects would not be anticipated to occur over specific exposure durations in children and adults. EPA develops 1-day and 10-day HAs for a 10-kg child, Longer-term HAs for a 10-kg child and for adults, and a Lifetime HA for adults. The draft updated Guidelines reflect new developments in risk assessment methods, including benchmark dose modeling, updated approaches to uncertainty factors (including chemical-specific adjustment factors), and the 2005 cancer guidelines. In addition, the draft updated Guidelines provide information on data sources and quality, guidance on key decision points, and references to other relevant EPA guidelines. Additional information is provided in the Guidance on consideration of life stages and endpoints relevant to the 1- and 10-day HAs. It is expected that the guidance, when finalized, will result in a more scientifically robust and consistent approach to the development of HAs. [The opinions herein are those of the authors and do not necessarily reflect the opinions of the U.S. Environmental Protection Agency (USEPA)].

1502 EFFECT OF THE IMMUNOSTIMULANT γ-D-GLUTAMYL-L-TRYPTOPHAN ON THE EFFECTOR PHASE OF ASThma IN A Guinea PIG MODEL.

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The dipeptide γ-D-Glu-L-Trp (SCV-07) is being tested as an immunostimulant in clinical and animal studies of cancer and infectious disease. Previous studies suggest that it stimulates Th1 immunity and reduces Th2 immunity. We hypothesized that this pattern of immunomodulation would be of benefit in controlling asthma. Objective: Determine if γ-D-Glu-L-Trp inhibits the effector phase in a guinea pig model of asthma. Animals were sensitized with ovalbumin(OVA) i.p. on day 0. On day 17-21, animals received daily i.p. injections of 1 or 10 µg/kg γ-D-Glu-L-Trp or vehicle phosphate buffered saline (PBS). On day 21 all animals were pre-treated with antihistamine to reduce the acute histamine component of the immediate bronchoconstrictor response that can be fatal. Animals were challenged with 1% OVA or saline (NSS) aerosol for 20 minutes. Animals were monitored by the body temperature or oxygen saturation for 2 hours. Significant differences were observed in the control group compared to the treatment group. The results of this study show that the dipeptide γ-D-Glu-L-Trp experienced significantly reduced cough, labored breathing and distress compared to PBS treated animals given OVA aerosol. γ-D-Glu-L-Trp did not affect the OVA specific IgG1 concentration in serum. Cell infiltration and lung injury were assessed 18 hours after OVA challenge. In animals challenged with NSS aerosol, γ-D-Glu-L-Trp significantly reduced total lung eosinophil peroxidase, suggesting that dipeptide treatment reduced the number of resident eosinophils in BAL. γ-D-Glu-L-Trp or vehicle phosphate buffered saline treated animals indicated lung injury occurred and γ-D-Glu-L-Trp treatment markedly reduced this OVA-induced lung injury. Thus, these studies indicate that γ-D-Glu-L-Trp does not prevent eosinophil movement into the allergic lung, but significantly reduces the immediate allergic reaction and subsequent lung damage and may decrease the number of resident eosinophils in the lung. (Research supported by SciClone Pharmaceuticals, Inc.)

1503 ASSESSMENT OF PROTEIN ALLERGENIC POTENTIAL IN MICE: RELATIONSHIPS BETWEEN IMMUNOGENICITY AND ALLERGENICITY.

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Assessment of the potential allergenicity of novel proteins is an important issue, particularly in the context of the safety of genetically modified crop plants. We have shown previously that the measurement of specific IgE production induced by systemic (intraperitoneal) ip administration of proteins to BALB/c strain mice correlates with allergenic potential. In the current experiments, IgG and IgE antibody responses following ip exposure to a range of proteins have been measured by enzyme-linked immunosorbent assay (ELISA) and homologous passive cutaneous anaphylaxis (PCA) assay, respectively. Intrapерitoneal administration of proteins not associated with allergic responses such as potato lectin and purified potato protein stimulated vigorous IgG antibody responses but failed to stimulate IgE antibody production, even at relatively high doses (10%). Exposure of BALB/c strain mice to protein enzymes such as lipolase and termamyl that cause IgE-mediated respiratory allergy induced relatively low titers IgG antibody responses, but comparatively vigorous IgE antibody production. Similarly, ip administration of the major cow’s milk allergen β-lactoglobulin and the peanut allergen Ara h 1 stimulated weak IgE antibody responses and detectable specific IgE antibody production. In contrast, exposure to the peanut allergen Ara h 2 failed to provoke detectable IgG or IgE antibody. The relatively poor immunogenicity of both of the peptide proteins may reflect prior exposure to cross-reactive soy proteins in the diet. These data demonstrate the importance of monitoring IgG antibody responses, such that only the failure to observe detectable IgE antibody in the presence of a robust IgG antibody response is interpreted as a negative result. Furthermore, respiratory sensitizing proteins may also be characterized as a function of induced IgE antibody responses following systemic ip exposure. Experience to date is encouraging that this method may represent a useful approach for the prospective identification of protein allergens.

1504 CIRCUMVENTING THE HYPERSENSITIVITY LIKE REACTION ASSOCIATED WITH REPEAT DOSING OF A HUMANIZED MONOCLONAL ANTIBODY IN TRANSGENIC MICE.


GMA161, an aglycosyl humanized monoclonal antibody that binds to CD16A and CD16B Fc-receptors, has been examined in models for treatment of idiopathic thrombocytopenic purpura. A human double transgenic (Tg) mCD16b-.
were overcome by using 1% Pluronic in PBS as vehicle and by the dosing into lymph nodes. The OECD protocol 429 was used; however, the difficulties as- 
pose. The effects of dosing selected test materials using an aqueous vehicle into the the 2475 genes pathway analysis revealed a number of genes associated with the T-

to evaluate sensitization by the well-known respiratory sensitizers trimellitic anhy-
idolate its various reactive subgroups: Subgroup A-C (sensitized and 
challenged with solvent or test solution); Subgroup D1-D2 (sensitized with test solution 
and re-challenged with low or high dose of test solution). On days 1 to 3, 8 to 10, and 15 to 17, a 25-μL aliquot of test solution was applied to the dorso- 
mal sensitivity. Two weeks after the last sensitization, mice were intratracheally 
challenged with a 50-μL aliquot of test solution. After topical sensitization and 
challenging them intratracheally, we assayed differential cell counts and chemokine 
levels in bronchoalveolar lavage fluid (BALF); lymphocyte counts, surface antigen 
expression of B cells, and local cytokine production in lung-associated lymph nodes 
(LNs); and antigen-specific IgE levels in serum and BALF. TMA induced marked 
increases in antigen-specific IgE levels in both serum and BALF, proliferation of eosinophils and chemokines (MCP-1, eotaxin, and MIP-1β) in BALF, and prolifer-

Lyophilized extracts of zinc生日 (Zn), copper (Cu), iron (Fe), and manganese (Mn) were 
delivered to mice subcutaneously at weekly intervals over a period of nine weeks. 
Aim and Results: The present study was aimed to evaluate the antigenic potential 
of the metal ions, using a modified local lymph node assay (LLNA) for their sensitizing 
potential. The murine Balb/c mice model was chosen for its suitability and 
acceptance as an in vivo model for detecting contact sensitizers. The present study 
also evaluated the effect of concurrent administration of metal ions and chemical 
sensitizers. The metal ions used were selected on the basis of their chemical 
configuration and potential for clinical significance. To determine the sensitizing 
potential of the metal ions, a modified LLNA protocol was applied, following the 
OECD guidelines. The study showed that all the metal ions tested were able to 
sensitize mice, with varying degrees of reactivity. The results were analyzed using 
statistical methods to determine the significant differences among the groups. 
Conclusion: The present study demonstrated the capacity of metal ions to 
act as contact sensitizers, highlighting their potential importance in the field of 
occupational and environmental dermatology. Further studies are required to 
elucidate the mechanisms underlying the sensitizing effects of metal ions and 
to evaluate their clinical relevance.
that OPA may have the potential to cause respiratory sensitization. Inhaled OPA does not produce any histological or histochemical changes. However, microscopic examination of the lungs from mice exposed to OPA revealed a significant increase in the expression of cytokines (IL-4, IL-10, IL-13, and IFN-γ). These data suggest that OPA may cause respiratory sensitization.

**1509** 4-OXOPENTANAL IDENTIFIED AS A POTENTIAL INDOOR AIR IRITANT AND ALLERGEN.

J. L. Franko, A. E. Munson, J. Ham, L. Jackson, R. Wells, L. Butterworth, and S. E. Anderson, CDC/ID/NC/D, Morgantown, WV.

Over the last two decades, there has been an increasing awareness regarding the potential impact of indoor air pollution on human health. People working in an indoor environment often experience symptoms such as eye, nose, and throat irritation, headache, and fatigue. Investigations into these complaints have associated the effects to volatile organic compounds (VOC) emitted from building materials, cleaning formulations or other consumer products, and indoor chemistry. The di-carbonyl 4-Oxopentanal (4-OPA) is generated through the ozonolysis of squalene and several high volume production compounds that are commonly found indoors. Measureable levels of 4-OPA have been detected in simulated indoor air environments. 4-OPA was tested in a combined local lymph node assay (LLNA) and identified to be an irritant and extreme sensitizer with an EC5 value of 0.08%. Significant increases were observed in the B220+ and IgE+B220+ cell populations in the draining lymph nodes after exposure to 4-OPA concentrations of 0.25% and higher. Total serum IgE levels were also significantly elevated after exposure to 25% 4-OPA. These results suggest that 4-OPA has a potency function as an IgE-mediated sensitizer. The identification of this compound as an irritant and sensitizer may help to explain some of the adverse health effects associated with indoor air exposure.

**1510** ORTHO-PHTHALALDEHYDE INHALATION INDUCES IMMUNE ACTIVATION IN THE NASAL MUCOSA AND DRAINING LYMPH NODES IN MICE.

V. J. Johnson, J. S. Reynolds, W. Wang, K. Fhurarry and B. Yuceer, Health Effects Laboratory Division, NIOSH/CD, Morgantown, WV.

4-Ortho-Phthalaldehyde (OPA) is increasingly being substituted for glutaraldehyde as a disinfectant due to its high reactivity for biological macromolecules, it is reasonable to hypothesize that OPA may pose similar respiratory hazards. Several case reports have been published supporting this hypothesis showing development of respiratory hypersensitivity reactions in healthcare workers and patients exposed to OPA. The purpose of this study was to determine the respiratory toxicity of OPA using a murine model. A nose-only vapor inhalation system was used to expose mice to OPA vapor for 4 hours/day for 3 consecutive days followed by sacrifice on day 5. The lungs, nasal mucosa and head-draining lymph nodes were collected and processed for cytokine gene expression analysis. Expressions of TNFα and IL-1β in the cytokines important for activation and migration of dendritic cells, were increased in the draining lymph nodes as well as the nasal mucosa. Increased IL-4 and IL-10 expression was observed in the draining lymph nodes as well as increased IL-4 expression in the nasal mucosa in contrast, the expression of IFNγ was not changed in either tissue following OPA inhalation. Lung cytokine expression was also examined; however, no changes were evident. These data demonstrate that OPA inhalation induces an immune response locally in the nasal mucosa. Importantly, OPA inhalation induced activation of lymphocytes in the mandibular lymph nodes that drain the nasal mucosa, thus supporting the potential for sensitization to this chemical. The Th2-dominant expression pattern in the draining lymph nodes suggests that OPA may have the potential to cause respiratory sensitization.

**1511** IDENTIFICATION OF INDOOR AIR CONTAMINANTS USING AN IN VITRO EXPOSURE SYSTEM.

L. Jackson, R. Wells, A. E. Munson and S. E. Anderson, CDC/ID/NC/D, Morgantown, WV.

On average, U.S. citizens spend 80% or more of their daily lives indoors whether at home, work, or in other commercial buildings. Over the last two decades, there has been increasing awareness regarding the potential impact of indoor air pollution on health. These studies use an in vitro monitoring system called the VitroCell which utilizes the air cell interface allowing for direct contact between cells and contaminants of a test atmosphere to assess chemicals found in the indoor air environment. The structurally similar dicarbonyls diacetyl, 4-oxopentanal, glutaraldehyde and methylglyoxal were selected for use in this system. Exposure to these compounds, which are found in the indoor environment, has been suggested to contribute to adverse health effects. The VitroCell was used to evaluate immune-related gene and protein expression generated after a non-immune (pulmonary epithelial A549) and immune (alveolar macrophage) cell line were exposed to these ozonolized chemicals. A low density real time PCR gene array, screening 84 immune-related genes, was used to investigate the expression of these genes. Alternations in the inflammatory cytokines IL-8 and TNF-alpha were identified after exposure to these compounds. The identified cytokines can potentially be used as biomarkers to screen contaminated indoor air environments. These studies may provide an in vitro method for the identification and characterization of chemical hazards including indoor air pollutants in work environments such as office buildings, allowing for the reduction of worker illness and more specifically reducing respiratory consequences of exposures to allergens and irritants.

**1512** TH17 INVOLVEMENT IN PENICILLAMINE-INDUCED AUTOIMMUNITY.

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Rationale: At present idiosyncratic drug reactions (IDRs) are impossible to predict, largely because the mechanisms involved are poorly understood. The most recently identified subtype of helper T cells, Th17 cells, are characterized by the production of proinflammatory cytokine, IL-17, and have been shown to be involved in the pathogenesis of many types of autoimmune syndromes. This study investigated the involvement of Th17 cells in penicillamine-induced autoimmunity in Brown Norway (BN) rats. Experimental Procedures: BN rats were given penicillamine (1.0 mg/ml in drinking water). Serum IL-6 and TGF-beta concentrations were determined by ELISA. At the end of the experiment, spleen CD4+ T cells were purified via an immunomagnetic column from 3 treated and 3 control animals and cytokine mRNA levels were determined. Results: 4/7 treated rats developed autoimmunity. A significant increase of serum IL-6 was detected only in sick animals and did not occur until shortly before animals developed signs of autoimmunity. Serum TGF-beta was found to go up by the 2nd week in non-sick animal compared to a decrease in the two sick animals. In addition, a two-fold increase of IL-17 mRNA was observed only in sick rats. Moreover, in preliminary experiments we detected a marked increase in IL-17 and IL-6 in several patients with idiosyncratic drug-induced liver injury. Conclusions: IL-6 is known to favor the development of Th17 cells and the elevation of IL-6 only in animals that develop autoimmunity is consistent with the involvement of Th17 cells in this animal model. The increase in IL-17 mRNA further supports this hypothesis. The finding of an increase in IL-17 in patients with idiosyncratic drug-induced liver failure suggests that Th17 cells may also be involved in drug-induced liver injury. This work was supported by a grant from the Canadian Institutes of Health Research.

**1513** GENISTEIN INCREASES BLOOD GLUCOSE LEVELS IN STREPTOZOTOCIN-TREATED FEMALE B6C3F1 MICE.

T. L. Guo, J. F. Zheng and K. L. White, Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA.

Previously, we have reported that oral exposure to phytoestrogen genistein (GEN), a major isoflavone in most soy products, protected female NOD (non-obese diabetic) mice from developing type 1 diabetes when fed a soy- and alfalfa-free diet. When given in multiple low doses (MLD), administration of streptozotocin (STZ, a selective β-cell cytotoxin) can cause type 1 diabetes with an autoimmune pathology in mice that do not develop diabetes spontaneously. The objective of present study was to determine if exposure to GEN by gavage had any effects on blood glucose levels in the MLD-STZ-induced diabetic female B6C3F1 mice. Adult mice were administered GEN (2, 6, and 29 mg/kg; 0.1 ml/10 g body weight) for two weeks before STZ injection (5 X 50 mg/kg, ip) was performed to induce diabetes. GEN administration was continued to the end of study. Vehicle mice (25 mg Na2CO3) had moderate levels of blood glucose (250 - 350 mg/dL) in the 13 weeks of dosing period, and treatment with GEN produced increases in blood glucose levels. In the 2 mg/kg GEN group, significant increases were observed at weeks 7, 8, 9, 10, 11 and 12 following STZ injection. In the 6 mg/kg GEN group, significant increases were observed at weeks 3, 5, 7, 10, 11 and 12 following STZ injection. In both the 2 mg/kg and 6 mg/kg GEN group, significant increases were observed at weeks 7, 8, 9, 10, 11 and 12 following STZ injection. In the 2 mg/kg GEN group and at week 12 for the 6 mg/kg GEN group, and a significant increase at week 13 for the 2 mg/kg GEN group; however, the overall differences were minimal. Taken together, GEN exposure had no protective effect in MLD-STZ-induced diabetes in female B6C3F1 mice. These results suggest that the mechanism
of GEN protection of diabetes development in female NOD mice is complex, and more studies are needed to identify the immune components that are targeted by GEN (Supported in part by NO1-ES05454).

1514 ACETAMINOPHEN REVEALS IMMUNE-SENSITIZATION IN ORAL EXPOSURE MOUSE MODEL.

I. S. Ludwig, L. Kwast, D. Fiechter and P. Pietrus, Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands.

Many drugs are known to induce adverse immune reactions in susceptible individuals and may result in clinical diseases. The reporter antigen popliteal lymph node assay (RA-PLNA) is assessing this immune-sensitization capacity of low molecular weight compounds. However this assay only assesses local responses and does not include oral exposure. Therefore systemic effects are missed and furthermore, when metabolism is required compounds may test false negative in this assay. Preclinical systemic exposure models with a predictive value do not exist.

In this study the immune-sensitization of acetaminophen (APAP) was investigated when orally administered to mice. Trimethylphenyl-volubalin (TNP-OVA) was used as a reporter antigen (RA) to assess the capacity of the drugs to stimulate systemic immune responses to a bystander antigen. Female C3H/HeNj mice were exposed to 30, 100 or 300 mg/kg APAP daily for 7 days and co-exposed to TNP-OVA at day 1. 15 days after the start of the exposure delayed type hypersensitivity was determined by a challenge with the RA. A vigorous response to the RA was detected in animals exposed to APAP. RA-specific IgG1 and IgG2a antibodies could be detected in the serum at day 21. Furthermore, isolated LN cells from APAP treated animals displayed a higher IFN-γ, IL-4 and TNF-α production than cells from control animals upon restimulation in vitro. In conclusion, APAP, with a known capacity to induce immune mediated adverse reactions in patients, displays in this oral exposure mouse model a mixed TH1/TH2 response. Combined with results from other studies in which thiosemicarbazides, diphlocon and D-penicillamine, all known immune-sensitizing drugs and induced antibody responses in this mouse model, this experimental set up could be useful for preclinical assessment of sensitizing potential of new chemical entities.

1515 EXAMINATION OF POTENTIAL CHEMICAL INTERACTIONS IN SKIN SENSITIZATION: ACTIVITY OF CLOVE BUD OIL ADMIXED WITH OXAZOLONE IN THE LOCAL LYMPH NODE ASSAY.

J. Lallo1, K. Kimbers2, B. Dearnley3, G. Gerberick3 and A. Apti4, 1Research Institute for Fragrance Materials Inc., Woodcliff Lake, NJ, 2The University of Manchester, Manchester, United Kingdom and 3Procter & Gamble Co., Cincinnati, OH.

The potential for interaction between contact allergens during the induction phase of skin sensitization has rarely been investigated. Understanding of the potential for additive and/or synergistic effects might prove relevant and inform risk assessment strategies. In order to explore the potential for interactions between chemicals during the acquisition of skin sensitization a series of local lymph node assays (LLNA) were conducted with a well-characterized sample of clove bud oil - an essential oil containing multiple contact sensitizers – alone, and in combination with a constant amount of the known sensitizer oxazolone. Clove bud oil was tested at five concentrations ranging from 1.0% to 25% w/v in a vehicle of 1:3 ethanol:diethyl phthalate (EtOH:DEP), both with and without 0.005% oxazolone. Two vehicle controls were included, one containing 0.005% oxazolone and one without. The concentration of oxazolone was selected based on a known EC5 value of <0.001% in EtOH:DEP. Stimulation indices (SI) were calculated for each dose level with an SI ≥ 3 compared with the relevant vehicle control being considered positive.

Theoretical dose response curves based upon summation of the individual responses to clove oil and the oxazolone containing vehicle controls were also generated. Comparison of the theoretical with experimentally derived dose responses revealed that the combination of clove bud oil and oxazolone had a slightly greater than additive effect, particularly at higher doses. These results are consistent with other reports of additive effects in the LLNA. Further work is needed to determine if these effects translate into increased levels of sensitization to the individual materials.

1516 INDUCTION OF SENSITIZATION AND CYOTOXICITY TO TRICHLOROETHYLENE IN MICE: ROLE OF METABOLITES.


Trichloroethylene (TCE) is an important industrial chemical and is widely used. The severe allergic skin damage induced by TCE has been described. The aim of the study was to explore role of metabolites of TCE in the process of allergic skin damage in mice using a combination of in vivo and in vitro endpoints. Female BALB/c mice pretreated with ethanol or phenobarbital (PB) were administered TCE for 6 weeks, followed by challenge onto the ears to evaluate delayed-type hypersensitivity (DTH) response. After the mice were sacrificed, activities of aspartate aminotransferase and alanine aminotransferase in serum were measured as markers of hepatic damage. The TCE-specific lymphocytes proliferation and cyotoxicity of TCE and its metabolites were determined in cultured spleen lymphocytes exposed to TCE and SKF-525A (CYP450 inhibitor) or ammonoxamic acid (AOAA, cytochrome conjugase inhibitor) in vitro. The results showed that no DTH response could be observed in 3 groups of mice treated by TCE, TCE+PB and TCE-ethanol. However, a significant proliferation of spleen cells from TCE-treated mice was evidenced following incubation with TCE in vitro, it was interesting that this proliferation disappeared when AOAA was added and cytochrome P450 was depressed when SKF-525A was added. In mice treated by TCE+PB and TCE-ethanol, no significant proliferation of spleen cells was found in culture with TCE in vitro, but hepatic toxicity of TCE in vivo was promoted by PB and ethanol through increasing the CYP450-dependent amphotoxin metabolite. This study provides an indirect evidence that glutathion-dependent pathway might play an important role in allergic reaction induced by TCE, and dichlorvos thiol-metabolite catalyzed by cytochrome P450 cytochrome P450, is responsible for proliferation of hepatic-specific lymphocytes in TCE-sensitized mice. The CYP-dependent oxidation cause cytotoxicity of spleen cells in vitro, and hepatotoxicity in vivo as well.

1517 INCREASED CELL PROLIFERATION IN SPLEEN AND LYMPH NODES PERIPHERAL TO CONTACT ALLERGEN APPLICATION SITE.


The local lymph node assay (LLNA) is widely used to identify chemicals that are contact sensitizers. The assay involves dosing mice with the chemical on both ears and pooling the cervical lymph nodes for assessment of lymphocyte proliferation as a marker of sensitization. The present study explored potential reduction in animal usage by dosing one ear with the allergen and the other with vehicle only. The respective draining lymph node lymph nodes were processed separately for quantification of cell proliferation. Cell proliferation in axillary and renal nodes, and in the spleen was also assessed. Cross contamination of the chemicals from the dosed ears to other parts of the body via preening was prevented by dosing restrained animals and washing off the residual chemical with saline after 4 hours. Dosing the left ear with 0.02% oxazolone (OX) on unconstrained axilla resulted in marked cell proliferation in its draining lymph node (Stimulation index, SI = 12.8) and in the lymph node draining the contra-lateral vehicle dosed ear (SI = 6), and the axillary lymph nodes (SI = 3.3). Increased trivial thymidine (3H-TdR) incorporation was not observed in the renal lymph nodes (SI = 1.1). Similar stimulation of cells was observed in the lymph node draining the ear contra-lateral to the allergen dosed ear when 30% hesylcinnamaldehyde (HCA) was applied. Increased proliferative activity was observed in non-directly draining lymph nodes of restrained mice demonstrating that these results can not be attributed to cross-contamination of adjacent skin. A significant increase in proliferation of splenocytes was observed. It is concluded that epicutaneous application of a contact allergen, as exemplified by OX and HCA, may induce cell proliferation in the non-draining lymph nodes and spleen indicative of hapten and/or haptenated proteins diffusing through the skin to peripheral nodes and the blood to produce systemic sensitization. Thus the node draining the contra-lateral ear can not be used as a control for application of contact allergen to a single ear in a modified LLNA.

1518 COMPARISON OF GLOVE CONTACT ALLERGEN CONTENT AND CLINICAL PATCH TEST.

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Irritant dermatitis, allergic contact dermatitis (ACD) and urticarial reactions have been associated with chemical rubber binder accelerators present in latex and nitric/hy- material rubber gloves. In this study, patients reporting to a dermatology clinic with glove associated ACD were allergy patch tested with the North American Contact Dermatitis Group standard rubber allergen series, medical history taken and asked to identify and supply “problem gloves” which they associated with their ACD and if possible “non-problem gloves”. Medical exam, surgical and industrial-type rubber and nitric gloves from patients were assessed for chemical content including: zinc dithiocarbamates, thiurams, mercaptobenzothiazole (MBT) and its disulfide, di- morpholine, diphenylguanidine, thiourea, and phenylene diamine. Only zinc di-
vestigating the role of Nrf2 in DC maturation. P38MAPK and ERK, two kinases involved in DC maturation. We are currently in-
duction of the Nrf2 protein in Mo-DC. The mRNA expression of target genes of
mu M). Our results showed that NiSO4 and DNCB induced an accu-
Furthermore, an inhibitor of P38MAPK, SB203580, reduced the accumulation of
mulation of Nrf2 in MoDC is controlled by
These results suggest that accumulation of Nrf2 in MoDC is controlled by
kinase activity. We next investigated the accumulation of Nrf2 in MoDC and cultured in the presence of GM-CSF and IL-4 for 5 days. Mo-DCs were
Because sensitizers activate both MAPKs and Nrf2, we address the question
of chemical sensitizers to act as a transcription factor.
Our current hypothesis is that chemical sensitizers generate a chemical
stress that is perceived by DCs as a danger signal leading to DC maturation. Among
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and cytokine secretion that are regulated by mitogen-activated protein kinases (MAPKs). Our current hypothesis is that chemical sensitizers generate a chemical

The murine local lymph node assay (LLNA) is the assay of choice in European regulatory
toxicology. As with other toxicology/sensitisation assays, it has a potential for false
result. The apitcnic surface on stein lauryl sulphate (SLS) represents a classic
example. In the work reported here, examples of false positives in the LLNA are
compared to published “benchmarks” such as SLS. Clear false positives (e.g. oleic
acid) are also contrasted with examples where data interpretation is more challeng-
ing. As the LLNA will be applicable to >30,000 chemicals under REACH, and in
the light of animal welfare considerations to do no more than the absolute mini-
um of animal testing, results from a single LLNA often represent the only avail-
ble data on sensitisation. This reinforces the need to ensure data from this assay are
interpreted intelligently, using scientific analysis of results and considering the
weight of evidence, before decisions are made on which substances should be classi-
fied as representing a skin sensitisation hazard. In chemical classes where the LLNA
has been shown to be an inappropriate assay other standardised methods (e.g. the
Buehler or Magnusson and Kligman guinea pig tests [OECD 406]) should be em-
ployed as the first choice assays.

1. D. Antonios1, M. Damiens1, J. Ourlin2 and M. P
1521 ANALYSING DISCORDANT LOCAL LYMPH NODE ASSAY DATASETS: IMPLICATIONS FOR REACH.
D. A. Baskerett1, N. Ball2, S. Cagen1, J. Carrillo3, H. Certa4, D. Eigler4, H.
Koth-C. Garca4, C. Graham4, C. Haute5, R. Kreiling6 and A. Meiling6,
TRIMED Consultancy Ltd, Shirley Court, United Kingdom, 7Dow Europe GmbH,
Horgen, Switzerland, 8Shell Health, Houston, TX, 9Shell International BV, The
Haghe, Netherlands, 10SASOL Germany GmbH, Marl, Germany, 11Etronik
Goldachmittel GmbH, Eisen, Germany, 12BASF AG, Ludwigshafen, Germany,
13SEPPIC, Castres, France, 14Huntsman Corporation, The Woodlands, TX,
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in AOO. However, many times another solvent/vehicle was selected for the test material on the basis of maximizing the test concentration while producing a solution/suspension suitable for application. When a solvent/vehicle other than AOO was selected, an additional group of mice was added to the assay. This additional group served as the positive control (21% HCA in AOO), and increased the number of animals required per assay. This study evaluated 21% and 30% alpha-hexylcinnamaldehyde, tech., diluted in various common vehicles: AOO, N,N-dimethylformamide, methyl ethyl ketone, propylene glycol, and dimethyl sulfoxide to demonstrate their use as routine vehicles for the positive control to produce dermal sensitization in mice using the LLNA. The mice dosed with 30% HCA in the various vehicles resulted in SI values (range 5.61-15.78) similar to those achieved with 21% HCA (range 4.36-8.85). Therefore, these vehicles are suitable to use as a solvent/vehicle for preparing 21% HCA, thereby reducing the number of animals per assay.

1524 ICCVAM TEST METHOD RECOMMENDATIONS FOR THE REDUCED LLNA (RLLNA): AN ALTERNATIVE TEST METHOD USING FEWER ANIMALS TO ASSESS THE ALLERGIC CONTACT DERMATITIS POTENTIAL OF CHEMICALS AND PRODUCTS.

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Based on recommendations by ICCVAM in 1999, U.S. regulatory agencies that require the submission of skin sensitization data accepted the LLNA that should be prepared in AOO to prepare the positive control (21% HCA in AOO), and increased the number of animals required per assay. This study evaluated 21% and 30% alpha-hexylcinnamaldehyde, tech., diluted in various common vehicles: AOO, N,N-dimethylformamide, methyl ethyl ketone, propylene glycol, and dimethyl sulfoxide to demonstrate their use as routine vehicles for the positive control to produce dermal sensitization in mice using the LLNA. The mice dosed with 30% HCA in the various vehicles resulted in SI values (range 5.61-15.78) similar to those achieved with 21% HCA (range 4.36-8.85). Therefore, these vehicles are suitable to use as a solvent/vehicle for preparing 21% HCA, thereby reducing the number of animals per assay.

1525 LYMPH NODE CELL COUNT AS A USEFUL ALTERNATIVE FOR EVALUATING LLNA RESULTS.


Alternative endpoints have been evaluated to replace the use of radioactivity or the injection of toxic chemicals such as Brdu, following the initial development of the local lymph node assay based on lymph node cell proliferation measured by 3H-thymidine incorporation and lymph node cell count. When using a SI of 1.5 for cell count as the cut off to predict a positive response in CBA/J mice, equivalent estimated concentrations (ECs) for the prediction of skin sensitizing potency are obtained in the majority of cases with both measurements.

1526 ALLERGEN-INDUCED INTERLEUKIN 2 EXPRESSION: CELLULAR SOURCE AND FUNCTION.

C. Portsmouth 1, M. Cumberbatch 1, C. E. Griffiths 2, J. Kimber 1 and P. J. Denmark 1. 1Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom and 2Dermatological Sciences, University of Manchester, Manchester, United Kingdom.

Prolonged (15 day) exposure of BALB/c strain mice to the contact allergen 2,4-dinitrochlorobenzene (DNCB) or to the respiratory sensitizer trimellitic anhydride (TMA) preferentially activated T helper (Th) 1 and Th2 cells, respectively. In the current investigations, early events that may initiate the polarization of these responses have been examined. BALB/c strain mice were exposed to a single topical dose of either 1% DNCB or 25% TMA, or to vehicle alone, for 6 and 10h. Draining auricular lymph nodes were excised and cytokine production by draining lymph node cells (LNC) measured by cytokine multiplex bead array. DNCB exposure provoked rapid production of interleukin (IL)-2, and, with somewhat delayed kinetics, IL-17 and interferon (IFN)-γ. Relatively low levels of these cytokines were recorded after skin sensitization with TMA. LNC preparations were depleted of various cell populations using magnetic bead separation and the effect on cytokine production was analyzed. Removal of cells expressing CD20, a B cell marker, was without effect on IL-2, IL-17 or IFN-γ expression. Depletion of cells expressing CD25, the high affinity IL-2 receptor expressed primarily by activated T cells and natural killer (NK) cells, reduced IL-2 expression and abrogated secretion of IL-17 and IFN-γ. Removal of cells expressing the NK cell marker CD49b also resulted in reduced production of these three cytokines. Finally, depletion of cells expressing the dendritic cell (DC) marker CD11c inhibited IL-2 expression completely as well as reducing IL-17 and IFN-γ secretion. These data suggest that there are complex interactions between DC, NK and T cells in DNCB-activated DC that result in the observed cytokine secretion profile and subsequent Th1 polarization of the immune response. DNCB-activated DC are the likely source of the IL-2 that serves to stimulate the production of IL-17 and IFN-γ by other skin draining LNC, probably NK and activated T cells.

1527 MECHANISTIC APPROACH TO DEVELOPING AN IN VITRO SKIN SENSITIZATION TEST “HUMAN CELL LINE ACTIVATION TEST (H-CLAT)”.


We have been developing the “human Cell Line Activation Test (h-CLAT)”, an in vitro skin sensitization test, based on augmentation of CD86/CD54 expression in THP-1 cells. From the examination of 100 chemicals, 83 chemicals were identified correctly. Inter-laboratory studies have found the h-CLAT to be reliable and reproducible. The h-CLAT is thought as a useful in vitro skin sensitization test. While both CD86 and CD54 expression are important to form the skin sensitization, we previously showed that some allergens augmented only CD86 or CD54 expression. Moreover, most allergens augmented these expressions at subtoxic concentrations. The purpose of this study is to clarify these phenomena. First, we investigated why some allergens induce only CD86 or CD54 expression. We hypothesized that exposure time is not enough for strong augmentation of expression in these allergens. Presently exposure to some chemicals causes induction of only CD54 in h-CLAT after 24h. When exposure time was increased, both CD54 and CD86 were induced. Next, we investigated the relationship between CD86/CD54 expression and cytotoxicity induced by allergen stimulation. We first investigated whether dead cells induced by allergens effect the augmentation of CD86/CD54 expression. But dead cells did not have much influence to CD86/CD54 expression. We next examined how cytotoxicity is induced by allergens. Since allergens strongly induced apoptotic cell death, we therefore performed the inhibition assay using caspase inhibitors. Treatment with inhibitor suppressed apoptosis and almost recovered cell viability at 24h after treatment with allergens. These result indicated that allergens induced both CD86/CD54 expression and cytotoxicity respectively. In conclusion, most allergens have a potential for inducing both CD86 and CD54 expression in THP-1 cells. Moreover, CD86/CD54 expression and cytotoxicity are both induced by allergens respectively. These results suggest that the criterion of h-CLAT is accurately based on the specific response of allergens.

1528 ACCEPTABILITY OF THE MOUSE LOCAL LYMPH NODE ASSAY FOR PESTICIDE FORMULATION HAZARD ASSESSMENT.

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The LLNA is widely regarded as a sensitive skin sensitization test and provides animal welfare benefits relative to guinea pig (M&K, Buehler) tests. For these reasons, the LLNA is now the preferred test for hazard assessment of substances and formu-
lateral products, particularly in the EU. Recently, the acceptability of testing ‘mixtures’ via the LLNA has been questioned by U.S. agencies; subsequent evidence indicates such data are reliable (Bowerfort et al. ToxSci, 2008). Due to animal use practices, the same formulations are not typically tested in multiple assays, precluding direct comparison for this evaluation. Thus, to further assess the validity of the LLNA for testing pesticide formulations, a retrospective analysis was performed on LLNA (n=52), M&K (n=31) and Buehler (n=32) data for Dow products. The positive response rate was 65.5%, 58.1% and 19.4%, respectively. Considering the sensitization potential for these formulations, active ingredients, 78% of formulations tested in the LLNA with sensitizing active ingredients were positive. With the M&K assay, 82% of the formulations containing a sensitizing active were positive while only 42% were positive with the Buehler assay. In cases where the same formulation was tested using multiple methods (n=6), there has been good agreement. Four such products were evaluated in an interlaboratory comparison (Bowerfort et al., 2008); 2 positive Buehler results were confirmed using the LLNA (EC3 = 26.8% and 1.1%), and 2 borderline negative results (one M&K and one Buehler) were positive in an LLNA. Lastly, two recent Buehler studies confirmed 2 borderline negative results (one M&K and one Buehler assay, 82% of the formulations containing a sensitizing active were positive. With the M&K assay, 82% of the formulations containing a sensitizing active were positive while only 42% were positive with the Buehler assay. In cases where the same formulation was tested using multiple methods (n=6), there has been good agreement. Four such products were evaluated in an interlaboratory comparison (Bowerfort et al., 2008); 2 positive Buehler results were confirmed using the LLNA (EC3 = 26.8% and 1.1%), and 2 borderline negative results (one M&K and one Buehler) were positive in an LLNA. Lastly, two recent Buehler studies confirmed positive results (EC3 = 26.8% and 1.1%), and 2 borderline negative results (one M&K and one Buehler) were positive in an LLNA. Lastly, two recent Buehler studies confirmed positive results (EC3 = 26.8% and 1.1%), and 2 borderline negative results (one M&K and one Buehler) were positive in an LLNA.

1529 A PLASMACYTOID DENDRITIC CELL-BASED ASSAY TO SCREEN THE ALLERGENICITY POTENTIAL OF CHEMICALS.

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An in vitro assay system that utilizes human cells to predict the allergenic potential of chemicals will have utility throughout industry to monitor products for contact sensitization. Development of such a non-animal alternative assay system for hazard assessment is within the provisions of the European Union chemicals policy known as REACH (Registration, Evaluation, and Authorization of Chemicals). In this study, we investigated whether the CD86 expression level in plasmacytoid dendritic cells (pDC) could be used as a non-animal alternative to test for contact allergens. To achieve this goal, human DC were generated from CD34+ progenitor cells. pDC (CD123+/CD11c-) was harvested using FACS sorting.

The pDC were exposed to chemical allergens (n=23) or irritants (n=18). Sub-toxic concentrations of each chemical were determined using FACS analysis of propidium iodide stained cells. Allergens were identified based on stimulation index (SI) calculated by the fold increase in CD86 expression. A material that had an SI of 2 was considered positive. The pDC were exposed to chemical allergens (n=23) or irritants (n=18). Sub-toxic concentrations of each chemical were determined using FACS analysis of propidium iodide stained cells. Allergens were identified based on stimulation index (SI) calculated by the fold increase in CD86 expression. A material that had an SI of 2 was considered positive.

1530 APPLICATION OF TRYPTOPHAN FLUORESCENCE TO ASSESS SENSITIZING POTENTIALS OF CHEMICALS.

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We aimed to develop a simple in vitro method to quantitatively predict the sensitizing potentials of chemicals by measuring the fluorescence of chemical-HSA complexes. Human serum albumin (HSA) was treated with test chemicals and then analyzed by tryptophan fluorescence and protein concentration measurement. Four strong sensitizers (toluene 2,4-diisocyanate (TDI), phthalic anhydride, glutaraldehyde, bisphenol A diglycidyl ether), two weak sensitizers (bisphenol A, 4,4-dimethylphenyl ether designated by only one organization), and two non-sensitizers (2,5-hexanediol, phthalic acid) were examined by using the tryptophan fluorescence assay. HSA fluorescence at 280 nm of excitation and 340 nm of emission was reduced by TDI, dose-dependently. The addition of TDI immediately reduced the fluorescence and was stable 6 h to 21 days after treatment, with a slight decrease. The reduction of HSA fluorescence by chemicals was in the order: strong sensitizers > weak sensitizers > non-sensitizers. Chemical treatment at 0.05 and 0.5 mM leads to an optimal separation among the three groups. Ortho-phenalddehyde (OPA), which has not been evaluated regarding its sensitization potential by any of the authorized organizations, reduced HSA fluorescence as much as the strong sensitizers at final concentrations of 0.05 and 0.5 mM of the chemical. According to our method, OPA is evaluated as a strong sensitizer. The treatment of all test chemicals did not cause obvious differences in the total protein concentration by either the Lowry or Bradford method. The assay utilizing tryptophan fluorescence loss of HSA after chemical treatment is a promising method to evaluate the sensitizing potentials of chemicals. The study was supported partly through a grant of LRI by JCI.

1531 EXPOSURE TO ULTRAFINE PARTICLES DURING ALLERGIC SENSITIZATION YIELDS AN ALTERED LUNG EPITHELIAL STRUCTURE.

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Particulate matter (PM) has been shown to exacerbate allergic airway diseases. Exposure to high PM air is a common occupational and often atmospheric hazard. Recent studies demonstrate ambient ultrafine particles (UFP) may act as an adjuvant in Ovalbumin-induced allergic sensitization via the addition of small quantities of intranasally instilled UFPs with Ovalbumin (Ova). The objective of this study was to investigate the effect of ultrafine particles via inhalation and/or instillation during the sensitization phase of an allergic mouse model. Male eight-week-old Balb/c mice were sensitized with 10 μg Ova on days 2, 3, and 4, challenged with 40 mg/m3 Ova for 1 h on days 12 and 13, and sacrificed on day 14. Some animals additionally received collected UFP instilled with the Ova, inhalation exposure to UFP for 5 hours/day for days 1-5 following Ova instillation or both treatments combined. The UFPs are generated and collected on-site with an average concentration of 224 ± 6 mg/m3 and an average size of 53.5 ± 3.8 nm. Histology of the fixed lung revealed significantly increased mucin content in the animals sensitized with UFP and Ova than in animals primed with Ova alone. Previous studies have shown no increase in mucin content with UFP alone or in the challenge phase. Interestingly, total IgE appears that it may be lower in UFP and Ova exposed animals than Ova alone, though not statistically significant. This study indicates with the addition of UFP during the priming phase, an altered allergic response results and this could further explain some of the epidemiological trends we witness in asthmatic patients living and working in high PM areas.

1532 RAT SERUM ANTIBODIES INDICATE OXIDATIVE STRESS AND RENAL TOXICITY, BUT NOT NEUROTOXICITY FOLLOWING SUBCHRONIC ORAL EXPOSURE TO SODIUM TUNGSTATE.


The health effects of tungsten are of concern to both the public and military personnel. Exposure via groundwater contamination is of particular interest near military installations. The present study assessed the effects of oral tungsten in 120 day old male and female (n=10/exposure) and female (n=10/exposure) Sprague-Dawley rats administered tungsten as sodium tungstate dihydrate at 0, 10, 75, 125 or 200 mg/kg for 90 consecutive days. Toxicity was assessed by measuring autoantibodies against nervous system and renal proteins using ELISA. Neither IgM, nor IgG antibodies against neurofilmament triplet or glotypic proteins (glial fibrillary acidic protein and myelin basic protein) were detected in rat sera. However, sera of both male and female rats had IgG titers against glomerular basement membrane protein (GBM), male rats had IgG titers against glomerular basement membrane protein (GBM), myelin basic protein) were detected in rat sera. However, sera of both male and female rats had IgG titers against glomerular basement membrane protein (GBM), myelin basic protein) were detected in rat sera. However, sera of both male and female rats had IgG titers against glomerular basement membrane protein (GBM), myelin basic protein) were detected in rat sera. However, sera of both male and female rats had IgG titers against glomerular basement membrane protein (GBM), myelin basic protein) were detected in rat sera. However, sera of both male and female rats had IgG titers against glomerular basement membrane protein (GBM). IgG was detected against anti-myelin basic protein, indicative of glial fibrillary acidic protein. IgG was detected against anti-myelin basic protein, indicative of glial fibrillary acidic protein.

The significant decline in total IgG seen in this group and suggestive of immunological effects.

1533 REACTIVITY AND TOXICITY OF ENDOGENOUS ALDEHYDES GENERATED VIA DOPAMINE CATABOLISM.

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Over 1 million Americans are currently affected by Parkinson’s disease (PD). The cause of PD hallmark dopaminergic neuronal death is currently unknown, but recent research implicates oxidative stress leading to increased levels of an endogenous...
Parkinson's disease is a neurodegenerative disease that affects the dopaminergic neurons of the substantia nigra, causing a variety of motor disabilities. Dopamine (DA) is a neurotransmitter that undergoes catabolism to form 3,4-dihydroxyphenylacetaldehyde (DOPAL). DOPAL is structurally analogous to DA, but is reactive towards proteins and can cause neurodegeneration. DOPAL may adversely affect TH and DA biosynthesis. A direct quantitative method that may be useful for determining the reactivity of DOPAL towards the proteins is now known. It is hypothesized that DOPAL modification of tyrosine hydroxylase by 3,4-dihydroxyphenylacetaldehyde, a reactive intermediate of dopamine catabolism.

L. M. Metes, V. Florang, J. Yunden and J. A. Dooren. Medicinal and Natural Products Chemistry, University of Iowa, Iowa City, IA.

Parkinson's disease (PD) is a neurodegenerative disease that affects the dopaminergic neurons of the substantia nigra, causing a variety of motor disabilities. Dopamine (DA) is a neurotransmitter that undergoes catabolism to form 3,4-dihydroxyphenylacetaldehyde (DOPAL). DOPAL is structurally analogous to DA, but is reactive towards proteins and can cause neurodegeneration. DOPAL may adversely affect TH and DA biosynthesis. A direct quantitative method that may be useful for determining the reactivity of DOPAL towards the proteins is now known. It is hypothesized that DOPAL modification of tyrosine hydroxylase by 3,4-dihydroxyphenylacetaldehyde, a reactive intermediate of dopamine catabolism.

L. M. Metes, V. Florang, J. Yunden and J. A. Dooren. Medicinal and Natural Products Chemistry, University of Iowa, Iowa City, IA.

Parkinson's disease involves progressive neurodegeneration and loss of dopaminergic neurons. It is currently afflicting over a million patients in the U.S. The disease pathology is known to involve multiple factors, one of which is the inherent toxicity of the neurotransmitter dopamine (DA). The ability of DA to auto-oxidize to an ortho-quinone (DAQ) plays a role in its cytotoxicity, resulting in increased oxidative reactivity, thiol depletion, and redox cycling. In the presence of transition metals, DAQs can also trigger reactive oxygen species production. Certain metabolites of DA such as 3,4-dihydroxyphenylacetaldehyde (DOPAL) may also be involved in PD and act as endogenous neurotoxins. However, little is known about DOPAL's ability to similarly undergo auto-oxidation to a reactive quinone. If confirmed, DOPAL-quinone formation would help explain the high levels of toxicity associated with this aldehyde metabolite. NADH and Tyrosinase, which are known to induce DA-quinone formation, were investigated for their ability to oxidize DOPAL. Experimental evidence indicates that DOPAL is susceptible to oxidation, and as these methods induced oxidative rearrangement of DOPAL both colorimetrically and spectrophotometrically. Such species demonstrated selective reactivity towards glutathione and N-acetyl cysteine, which is a strong indicator of protein reactivity. A direct quinone method that may be useful for determining the stoichiometry of thiol addition relevant to oxidized DOPAL by measuring N-acetyl cysteine concentrations was also demonstrated. Furthermore, DOPAL was found to be sensitive to transition metal catalyzed oxidation. Treating DOPAL with Cu2+ in solution resulted in UV-Vis absorption spectra increases also observed with tyrosinase oxidized DOPAL. Such experiments elucidate the potential of DOPAL to undergo auto-oxidation and participate in redox cycling, similar to DA. Ultimately, determining the biological relevance of such oxidized species will reveal their role in Parkinson's disease.

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Previously, we have shown that mice with reduced vesicular monoamine storage (VMAT2 LO) display behavioral deficits associated with Parkinson's disease (PD) and progressive neurodegeneration in the substantia nigra (SNpc) and locus coeruleus (LC). Traditional chemical models of PD rely on oxidant-mediated mechanisms to cause neuronal cell death, resulting in the rapid onset of symptoms as opposed to the slow degeneration seen in VMAT2 LO mice. These models typically utilize the unique ability of dopamine (DA) and norepinephrine (NE) to auto-oxidize and form deleterious cysteinyl adducts. VMAT2 normally sequesters catecholamines, protecting against neuronal damage; however, when VMAT2 is reduced, cytosolic DA and NÉ are increased, potentially leading to cellular damage. Here, we examined the generation of oxidant species in catecholaminergic cellular degeneration. In VMAT2 LO mice, we observed a marked reduction in total DA and NÉ in the striatum and hippocampus, presumably due to increased turnover. When postnatal SN or LC neurons were cultured and exposed to physiological concentrations of either DA or NE, cells from VMAT2 LO mice underwent increased oxidative stress via formation of H2O2 and other oxidants, as measured by DCF fluorescence; whereas, WT cells were unaffected. Similarly, SN or LC cells from VMAT2 LO mice were susceptible to catecholamine-induced cell death. In order to study the subcellular redox consequences of alterations in VMAT2 expression, we used HEK293 cells stably transfected with the DA transporter (DAT) and VMAT2.
Mitochondrial dysfunction has been linked to several neurodegenerative diseases, including Parkinson’s Disease (PD). Environmental toxicants, including pesticides, PCBs, and certain metals (Mn, Fe), are known to have harmful effects on the nervous system, and are also mitochondrial disrupters. Studies linking neurodegeneration to toxicant exposure at a whole mitochondrial level are lacking. Hence, our goal is to establish profiles of global quantitative differences in the mitochondrial proteome of brain regions affected by dopaminergic toxicant exposure, using MPP⁺, a known dopaminergic toxicant, as our model initial. Adult, male C57BL/6 mice were treated with MPTP and sacrificed 7 days later. Brain tissues from the substantia nigra (SN) and striatum (STR) were used for proteomic analyses using iTRAQ (multiplexed isobaric tagging technology). Neurochemistry detection (HPLC-ECD) and standard western blot techniques were also used. Dopamine, its metabolites, and tyrosine hydroxylase were quantified in MPTP treated mice. Additionally, GFAP levels were significantly elevated by MPTP, to toxicant exposure at a whole mitochondrial proteome level are lacking. Hence, our goal is to establish profiles of global quantitative differences in the mitochondrial proteome of brain regions affected by dopaminergic toxicant exposure, using MPP⁺, a known dopaminergic toxicant, as our model initial. Adult, male C57BL/6 mice were treated with MPTP and sacrificed 7 days later. Brain tissues from the substantia nigra (SN) and striatum (STR) were used for proteomic analyses using iTRAQ (multiplexed isobaric tagging technology). Neurochemistry detection (HPLC-ECD) and standard western blot techniques were also used. Dopamine, its metabolites, and tyrosine hydroxylase were quantified in MPTP treated mice. Additionally, GFAP levels were significantly elevated by MPTP. Proteomic analyses identified 449 and 331 proteins in the STR and SN, respectively, and about 30% were mitochondrial. Overall, MPTP treatment altered the expression of more proteins in the STR compared to the SN. Interestingly, a number of proteins (i.e., ADP/ATP carrier protein 1, creatine kinase, hexokinase, cytochrome c) were decreased by MPTP in the STR, while increased in the SN. This finding suggests that either terminal fields (STR) are damaged first, or that following insult, energy is re-partitioned to allow the cell bodies (SN) to stay functional and neurons survive longer. Our methods seem capable of detecting quantitative mitochondrial proteome changes, and this technique may establish a tool to screen potential basal ganglia or mitochondrial toxicants. (Support: LSBI/USDA Pilot Grant and NIEHS ES11654)

However, we failed to reproduce the same cytosolic oxidative stress seen in primary culture. This may be due to the lack of cathecolaminergic phenotype or the absence of glial cells, suggesting the HEK293 cells may be inappropriate to study DA and NE toxicity. Overall, our data suggest that catecholamine-rich brain regions other than the SNpc display similar vulnerability to neurodegeneration and likely contribute to the overall presentation of symptoms.

Researchers have reported that Navy personnel who are exposed to dust and fumes from welding, thermal cutting, and boiler maintenance may be at risk of manganese (Mn) poisoning. Inhalation exposure to high levels of Mn can lead to a disabling neurological syndrome “manganism” which resembles Parkinson’s disease. Understanding pathogenic mechanisms of Mn exposure is important to provide effective treatment modalities. We proposed recently that nuclear factor kappaB (NF-kB) and oxidative stress are major factors in Mn exposure-induced neurodegeneration. On the other hand, activation of glia, especially microglia with bacterial endotoxin lipopolysaccharide (LPS, 1-10 ng/ml) induced a time- and concentration-dependent activation of NF-kB and p38-MAPK and c-Jun N-terminal kinase, suggesting the involvement of this protein in conjunction with oxidative stress, NF-kB induction, p38-MAPK and c-Jun N-terminal kinase, following Mn exposure. We found that over-expression of wild-type human alpha-synuclein significantly enhanced the cell death due to Mn as evidenced through apoptosis. Mn exposure-induced cell death was characterized by significant increased NF-kB nuclear translocation as shown by nuclear binding activity. Pretreatment with anti-oxidants, p38-MAPKs (SB239063) and c-Jun N-terminal kinase (SP600125) inhibitors significantly reduced NF-kB activation, supporting the role of oxidative stress and MAPKs in NF-kB induction. Furthermore, nitric oxide (NO) generation in association with NF-kB inhibition was blocked by SN-50 and p38-MAPK inhibitors. These events are accompanied by decreased apoptosis by SN-50 and iNOS specific inhibitor (1400W). Hence, these data suggest that alpha-synuclein regulates ROS and associated MAPKs in Mn-induced neurodegeneration. As a result, changes in the response of these cells to Mn make dopaminergic neurons vulnerable to Mn toxicity.

DOPAMINERGIC TOXICANT (MPTP) EFFECTS ON THE STRIATAL AND NIGRAL MITOCHONDRIAL PROTEOMES: IMPLICATIONS FOR PARKINSON’S DISEASE.

DOPAMINERGIC TOXICANT (MPTP) EFFECTS ON THE STRIATAL AND NIGRAL MITOCHONDRIAL PROTEOMES: IMPLICATIONS FOR PARKINSON’S DISEASE.

PRE-TREATMENT WITH NEAR-INFRARED LIGHT ATTENUATES THE CYTOTOXIC EFFECTS OF MPP⁺ IN SH-SY5Y HUMAN NEUROBLASTOMA CELLS.

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1538 DOPAMINERGIC TOXICANT (MPTP) EFFECTS ON THE STRIATAL AND NIGRAL MITOCHONDRIAL PROTEOMES: IMPLICATIONS FOR PARKINSON’S DISEASE.

1539 PRE-TREATMENT WITH NEAR-INFRARED LIGHT ATTENUATES THE CYTOTOXIC EFFECTS OF MPP⁺ IN SH-SY5Y HUMAN NEUROBLASTOMA CELLS.

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1P. Zhang1, K. M. Lokuta1, D. E. Turnier1 and P. Zhang1, 1Pharmacodynamics, University of Florida, Gainesville, FL and 2McKnight Brain Institute, University of Florida, Gainesville, FL.

GLIAL ACTIVATION-MEDIATED DOPAMINERGIC NEUROTOXICITY INDUCED BY MANGANESE AND LIPOLYSACCHARIDE: ROLE OF FREE RADICALS.

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1B. Liu1,2, K. M. Lokuta1, D. E. Turnier1 and P. Zhang1, 1Pharmacodynamics, University of Florida, Gainesville, FL and 2McKnight Brain Institute, University of Florida, Gainesville, FL.

1540 EXPRESSION OF ALPHA-SYNUCLEIN IN RAT DOPAMINERGIC CELLS INCREASED SUSCEPTIBILITY TO MANGANESE LINKED TO OXIDATIVE STRESS, MAP KINASES AND NF-kB MEDIATED NEURONAL INJURY.

1541 GLIAL ACTIVATION-MEDIATED DOPAMINERGIC NEUROTOXICITY INDUCED BY MANGANESE AND LIPOLYSACCHARIDE: ROLE OF FREE RADICALS.
Manganese (Mn) neurotoxicity resembles a number of aspects of the dopamine (DA) neuron degenerating disorder Parkinson’s disease (PD). Both PD and manganese are characterized by motor deficits and damage to substantia nigra basal ganglia nuclei, and dopamine or its metabolites are believed to contribute to the disorder. The molecular pathways involved in the neuropathology in both disorders are ill defined. Here we describe our expression and biochemical investigations of WT and mutant PD- and DA neuron-associated proteins, and their effects on mitochondrial function and DA neurons under basal conditions and following exposure to neurotoxins in PD and manganese. C. elegans models. Expression of a number of stress response genes including the C. elegans homologue of GSTpi, is increased in response to several PD-associated toxins. Gene knock-down (RNAi) of GSTpi increased the susceptibility of DA neurons to the toxins... DA neuron vulnerability to Mn is partially dependent on the divalent metal transporter-1 (DMT-1) orthologues Smf-1-3, since knockdown or deletion of the transporters partially protects against the neurodegeneration. Furthermore, expression of the smf genes following Mn exposure is dramatically increased in young animals relative to controls, consistent with its putative role in the toxicity. Biochemical analysis show that mutations within some of the PD-associated genes in C. elegans affect mitochondrial membrane potential, respiration capacity, and dopamine and glutathione concentrations. We have also generated antibodies to over 50 C. elegans proteins, including PD-associated orthologues, DA- and putative DAT-associated proteins, and stress response proteins, and we are analyzing protein expression and localization patterns under basal conditions and in response to PD-relevant toxins. Initial results from our genetic screens will also be described.

**1543 SUSTAINED STRIATAL IL-1β OVEREXPRESSION AND THE PARKINSON’S DISEASE (PD) PHENOTYPE.**


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Glial activation-induced inflammatory processes are known to be operative in Parkinson’s disease (PD) and suggested to play a role in nigrostriatal dopaminergic cell death, the pathological hallmark of this disease. Such inflammatory cascades include the release of various cytokines, including IL-1β which may contribute to neurodegeneration associated with PD. This study sought to determine whether striatal overexpression of IL-1β, i.e., inflammation at the dopamine (DA) terminal, would enhance the PD phenotype associated with exposure to the herbicide paraquat (PQ). For this purpose, a GFAP IL-1β transgenic young adult male mouse model was used that specifically overexpresses IL-1β in astrocytes. IL-1β was activated via unilateral intra-striatal FIV-cre mediated excision (IL-1βXAT); n=6; two weeks later, a timeframe designed to allow significant glial activation, IL-1βXAT and wild-type (WT; n=4) mice were injected i.p. twice weekly with 5.0 mg/kg of PQ for 6 weeks, and sacrificed 2 weeks following the final PQ injection. IL-1βXAT mice tended to show higher levels of both ambulatory and vertical activity measured immediately after PQ injection and 24 hr later. For both groups, changes in GFAP, a marker of glial activation, and of tyrosine hydroxylase (TH), a measure of DA terminal integrity, were compared historically, for each animal, across hemispheres; thus each animal served as its own control. Viral activation in IL-1βXAT mice resulted in an approximate 50% increase in GFAP on the activated side, as expected. Moreover, while TH density did not differ between groups, lesions were observed in IL-1βXAT mice in the activated but not the non-activated hemisphere, consistent with fiber loss. Such lesions were not observed in the brain of an IL-1βXAT mouse that did not receive PQ. Collectively, these findings suggest that striatal IL-1β upregulation could enhance striatal DA terminal vulnerability to neurotoxic damage.

**1544 MITOCHONDRIAL MECHANISMS OF ROS PRODUCTION AND OXIDATIVE STRESS BY REDOX CYCLING HERBICIDES IMPLICATED IN PARKINSON’S DISEASE.**

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Exposure to environmental pesticides causes significant brain damage and has been linked with the etiology of Parkinson’s disease (PD). Bipyrindyl herbicides, such as paraquat (PQ) and diquat (DQ) and benzyl viologen (BV), are redox cycling agents (RCAs) known to exert cellular damage through the production of reactive oxygen species (ROS) leading to oxidative stress. However, the site and mechanism of action within the brain of these compounds has not been resolved. The goal of this study was to determine the mechanisms by which redox cycling compounds exert neurotoxicity. Previous work in our laboratory has shown that brain substantia nigra were a major cellular site for PQ-induced H2O2 production. Here, we further characterized the role of mitochondria in redox cycling-induced ROS production and determined if in vivo treatment with PQ results in mitochondrial oxidative stress. Following redox treatment with redox cycling agents, H2O2 production was measured in mitochondria isolated from respiratory-deficient SH-SY5Y dopaminergic cells compared to controls. In isolated rat brain mitochondria, DQ and BV stimulated H2O2 production at rates significantly greater than PQ at similar concentrations. RCA-induced ROS production was inhibited by antimycin A suggesting a role for Complex III of the mitochondrial respiratory chain. Additional mechanistic studies further identified the Qi site of cytochrome b within Complex III as the potential site of ROS production by these RCAs. Administration of 10 mg/kg PQ bi-weekly for three weeks significantly increased mitochondria DNA damage assessed by gpcR in PQ-treated mice. Additionally, reduced coenzyme A, an index of the mitochondrial redox status was significantly decreased in the ventral midbrain of PQ-treated mice in comparison with saline-treated mice. These data provide evidence that bipyrindyl herbicides may play a role in the development of Parkinson’s disease.

**1545 EFFECTS OF MIXED TOCOPHEROLS IN ANIMAL MODELS OF PARKINSON’S DISEASE.**

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Parkinson’s disease (PD) is a progressive neurodegenerative disorder that affects more than 1.5 million people in the United States alone. An abundance of evidence indicates that oxidative stress leads to the loss of the dopaminergic neurons. Vitamin E (α-tocopherol, αT) has been tested both in animal models of PD as well as in human clinical trials, but with equivocal results. Although αT is the biologically most active form of vitamin E, γ-tocopherol (YT) is the most abundant form found in the U.S. diet and is also a better antioxidant and anti-inflammatory agent. Thus, to determine the efficacy of γT to protect against dopaminergic cell death, animals were pre-treated for 2 months with either a 0.5% mixed tocopherol diet (containing about 60% YT) or control diet and subsequently treated with either saline or 10mg/kg paraquat (PQ) + 30 mg/kg maneb (MB) twice a week for 6 weeks or 20 mg/kg MPTP everyday for 5 days. Both MPTP and PQ+MB exposure caused a significant decrease in striatal dopamine (DA) and metabolite levels in mice on the control diet. However, mice on the mixed tocopherol diet treated with either PQ+MB or MPTP were partially protected against decreases in striatal DA and metabolite levels. In a subsequent experiment, however, mice that were pre-treated for only 1 month with 5 different tocopherol enriched diets were not protected against decreases in striatal DA levels from exposure to 20 mg/kg MPTP everyday for 5 days. Interestingly, the diet with the highest tocopherol concentration tested (0.5% mixedT) proved to be neuroprotective, with saline-treated mice showing significantly decreased striatal DA levels compared to mice on the control diet. The data suggests that a diet enriched with γT may provide protection dopaminergic neurons against oxidative stress generated by exposure to dopaminergic neurotoxins.

**1546 DIFFERENTIATION OF BONE MARROW-DERIVED STEM CELLS INTO DOPAMINERGIC PROGENITOR CELLS INCREASES THEIR SENSITIVITY TO PARAGUAT.**

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Dopaminergic neurons of the substantia nigra are known to be sensitive to paraquat, an herbicide associated with increased risk of Parkinson’s disease in humans. Several animal studies have demonstrated that dopaminergic progenitor cells are particularly vulnerable to paraquat. However, the mechanism responsible for this selective vulnerability is unknown. Paraquat is known to undergo redox cycling to generate reactive oxygen intermediates (ROI) that are thought to mediate its toxicity. Thus, it is possible that differences in enzymatic generation of ROI may play a role in this selective vulnerability. In the present studies we utilized human adult-derived mesenchymal stem cells (MSCs) that were differentiated into dopaminergic progenitor cells to determine how differentiation affects the neurotoxicity of paraquat. Following differentiation, cells progressively display characteristics of...
dopaminergic neurons. Little or no caspase-3 activation was observed in undifferen-
tiated (day 0) or partially differentiated (day 6) MSC treated with paraquat (100
or 400 μM). However, like cells of the substantia nigra, cells differentiated for 12
days showed significant caspase-3 activation. Differences in the generation of ROI
were responsible for the increased toxicity in differentiated cells; MSC lysates from 6
day- and 12-day differentiated cells produced 2.4- and 5.3-fold more hydrogen
peroxide than non-differentiated cells in the presence of paraquat (100 μM) when
supported by the reducing agent NADPH. Taken together, these data demonstrate
that differentiation of MSCs into dopaminergic progenitor cells increases their sus-
cceptibility to paraquat and suggest that this is due to in part enhanced expression of
NADPH-dependent oxidoreductase activity and paraquat redox cycling.

**1547** FYN KINASE DEPENDENT PROTEOLYTIC ACTIVATION OF PROTEIN KINASE C-DELTA PHOSPHORYLATES ANTI-APOPTOTIC KINASE PKD1 DURING OXIDATIVE INSULT IN CELL CULTURE MODELS OF PARKINSON’S DISEASE.

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Oxidative stress is a prominent pathophysiological mechanism contributing to neu-
rodegeneration in Parkinson’s disease (PD) and therefore, unraveling cell signaling
mechanisms underlying oxidative neuronal damage will provide new insights into mechanisms of nigral degeneration as well as foster development of novel treat-
ment modalities in PD. We previously showed that PKCδ proteolytic activation mediates oxidative stress-induced apoptosis in dopaminergic neuronal cells (N27).

In the present study, we identified that Protein kinase D1 (PKD1) functions as a key anti-apoptotic kinase to protect dopaminergic cells against oxidative insult. Treatment of N27 cells with H2O2 (100-300μM) or MPP+ (300μM) induced phosphorylation of PKD1 activation loop in a dose- and time-dependent manner. Inhibition of PKCδ with rottlerin or siRNA significantly attenuated H2O2-in-
duced PKD1 phosphorylation, suggesting that PKCδ regulates PKD1 activation.

Treatment with Z-VADfmk or overexpression of cleavage-resistant PKCδ mutant e
terf ciently attenuated PKD1 activation, indicating that the PKCδprof ective ac
tivation contributes to PKD1 phosphorylation. Overexpression of PKCδ catalytic fragment, but not regulatory fragment, also increased PKD1 phosphorylation.

Additionally, Fyn kinase inhibitors block both PKCδ activation and PKD1 phos-
phorylation, indicating that FYN kinase is a upstream regulator of the PKCδ-
PKD1 signaling. RNAi-mediated PKD1 knockdown augmented oxidative stress-
induced apoptosis, suggesting an anti-apoptotic role for PKD1 during oxidative injury. Collectively, our results suggest that Fyn-PKCδ-PKD1 activation represents a key intrinsic protective response in counteracting early stages of oxida-
tive damage in dopaminergic cells. A positive modulation of PKD1 signaling may prove to be a novel neuroprotective strategy against oxidative damage in PD (sup-
port NIH grants ES10586 & NS54153).

**1548** ELEVATED HISTAMINE AS A VULNERABILITY FACTOR IN THE PARKINSON’S DISEASE PHENOTYPE.

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Brain histamine levels are elevated in Parkinson’s disease (PD) patients. There is a significant interaction between brain histamine and dopamine (DA) systems. Given the inflammatory processes evoked by histamine, and the presumed role of inflammation in PD, this study sought to generate a mouse model of increased brain histamine that could be used in subsequent experiments to determine its abili-
ty to enhance risk in PD animal models. Male C3H/B6 mice were fed standard lab
chow that was fortified with 0, 6 or 10% (n=2, 3 and 3, respectively) of the histamine
containing foodstuff. Male C57Bl6 mice were fed standard lab
chow that was fortified with 0, 6 or 10% (n=2, 3 and 3, respectively) of the histamine
containing foodstuff. Male C57Bl6 mice were fed standard lab
chow that was fortified with 0, 6 or 10% (n=2, 3 and 3, respectively) of the histamine
containing foodstuff.

Brain histamine levels were found to be significantly elevated in the 6 and 10% treatment groups, with the 10% treatment group exhibiting both higher levels of histamine and a more pronounced antiparkinsonian phenotype. Histamine levels were also found to be significantly correlated with decreased activity in the DA-rich nigrostriatal pathway, as measured by the Open Field Test. In addition, histamine levels were found to be significantly correlated with increased sensitivity to L-DOPA, a known antiparkinsonian drug.

**1549** MITOCHONDRIA-TARGETED TRIPHENYLPHOSPHONIUM CONJUGATED NITROXIDE FUNCTION AS A RADII PROTECTOR.

L. Jiang, D. Stoyanovsky, N. A. Belikova, Q. Zhao, V. Y. Tyrina, M. A. Tungekar, V. Kapralova, J. S. Greenberger and V. E. Kazan.

Removal of intracellular reactive oxygen species (ROS), particularly mitochondrial ROS, is important in protecting against oxidative damage in PD. We previously showed that mitochondria-targeted nitroxides act as protective agents against oxidative stress by blocking mitochondrial ROS production and preventing their release into the cytosol. In the present study, we investigated the protective effects of mitochondrial-targeted nitroxides on mitochondrial ROS production and their ability to preserve mitochondrial function in a model of light-induced PD.

We used the MPP+ model of PD to evaluate the protective effects of mitochondrial-targeted nitroxides. MPP+ is a mitochondrial toxin that blocks mitochondrial ATP production and induces oxidative stress. We found that mitochondrial-targeted nitroxides significantly reduced MPP+-induced mitochondrial ROS production and preserved mitochondrial function, as evidenced by increased ATP production and decreased apoptosis.

Our findings suggest that mitochondrial-targeted nitroxides have potential for use in the treatment of PD and other neurodegenerative diseases characterized by increased mitochondrial ROS and oxidative stress.
result of their chemical reactivity and/or because these biliary metabolites are the major transport form of the drug to the small intestine where the aglycone is released. Because DCF induces mitochondria-mediated apoptosis in other cell types including hepatocytes, we hypothesized that drug-induced mitochondrial permeabilization may be involved in intestinal mucosal damage induced by DCF. To test this hypothesis, we first developed a mouse model of DCF enteropathy. Male C57BL/6 mice (fed) were injected with a single dose of DCF (60 mg/kg, ip) or vehicle (10% solanol HS-15 in PBS), and the extent of injury to the jejunum and ileum was quantified at various time points by estimating the number and size of ulcers, as well as by determining decreases in serum alkaline phosphatase (ALP) activity and total protein levels. Next, to explore the role of mitochondrial permeabilization, mice were post-treated (1 h) with low doses (<10 mg/kg, ip) of cyclosporin A (CsA), a well-known inhibitor of the mitochondrial permeability transition pore. Post-treatment was chosen to minimize possible interactions of CsA with the hepato-biliary transport of DCF. Treatment with CsA greatly reduced both the number and size of small intestinal ulcers, indicating significant protection from DCF-induced enteropathy. Also, ALP activity and total serum protein levels were restored to normal values. These results are compatible with our concept that diclofenac causes enteropathy via a topical effect involving mitochondrial permeabilization and cell death.

1552 KNOCKDOWN OF SUPEROXIDE DISMUTASE 2 IN BRL3 CELL AND RAT TO EVALUATE THE DURG-INDUCED HEPATOTOXICITY.

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Drug-induced hepatotoxicity is a major problem in drug development, and oxidative stress is known as one of its causes. Superoxide dismutases (SODs) are important antioxidant enzymes against reactive oxygen species (ROS). Mitochondria are the major source of superoxide production, and SOD2 is mainly located in mitochondria, suggesting that SOD2 plays an important role in scavenging superoxide among other SODs. In this study, we established SOD2 knockdown cells and animal models. An adenovirus vector with short hairpin RNA against rat SOD2 (AdSOD2-shRNA) was constructed, and infection of AdSOD2-shRNA resulted in significant decrease of SOD2 activity by 50% after 3 days infection. We previously constructed an adenovirus expressing CYP3A4 (AdCYP3A4). Co-infection of AdSOD2-shRNA and AdCYP3A4 to BRL3A cells was performed to evaluate the superoxide- and CYP3A4-mediated oxidative stress. As a result, rats treated with AdSOD2-shRNA and AdCYP3A4 in the liver resulted in significant decrease of SOD2 activity by 50% after 3 days treatment. These results suggest that diclofenac causes enteropathy via a topical effect involving mitochondrial permeabilization and cell death.

1553 IDENTIFICATION OF SMALL MOLECULE INDUCERS OF HEAT SHOCK RESPONSE.

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Heat shock response (HSR) is triggered by cellular and environmental insults such as increased temperature, oxidative stress and chemical exposure, which results in the over expression of heat shock proteins (HSPs). As an integral component of HSR, HSPs function as molecular chaperones that assist in protein folding and protein homeostasis during cellular stress. HSP expression is regulated by heat shock transcription factors (HSF), such as HSF1, which can serve as prognostic indicators of several diseases associated with misfolded/aggregated proteins, such as neurodegenerative disorders or cancer. This study was designed to identify novel small molecule inducers of HSR that promote the activation of HSF1 and subsequent up-regulation of HSPs. A Hi4 cell line that stably expresses a β-lactamase reporter gene under the control of a heat shock response element (HSE) was developed, optimized, and miniaturized into a 1536-well plate format for high-throughput screening (HTS). Thus far, approximately 2000 approved drugs from the NCGC Pharmaceutical Collection (NPC) have been screened with this assay. Several known HSR inducers, such as 17-AAG (via HSP90 inhibition), bortezomib (via proteasome inhibition) and curcumin (via Keap1/Nrf2/ARE pathway) were identified, as well as several novel HSR inducers. Mechanistic and pathway specificity associated with identified compounds were further studied in other stress related pathways including NFkB signaling. Identification of novel HSR inducers from the NPC using our HTS approach sheds new light on the mechanism of action of these drugs and their potential for producing both toxicological and novel therapeutic effects.

1554 LOW LEVEL LIGHT THERAPY ENHANCES RENAL FUNCTION AND ANTIOXIDANT DEFENSE SYSTEM IN STREPTOZOTOCIN-INDUCED DIABETIC RAT MODEL.

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Hyperglycemia-induced oxidative damage contributes to the progression and severity of diabetic complications. Levels of oxidative stress are controlled by antioxidant defense systems. However, diabetes generates excessive oxidative stress in tissues and consequently results in renal damage. Low level light therapy in the range of 630-1000 nm has been shown to accelerate wound healing, thereby increasing the recovery rate from ischemic injury in heart tissue and attenuating degeneration in the injured retina and optic nerve. The present study tests the hypothesis that 670 nm light therapy increases antioxidant defense capabilities against renal oxidative stress in the streptozotocin-treated rat model of type 1 diabetes. Male Wistar rats were made diabetic with streptozotocin (50 mg/kg, ip), and subsequently exposed to 670 nm light at a dose of 9 J/cm2 daily for 15 weeks. Kidneys were harvested, flash frozen, and assayed for markers of oxidative stress, including glutathione reductase, glutathione peroxidase, superoxide dismutase, catalase, reduced/oxidized glutathione, lipid peroxidation, cytochrome c oxidase, and 8-hydroxy-2’-deoxyguanosine. Markers of renal function (Na+ K+ ATPase, blood urea nitrogen, and creatinine levels) were also evaluated. Both renal antioxidant defense systems and kidney function were enhanced by light therapy compared to diabetic groups, while the levels of oxidative stress in diabetic animals showed no significant changes compared to control groups. Our findings suggest that low-level light therapy may attenuate the effects of diabetes on renal function and stimulate antioxidant protective pathways in the diabetic rat model.

1555 ANTIOXIDANT ACTIONS OF TAURINE AND STRUCTURALLY-RELATED SULFUR-CONTAINING COMPOUNDS IN THE BRAIN OF DIABETIC RATS.

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This work has assessed the ability of taurine (TAU) and of the structurally-related compounds aminomethanesulfonic acid (AMSA), homotaurine (HMTAU) and pantoyltaurine (PTAU) to protect the brain against diabetes-induced oxidative stress in male Sprague-Dawley rats. The rats were divided into three groups: control group, diabetic group, and diabetic group treated with a sulfur compound. Diabetes was induced using a single, 60 mg/kg, intraperitoneal (i.p.) dose of streptozotocin (STZ). A sulfur compound was administered as two 1.2 mM/kg i.p. doses, 75 min and 45 min before STZ. Control animals received only physiological saline. At 24 hr post-STZ, animals were sacrificed and their brain removed. Enzymatic (catalase, glutathione peroxidase, superoxide dismutase) and nonenzymatic (malondialdehyde, reduced and oxidized glutathione, nitric oxide) indices of oxidative stress were measured in brain stem, cerebellum, cortex and spinal cord. While diabetes lowered the values of all enzymatic parameters and of the reduced/oxidized glutathione ratio, it increased those of malondialdehyde and nitric oxide. Without exceptions, all the aminosulfonic acids tested here were found to effectively protect the brain against oxidative stress due to diabetes. In general, the magnitude of the protective effect was found to vary in a rather consistent manner in the various areas of the brain, with HMTAU and AMSA usually being more potent than TAU and PTAU. The only exception was the nitric oxide values, for which TAU and PTAU appeared more protective than HMTAU and AMSA in all brain areas but the spinal cord.

1556 ALDH7A1 IS A NOVEL ALDEHYDE DEHYDROGENASE WITH MULTIPLE FUNCTIONS INCLUDING PROTECTION FROM OXIDATIVE STRESS.

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ALDH7A1 is a novel aldehyde dehydrogenase (ALDH) that plays a major role in lysine catalolism through the NAD+ dependent conversion of α-aminoacrylic semialdehyde (AAS) to α-aminoacrylic acid (AAA). In humans, deleterious muta-
tions in ALDH7A1 are responsible for Pyridoxine-Dependent Epilepsy (PDE). The continuing aim of this study is to characterize the subcellular localization, tissue distribution, biochemical properties and functions of mammalian ALDH7A1. Human ALDH7A1 protein, baculovirus expressed and affinity purified, was found to be co-localized with various ald(oh)dehydrogenases. Western blot analyses revealed high expression of ALDH7A1 protein in mouse kidney, liver, brain, and tongue and low expression in the heart, stomach, and lung. Subcellular fractionation studies, immunohistochemistry and confocal microscopy revealed ALDH7A1 protein was found in the cytosol, nucleus and mitochondria. Through sequence analysis and RT-PCR we identified a second novel ALDH7A1 transcriptional variant, which was found to contain a mitochondrial leader sequence. Both ALDH7A1 transcript variants were expressed at different ratios in a tissue specific manner. The mitochondrial variant was the predominant transcript found in the liver, kidney, stomach and brain. Finally, ALDH7A1 protects against osmotic stress-induced apoptosis. In conclusion, ALDH7A1 is a novel ALDH found in the cytosol, nucleus and mitochondria, which along with its substrate specificities suggest potential tissue- and cell-specific biological roles against oxidative damage.

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**1557 EFFECTS OF SELENOCOMPOUNDS ON THIOREDOXIN REDUCTASE-1 IN NON-TUMORIGENIC AND TUMORIGENIC HUMAN LUNG CELLS.**

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Selenium-based cancer prevention strategies have demonstrated reduced cancer mortality and efficacy for some cancer types, but the mechanisms of selenium-dependent cancer prevention are not fully understood. Considerable differences in efficacy exist among the cancer prevention activities of organoselenocompounds, and the amino acid selenomethionine is currently the favored selenocompound used in clinical trials. However, other selenocompounds have demonstrated better efficacy in the NNK murine model of lung cancer. Based on a microarray study using the NNK model and selenium sufficient mice, we found evidence of activation of genes involved in oxidoreductase activity, including the selenoprotein thioredoxin reductase (Trxr1), when the mice were supplemented with selenocysteine. We have also evaluated the cellular redox consequences of selenocysteine in human lung cell lines and found that it had an oxidizing effect on a lung cancer cell line, A549, but minimal effect on a non-tumorigenic lung cell line, BEAS-2B. Therefore, we set out to determine if selenocysteine, as well as other organoselenocompounds, influenced the expression of Trxr1 in these human lung cell lines after 12, 24, and 48 hour treatments. Using QPCR, we found that selenocysteine, methylecysteinic acid, and selenocysteine prodrugs (selenazolidines) all elevate Trxr1 mRNA expression in BEAS-2B cells after treatment for 24 hours, but we did not observe this same change in expression in A549 cells. These results appear consistent with the Nrf2-KEAP1 status of these cell lines, as A549 cells have a mutated KEAP1. Of interest, selenomethionine did not induce expression of Trxr1 in either cell line at 24 hours. Trxr1 protein expression and activity in the A549 and BEAS-2B cells were also evaluated following selenocompound treatment using Western blotting and an NADPH oxidation assay, respectively. These data taken together suggest that selenocysteine, as well as other selenocompounds, may modulate Trxr1 as part of their mechanism of action in human lung cells.

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**1558 MANIPULATION OF CELL GLUTATHIONE (GSH), GLUTAMYL TRANSPEPTIDASE (GGT) & GLUTATHIONE S-TRANSFERASE (GST) ACTIVITY SENSITIZES NORMAL BUT NOT VITILIGO MELANOCYTES AGAINST 4-TERTIARY BUTYLPHENOL (4-TBP) TOXICITY.**

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Exposure to 4-TBP is associated with onset of occupational vitiligo (patchy depigmentation) and is considered to be a risk factor in non-occupational vitiligo. Skin lesions in ALDH7A1 deficient mice develop and progress at a faster rate than in the wild type control. When exposed to 4-TBP, the ALDH7A1 deficient mice are more sensitive to subsequent treatments with mercaptopurine. In our previous studies, we found that selenomethionine and ACV increased sensitivity of PIG1 cells to 250μM 4-TBP but not in PIG3V cells. Since selenomethionine and ACV increased sensitivity to GST inhibition by EA alone or in combination with 4-TBP compared to PIG3V cells. Data describe inherent deficiency in GSH content, GGT and GST activities in PIG3V compared to PIG1 cells that influence sensitivity to 4-TBP induced toxicity.

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**1559 DETA NONOATE RESCUES HYPEROXIA-INDUCED MACROPHAGE DYSFUNCTION.**

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Mechanical ventilation with hyperoxia is a life-saving therapy for the management of patients with acute respiratory failure. However, it carries many potential complications including inflammatory lung injury and ventilator-associated pneumonia (VAP). Previous studies show that elevated levels of ROS under hyperoxic conditions lead to the compromised ability to phagocytose bacteria by macrophages, thus predisposing the patient population to VAP. Despite of advances in antimicrobial therapy, VAP remains a significant morbidty and mortality in patients on ventilation. Nitric oxide (NO) has been shown to be involved in vasodilation and immune modulation. In vitro studies in other laboratories have shown that NO, in various forms, has antimicrobial activity against bacteria, yeasts and mycobacteria. NO can also act as a NO donor, can spontaneously and nonenzymatically release NO.

In this study, we tested the effects of DETA NONOate on hyperoxia-induced impairment of macrophage function. RAW 264.7 cells were treated with the combination of treatment of hyperoxia and varying concentrations of DETA NONOate (5 μM to 300μM). Moderate concentrations (up to 150 μM) of DETA NONOate significantly rescued hyperoxia-suppressed phagocytosis and reversed hyperoxia-induced inhibition of cell migration. However, DETA NONOate at concentrations of 300μM or higher caused the death of macrophages. These results suggest that NO at concentrations that are comparable to those used clinically for treating pulmonary hypertension may also be useful in preventing VAP.

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**1560 RNA INTERFERENCE OF HGST4A IN HUMAN AORTIC VASCULAR SMOOTH MUSCLE CELLS RESULTS IN SIGNIFICANT CHANGES IN GENE EXPRESSION.**

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Our previous work in endothelial and smooth muscle cells showed that overexpression of glutathione S-transferases (GSTs) protects against oxidative damage from aldose (such as 4-hydroxynonenal (HNE) and hydrogen peroxide (H2O2). Over expression of mGSTA4-4 in endothelial cells plays also a key role in upregulation of iNOS, NF-κB.

In this study, we investigated whether knocking down hGSTA4a gene in human aortic vascular smooth muscle cells (HASMVCs) by RNAi is deleterious to the defensive mechanism of these cells against oxidative stress. Cell culture of HASMVC, siRNA of hGSTA4 transfection, Microarray and Ingenuity data analysis, Real-time RT-PCR analysis, cytotoxicity assay, enzyme assay and Western blot were carried out. The Affymetrix GeneChip Human Genome U133A2.0 microarray chip was used in the present studies; a total of 309 genes in siRNA of hGSTA4a transfected HASMVC were either up-regulated or down-regulated (p<0.05 by Ingenuity software). Our focused on 15 altered genes with a possible relevance to cardiovascular disease, especially atherosclerosis and those genes related to apoptosis, lipid peroxidation, or tone of vascular wall. The up-regulated genes in this group include: AIFM1, BDKRB2, BINS3, Cytochrome c, HMOX1, D2, PBRM1, and TFE3. The down-regulated genes include: Bcl2, CYB61, ITGB5, OPAL1, POU4F1, and PSEN2. Down-regulating hGSTA4-4 by siRNA raised the basal level of lipid peroxidation products, MDA and HNE in cells. Cytotoxicity assay proved that hGSTA4-4 is a necessary defensive enzyme against oxidative stress in vascular smooth muscle cells. When the enzyme was decreased, the cells were more easily damaged by toxics.

These results demonstrate that hGSTA4-4 is an important defensive enzyme in the vascular wall. Knocking down this gene greatly influences cellular function by altering 4-HNE levels that modulate the expression of the other genes.
**1561 COMPARATIVE DISTRIBUTION AND RETENTION OF ARSENIC IN ARSENIC (± OXIDATION STATE) METHYLTRANSFERASE KNOCKOUT AND WILD TYPE MICE.**


The mouse arsenic (± oxidation state) methyltransferase (A3mt) gene encodes a ±43 kDa protein that catalyzes conversion of inorganic arsenic into methylated products. Heterologous expression of A3mt or its silencing by RNA interference controls arsenic methylation phenotypes in cultured human cells, suggesting a critical role for the enzyme in arsenic metabolism. Because methylated arsenicals mediate some of the toxic or carcinogenic effects associated with exposure to inorganic arsenic, studies of the fate and effects of arsenicals in mice which cannot metabolize arsenic could be a valuable experimental tool. The mouse A3mt gene was disrupted by homologous recombination and transferred into the C57BL/6 mouse strain. Homozygous A3mt−/− mice are viable and fertile. This study compared retention and distribution of arsenic in A3mt knockout mice and C57BL/6 mice in which expression of the A3mt gene is normal. Male and female mice of either genotype received an oral dose of 0.5 mg of arsenic as arsenate per kg containing [131]Arse-nate as a label. Mice were radioassayed for up to 24 or 96 hours post dosing and tissues were collected at term. Whole body clearance of [131]As in A3mt knockouts is substantially slower than in C57BL/6 mice. At 24 hours post dosing, A3mt knockouts retain about 90% of the dose; C57BL/6 mice retain about 60%. After 96 hours, A3mt knockout mice still retain 20% and C57BL/6 mice retain less than 2% of the dose. At 24 hours post dosing, A3mt knockout mice retain significantly higher percentages of arsenic dose in liver, kidney, uterine bladder, lungs, heart, and carcass than do C57BL/6 mice. These data confirm a central role for A3mt in metabolism of inorganic arsenic and indicate that phenotypes for arsenic retention and distribution are markedly affected by the null genotype for arsenic methyltransferase. This abstract does not reflect US EPA policy.

**1562 COMPARISON OF TOXICOGENOMIC RESPONSES TO INORGANIC AND ORGANIC MERCURIES IN CAENORHABDITIS ELEGANS.**

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Humans are exposed to methylmercury through consumption of fish, and inorganic mercury through occupational exposures and dental amalgams. While the toxicity of mercury is well established, much remains to be elucidated about the mechanisms of mercury toxicity. In particular, little is known about the extent to which different species of mercury behave similarly or differently at the cellular level. In order to address this issue, we employed a toxicogenomics approach using the nematode Caenorhabditis elegans. We first established toxic responses in C. elegans to methylmercury chloride (MeHg) and mercuric chloride (HgCl2) using the nematode Caenorhabditis elegans. We first established toxic responses in C. elegans to methylmercury chloride (MeHg) and mercuric chloride (HgCl2). Analysis of the microarray data high toxic HgCl2 exposures and 44, 424 and 2835 differentially expressed genes in off we identified 8, 76, and 411 differentially expressed genes in the sub-, low- and high toxic MeHg exposures. Additionally, comparison of significantly expressed genes indicated that C. elegans have vastly different transcriptional responses when exposed to different mercury species. Furthermore, in C. elegans, the zinc transporter ZnT-2, which is highly expressed in the intestine, is not expressed in the liver or other tissues. The solute carrier gene (SLC) superfamily comprises 55 families and at least 562 putative protein-coding genes. Members within each family are evolutionarily related, whereas families in the superfamily have been assigned mostly on the basis of function: transmembrane transporters that move ions and other molecules in or out of cells, or across vesicular and mitochondrial membranes. SLC39 genes are members of the ZIP family of metal ion transporters, which transport essential metals such as Zn, Fe, Cu and Mn. For example, mutations in the human SLC39A4 gene are responsible for acrodermatitis enteropathica, Zn deficiency (AE). Our lab has shown that ZIP8 is a Zn2+/(HCO3−) symporter moving both ions into the cell as an electroneutral complex. Zn is well known to be critical during embryo implantation and development. While creating a SLZ8 knockout construct, we inserted a neomycin-resistance gene (neo) into intron 2 with lnp8 sites in introns 2 and 5. Crossing, SLZ8(+/-) heterozygotes produced offspring having a normal Mendelian distribution through gestational day (GD)16.5; however, all SLZ8(-/-) homozygotes were pale, growth-retarded and succumbed between GD16.5 and postnatal day (PND)2. ZIP8 mRNA was significantly decreased in SLZ8(-/-) embryos, yolksac, and placenta. At PND1, SLZ8(-/-) Zn2+ concentration in the embryo was 60.3% (P<0.016) and Fe 46.8% (P=0.011) of littermate controls, liver showed far fewer hematopoietic islands, and hepatocytes contained much less glycogen; the RBC was 68% (P=0.06), Hgb 60% (P=0.008) and Hct 64% (P=0.03) of littermate controls. Crossing SLZ8(-/-) on our BAC-transgenic BTZIP8-3 line (having 3 extra copies of SLZ8), we successfully rescued the SLZ8(-/-) offspring pups. These studies identify that ZIP8 serves an essential function of transporting Zn into the embryo, needed for normal hematopoiesis and development. — Supported, in part, by NIH grants R01 ES010416 and P01 ES060696.

**ROLE OF MELATONIN IN ENZYMATIC STRESS MARKERS AND GEN EXPRESSION IN ABETAPN TRANSGENIC MICE EXPOSED TO ALUMINUM.**

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Esparza JL1, Garcia T1, Gómez M1, Nogués MR2, Romeu M2, Domingo JL1 Laboratory of Toxicol & Environ Health, and 2Pharmacology Unit, School of Medicine, URV, 43201 Reus, Catalonia, Spain, Aluminum (Al), a well known neurotoxic agent, induces free radicals in brain, while melatonin (Mel) can act as a free radical scavenger, or induce the expression of some genes linked to the antioxidant defence. In this study, AbetaPP transgenic (Tg2576) (Tg) and wild-type mice of 5 months of age, received a supplement of Al lactate (1 mg Al/g diet). They also received oral melatonin (Mel)(10 mg/kg) until 11 month of age. For Tg and wild-type mice, there were 4 groups: control, Al, Mel, and Al+Mel. At the end of the treatment, hippocampus was removed and processed to examine: GST, GSH, GSG, SOD, GR, GPx, CAT and TBARS. The expression of Cu-ZnSOD, GR and CAT was evaluated by real-time PCR. Al concentration in hippocampus was also determined. Al levels increased in all Al-exposed groups with independence of the genotype. When all groups were compared, stress oxidative markers showed differences in GSH, SOD and GR, but not in GSG, CAT, GPx and TBARS. GSH levels increased in Al+Mel Tg/wild-type mice, while SOD and GR decreased in Al+Mel wild-type and in Al+Mel Tg/wild-type groups, respectively. In relation to mRNA levels of Cu-ZnSOD, CAT and GR, results show that concomitant administration of Al and Mel increases the gene expression of SOD and CAT, but not GR mainly in Tg animals. In turn, Al decreased similarly the m-RNA levels of CAT in wild-type and Tg exposed groups. Mel wild-type groups showed a decrease in the expression of Cu-ZnSOD. Overall, Al decreased similarly in all groups exposed to Al only. The biochemical changes observed in hippocampus indicate that Al acts as pro-oxidant agent, while Mel exerts an antioxidiant action by increasing the mRNA levels of the antioxidant enzymes SOD and CAT in presence of Al and Mel, and with independence of the animal model.

**BLOOD CADMIUM AND LEAD AND CHRONIC KIDNEY DISEASE IN U.S. ADULTS.**

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Environmental exposures to cadmium and lead are widespread. Recent data indicate that environmental lead exposure increases risk for chronic kidney disease (CKD). However, few studies have evaluated associations between low-level cadmium and clinical renal outcomes, particularly with respect to joint cadmium and lead. Therefore, we calculated odds ratios for albuminuria (albumin ≥ 15 mg/g creatinine), reduced estimated glomerular filtration rate (eGFR: ≤ 60 ml/min/1.73 m2), and both outcomes combined by quartile of blood cadmium and lead in
adults who participated in the 1999-2006 National Health and Nutrition Examination Survey (n=14,778). Models were adjusted for survey year; socioeconomic and CKD risk factors; and the other metal as a continuous variable. The geometric means of blood cadmium and lead were 0.41 μg/L and 1.58 μg/dL, respectively. The adjusted odds ratios (95% confidence interval) for albuminuria, reduced eGFR, and both albuminuria and reduced eGFR were 1.92 (1.53, 2.43), 1.32 (1.04, 1.68) and 2.91 (1.76, 4.81), respectively, comparing the highest to the lowest blood cadmium quartiles, and 1.19 (0.96, 1.47), 1.56 (1.17, 2.08) and 2.39 (1.59, 3.64), respectively, comparing the highest to the lowest blood lead quartiles. The odds ratios comparing participants in the highest quartile of both metals to those in the lowest quartile of both metals were 2.36 (1.49, 3.73) for albuminuria, 1.88 (1.24, 2.84) for reduced eGFR and 7.80 (3.04, 19.98) for both outcomes. These findings provide strong support for consideration of lead and both metals as a risk factor for the development of CKD in populations where exposure to both metals is common.  

**1566 MICROARRAY ANALYSIS OF THE PULMONARY EFFECTS OF STAINLESS AND MILD STEEL WELDING FUMES IN A/J AND C57BL/6J MICE.**

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Our earlier studies found supportive evidence for a tumorigenic effect of carcinogenic metal-containing welding fume in A/J mice. A/J mice are genetically predisposed to spontaneous and/or chemically-induced lung tumors while C57BL/6j (B6) mice are resistant. This genetic disparity provides a unique scenario to identify molecular mechanisms associated with the lung response to welding fume at the transcriptome level. Mice were exposed four times by pharyngeal aspiration to 5mg/kg mild steel (MS) fume, stainless steel (SS) fume, or saline vehicle. Mice were necropsied 28 days after the last exposure and whole lung microarray using Illumina Mouse Ref-8 expression beadchips was done. Ingenuity pathway analysis (cut-off: p<0.05; fold change >1.3) of the microarray data revealed the top global molecular network involved in the AJ response to MS fume was behavior, nervous system development and function, and gene expression. In contrast, the connective tissue disorders, immunological disease, inflammatory disease network was most significant in the B6 strain. In the AJ, 75% of the focus molecules that met the cutoff were up-regulated as compared to 40% in the B6. Six genes were common between the strains such as KLFL2, KLFL4 and MARCCO. SS fume exposure in the AJ induced genes primarily involved in connective tissue disorders, immunological disease, and inflammatory disease. Genes regulating cellular movement, hematopoietic system development and function, and immune response were most involved in the B6 response. Of the significant focus molecules, 88% were up-regulated in the AJ compared to 45% in the B6. Only five common genes were found between the strains such as HSPH1, MMP12 and CTSK. Overall, these data confirm our previous observation that strain-dependent differences in response to welding fume occur in the AJ and B6 lung. Also, in contrast to the B6, the AJ strain exhibited a persistent up-regulation of welding fume-induced gene transcription suggesting that chronic lung cell activation may play a role in the tumorigenic effects of welding fume.

**1567 EXPOSURE TO ARSENIC COMBINED WITH HIGH FAT DIET PROMOTES THE IMPAIRMENT OF GLUCOSE TOLERANCE IN C57BL/6 MICE.**

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Obesity is the single most important risk factor for the development of insulin resistance and type 2 diabetes. However, chronic exposures to inorganic arsenic (iAs) have also been associated with an increased prevalence of type 2 diabetes mellitus. The current study examines diabetogenic effects of iAs combined with consumption of a high fat diet (HFD). Here, weanling, male, C57BL/6 mice were divided into 6 groups which drank deionized water containing arsenite (iAsIII) (25 or 50 ppm) or water without iAsIII, ad libitum, for 20 weeks while consuming either a HFD (58% fat) or a low fat diet (LFD) (11% fat), also ad libitum. Body weight, adiposity, food and water consumptions were measured throughout the entire study. At 20 weeks fasting blood samples were collected and oral glucose tolerance tests were administered to all mice. In general, the 25 and 50 ppm groups consumed less water than control mice. iAsIII intake was estimated at 57 μg/d for 25 ppm groups and 81 μg/d for 50 ppm groups. In general, HFD groups gained significantly more body fat mass and had higher fasting blood glucose and serum insulin levels than did their respective LFD groups. However, these measures decreased with iAsIII intake in a dose dependent manner. Oral glucose tolerance tests showed an impairment of glucose tolerance for HFD groups compared to their respective LFD groups. The degree of glucose intolerance increased with iAsIII intake in a dose dependent manner in spite of the observed decrease in adiposity, fasting glucose and fasting insulin levels. These data suggest that the diabetogenic effects of iAsIII are independent of the mechanisms traditionally associated with consumption of high fat diets and/or obesity in mice.

**1568 CADMIUM INDUCES REDUCED PLACENTAL ENOS ACTIVATION, REDUCED UMBILICAL ARTERY BLOOD VELOCITY AND FETAL GROWTH RESTRICTION IN MICE.**

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Cadmium is an environmental toxin present in industrial wastes, road and house dust, and food crops grown in cadmium-polluted soil. The most significant route of cadmium exposure is via cigarette smoke. Women who smoke during pregnancy have a higher incidence of placental insufficiency and intrauterine growth retardation (IUGR) than their nonsmoking counterparts. We hypothesized that cadmium could induce fetal growth restriction and placental insufficiency through a reduction of placental blood vessels and reduction of blood flow through the umbilical artery. Methods: Cadmium chloride (40ppm) was administered via drinking water to female C57BL/6 mice from 2-4 weeks prior to conception. Due to the reduction of placental surface area, the treatment was adjusted to 20ppm starting at day 1 until E15 of pregnancy. Umbilical artery blood velocity was assessed on E15 via ultrasound using the Doppler pulse method before euthanasia. Fetuses and placentas were weighed and evaluated by histopathology; TUNEL staining and western blotting. Results: Mice treated with cadmium chloride demonstrated a significant reduction of umbilical artery velocity with control mice having 59.67±14.15 mm/sec versus 35.84±1.89 mm/sec in the cadmium group. Mean weights for fetuses of cadmium-treated mice were reduced 0.119±0.007g compared to the controls 1.549±0.119g. Fetal blood lead levels were also reduced from 0.164±0.0898μg/g in the controls to 0.131±0.0808μg/g in the cadmium group. Fewer blood vessels and more TUNEL positive cells were found in the placenta with cadmium treatment. Western blots determined that endothelial nitric oxide synthase, pENOS and heat shock protein 90 (chaperone of eNOS) were decreased in placentas from cadmium exposed mothers. In conclusion, cadmium compromises HSP90 and eNOS pathways leading to reduced placental blood flow with subsequent fetal growth restriction suggesting that this metal may be one of the compounds in cigarette smoke that induces IUGR in pregnant women who smoke.

**1569 HYPOXIA INDUCIBLE FACTOR 1 ALPHA (HIF1α) PROTECTS MICE LUNGS FROM COBALT-INDUCED INJURY.**

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Millions of American workers are exposed to cobalt, primarily through inhalation, leading to several disorders including respiratory tract hyperplasia, fibrosis, and asthma. Cobalt is a known hypoxia mimic, attributed to its ability to stabilize hypoxia-inducible factor (HIFs). HIFs are oxygen-regulated transcription factors that are critical to the development of the lung and processes such as inflammation. The role of HIFs in coal-based induced lung injury has not been determined. To address this knowledge gap, a dosycycline-inducible, lung-specific HIF1α knockout mouse model was generated. In this study, wild type (WT) and HIF1α lung-deficient male mice were treated for two weeks (5 days on, 2 days off, 5 days on, 2 days off) with saline, 5 mM CoCl2, or 10 mM CoCl2 via oropharyngeal aspiration. HIF1α-deficient mice were more prone to cobalt-induced toxicity when compared to WT controls and the severity of the lesion correlated with the level of loss of HIF1α. In comparison to WT mice, HIF1α deficient mice had greater numbers of eosinophils and neutrophils consumption was subovaular lavage fluid following cobalt exposure with greater eosinophil infiltration in affected alveolar tissues. In addition, there was greater airway mucous cell metaplasia in the bronchiolar epithelium and greater chitinate-like protein (TIM1) expression in the airway epithelium and alveolar macrophages (MPB) in the lungs of HIF1α deficient mice following metal challenge, when compared to similarly treated WT mice. Finally, inflammatory cytokine profiling revealed significant induction of KC and IL-4 only in metal-treated HIF1α deficient mice. These results suggest that HIF1α regulates the coal-induced inflammatory response in the lung with asthma-like phenotype.
**1570** LYMPHOCYTE NUCLEAR CHANGES AFTER INHALED V2O5 EXPOSURE. B1-LAMIN IMPLICATION.

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It has been reported that vanadium pentoxide (V2O5) exposure produces cytotoxic effects such as genotoxicity, cell cycle modifications, apoptosis, as well as structural and functional cytoskeleton alterations. Vanadium adverse effects help to explain its potential in producing pathologies such as cancer. Previously our laboratory observed that vanadium pentoxide inhalation induced nuclear morphological changes in lymphocyte similar to those found in neoplastic cells. Owing to the fact that nuclear lamins keep nuclear structure and function integrity, and have been associated with neoplastic transformation, we investigated the alterations in nuclear B1-lamin from spleen lymphocytes after vanadium pentoxide inhalation. CD-1 male mice were exposed twice a week for 4 weeks to vanadium pentoxide (0.02M). Mice were sacrificed at 1, 2, 3 and 4 weeks; spleen was removed. Changes in nuclear B1-lamin concentration in lymphocytes subpopulations were evaluated by flow cytometry. Our results demonstrated that vanadium exposure produce a decrease in B1-lamin concentration in all lymphocyte populations analyzed (CD19, CD4, CD8) compared to controls. Moreover, this effect was time dependent in all cases. Our findings explain the morphological nuclear changes observed previously in our laboratory. Additionally, these results help to elucidate the potential carcinogenic effect of vanadium pentoxide due to the decrease in nuclear lamins, as it has been observed in cancer.

**1571** S. CEREVISIAE Genomewide Mutant Screen for Altered Sensitivity and Resistance to NISO4,

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Although nickel compounds are extremely important commercial elements, they are also occupational and environmental pollutants classified by both the International Agency for Research on Cancer and the U.S. National Toxicology Program as carcinogens to humans. However, the mechanism(s) of nickel toxicity and carcinogenesis remains unclear. Here, we performed a systematic screen of the gene deletion strains (the entire set of nonessential genes for this organism) and have identified 111 genes whose gene deletion causes a set of 4,733 haploid transcriptional repression, cell cycle arrest, and DNA recombination. This study takes a whole genome approach to understanding the toxicity and carcinogenesis of nickel compounds and sheds light on previously underestimated molecular mechanisms/pathways associated with the tolerance of eukaryotic cells to Ni2+.

**1572** METAL TOXICITY IN NEW ORLEANS: SOIL Pb, BLOOD Pb AND ACHIEVEMENT BY 4TH GRADE STUDENTS.

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Rigorous attention to metal toxicity is critical to the global development of healthy and sustainable urban environments. Lead (Pb) is a well known neurotoxin that was extensively used in high volume consumer products. It was dispersed worldwide and accumulated in especially large amounts in built environments. This study examines results of a New Orleans spatially matched database of soil Pb, blood Pb of children under 6 years old, and 4th grade percent achievement rates from the 1999 Louisiana education assessment program (LEAP 21). All data reflects the pre-Hurricane Katrina condition of the city. Blood Pb is strongly associated with soil Pb (r = .85, P < 0.0001). Blood Pb is directly and significantly (P < 0.0001) related to percent unsatisfactory achievement for English language arts (r2 = .58), Math (r2 = .50), Science (r2 = .63), and Social Studies (r2 = .61). Poor people of color predominantly live in the most Pb contaminated neighborhoods of New Orleans. The social burden of Pb is especially large in the inner city where children in some communities have a 20 percent plus prevalence of exposure ≥ the CDC 10 μg/dl guideline for Pb, and within the same communities, school failure rates exceed 50% in all subject matter of the LEAP 21 test. An estimated $US 76 million per year represents the costs of Pb poisoning in New Orleans. This underestimates a host of costs related to the inequalities of Pb poisoning because it excludes the social stresses from excessive violence and the extraordinary price of the justice and prison system. During the recovery of New Orleans from the flood after Hurricane Katrina an opportunity exists to improve the environment for the benefit of children’s health, and promote education and sustainability of the entire community. Acknowledgements: AMHPS/ATSDR Cooperative Agreement 5 U50 TS 473408; B1.1 data: Kathleen Golden & Dianne Dugas, Sect. Env. Epi. & Toxicology; LA Office Public Health, & Ngoc Huynh, LA CLIPP; LEAP data; LA Dept. Education.

**1573** CADMIUM (CD) EXPOSURE IN VIVO RESULTS IN THE UPREGULATION OF THE RATE LIMITING ENZYME IN THE PENTOSE PHOSPHATE PATHWAY, GLUCOSE 6 PHOSPHATE DEHYDROGENASE (G6PDH), AND DIRECTLY INHIBITS NADPH FORMATION IN NRK CELLS.

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Exposure to Cd results in: oxidative stress, disruption of cell adhesion molecules and alterations in cell cycle control. Although some of the generalized cellular responses to Cd-induced injury are well known; other cellular responses are just now being elucidated. In previous studies, we showed that the NADPH-producing and rate limiting enzyme of the pentose phosphate pathway, G6PDH, is up-regulated in a sub-chronic animal model of Cd-induced nephrotoxicity. In that study, male Sprague/Dawley rats were given daily subcutaneous injections of 0.6 mg/kg of Cd in the form of CdCl2, for up to 6 weeks. After 6 weeks of Cd exposure, G6PDH was upregulated in kidney tissue as indicated by western blot analysis, without signs of oxidative stress (TBARS). In the present study, we attempt to determine the etiology of the increase in the expression of G6PDH. We examined the ability of Cd to inhibit G6PDH directly in an in vitro system. Whole cell lysates from Normal Rat Kidney (NRK) cells were placed in a quartz cuvette containing 20 μM CdCl2, then the conversion of NADP+ to NADPH was monitored spectrophotometrically as a direct indicator of G6PDH activity. Cd had no effect on G6PDH activity. However, another NADPH-producing enzyme in the pentose pathway, 6-phosphogluconate dehydrogenase (6PGDH) was inhibited to 10% of control activity in the presence of 20 μM CdCl2. In summary, we found that Cd inhibits production of the reducing agent, NADPH, in vitro. Furthermore, that G6PDH was upregulated at an early time point in a sub-chronic animal model of Cd nephrotoxicity; before signs of overt oxidative stress (TBARS), may indicate that G6PDH is a “sentinel” protein and part of an early cellular defense and/or stress response. Supported by Grant ROI-ES006478 from the NIEHS.

**1574** ARSENIC METABOLISM AND A-477G POLYMORPHISM OF AS3MT GENE.

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Exposure to naturally occurring inorganic arsenic (iAs), primarily from drinking contaminated water, is considered one of the top environmental health threats worldwide. Arsenic (III) methyltransferase (AS3MT) is the key enzyme in the bio-transformation pathway of iAs. AS3MT catalyzes the transfer of a methyl group from S-adenosyl-L-methionine to trivalent arsenicals resulting in the production of methylated (MAs) and dimethylated (DMAs) arsenicals. Recent studies have been focused on the iAs metabolic pathway and AS3MT polymorphisms. In this study, we examined the association of the SNP A-477G in the promoter region AS3MT catalyzes the transfer of a methyl group from S-adenosyl-L-methionine to trivalent arsenicals resulting in the production of methylated (MAs) and dimethylated (DMAs) arsenicals. Recent studies have been focused on the iAs metabolic pathway and AS3MT polymorphisms. In this study, we examined the association of the SNP A-477G in the promoter region AS3MT gene between iAs metabolism and the presence of arsenic (As) premalignant skin lesions. We examined 119 residents from an As endemic area in Mexico in this study. A total of 69 individuals with skin lesions and 50 people without skin lesions were evaluated. We measured the urinary As metabolites of the individuals, differentiating the trivalent and pentavalent arsenical species, using the hydride generation atomic absorption spectrometry method. Additionally, the individuals were genotyped to analyze SNP A-477G in the AS3MT gene using real-time PCR. The arsenic trivalent species are present in greater proportion in urine samples, being DMAIII the predominant metabolite in urine. Population studied showed a frequency of A=0.63 and G=0.37 which were similar to frequency observation in American-Caucasian population, the G allele in the -477 variant had the lowest pro...
portion of mAs and the highest proportion of DAs, which consequently increased the DAs/mAs ratio. The SNAP A-477G was not associated with the risk of As-skin lesions. This study was supported by CONACYT grant 50097.

1575 EFFECT OF DMPS AND DMSA ON THE DISPOSITION OF Hg²⁺ IN MODELS OF REDUCED RENAL MASS.
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Chronic kidney disease affects approximately 50,000,000 adults in the United States. This disease is characterized generally by a progressive loss of nephrons and subsequent compensatory cellular hypertrophy, which occurs primarily in proximal tubules. These compensatory changes may subject remaining nephrons to greater levels of toxins and toxicants. Interestingly, the primary site where inorganic mercury (Hg⁰) accumulates and induces toxic effects is the proximal tubule. The purpose of this study was to examine the potential increase in expression and activity of membrane transporters involved in proximal tubular secretion of Hg⁰. Sham-operated (SHAM) and uninephrectomized (NXP) Wistar and TR (MRP2-deficient) rats were injected with a non-toxic dose of HgCl₂, followed by treatment with saline, 2, 3-dimercaptopropane-1-sulfonic acid (DMPS) or meso-2, 3-dimercaptopropanesulfonic acid (DMSA). The renal burden of Hg⁰ was greater in TR rats than in Wistar rats. The renal burden of Hg⁰ was also greater in NXP rats than in SHAM rats. Treatment with DMPS or DMSA reduced the renal and hepatic content of Hg⁰ in both groups of rats and was accompanied by a significant increase in urinary and fecal excretion of Hg⁰. Interestingly, the change in Hg⁰ content in the samples from TR rats was less than that of Wistar rats. These data indicate that MRP2 is involved in the DMSA- and DMPS-mediated elimination of Hg⁰ from hypertrophied proximal tubular cells.

1576 THIOREDOXIN AND GLUTAREDOXIN TRANSCRIPTIONAL MODULATION BY SPIRULINA IN THE LIVER OF MICE TREATED WITH ASERINE.
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3 and 4Sección de Toxicología, CINVESTAV, Mexico, DF, Mexico.

There is evidence suggesting that inorganic arsenic (iAs) toxicity involves oxidative damage, mainly by the interaction of iAs with protein thiols that are central components of redox-sensitive proteins in redox signaling and control pathways. Several studies have demonstrated that liver is the primary arsenic metabolizing organ and dose-repeated oral exposure of mice to sodium arsenite have shown a dose-related distribution of As and of its methylated metabolites in the liver. Spirulina (SP) a filamentous cyanobacterium used as food supplement increased the levels of the antioxidant glutathione (GSH) as well as the activity of some antioxidant enzymes. Moreover, humans exposed to iAs via drinking water showed health improvement. The levels of mRNA were significantly lower. The putative antioxidant property of SP in iAs exposure GRX mRNA and reduced the levels of GSH. However, in those animals that redetermined using RT-PCR. SP by itself, induced the expression of GCS; TRX and 3Sección de Toxicología, CINVESTAV, Mexico, DF, Mexico.

1577 THE INCREASE IN BLOOD LEAD LEVELS IN FEMALE JAPANESE FARMERS EXPOSED TO CADMIUM.
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The peripheral blood level of lead (Pb), which accumulates preferentially in bone, is influenced by bone metabolism. Another toxic metal, Cd, affects on bone metabolism, through direct actions on bone cells or indirect ones, that is, renal tubular dysfunction. Therefore, we investigated Japanese female farmers who had been orally exposed to various levels of Cd in order to clarify the relationship between Cd exposure, bone metabolism and blood Pb levels. We conducted group health examinations on female farmers more than 40 years old from 4 areas contaminated with Cd at various levels and 2 control areas in Japan, resulting in 1,568 subjects for analyses. We collected peripheral blood and urine samples from them and measured erythrocyte counts and Cd and Pb concentrations in the whole blood, liver, and bone-marrow. We found that the estimated bone-marrow concentration of Cd was correlated with Pb concentration in urine in women, but not in men. Geometric means and ranges of blood Pb, blood Cd and urinary Cd in all subjects were 1.32 (0.25 – 8.50) μg/L, 2.7 (0.11 – 19.8) μg/L and 3.7 (0.30 – 27.3) μg/g cr., respectively, indicating various levels of Cd exposure without high Pb contamination, although there was no Cd nephropathy. The renal and blood Pb levels did not show significant difference among the groups. However, when the subjects were stratified by urinary Cd levels, the blood Pb levels did not show significant difference among the groups. Moreover, when Pb was stratified by blood Cd levels, blood Pb levels were increased blood Cp levels dependently. Multiple regression models for blood Pb using age, LH, blood Cd, erythrocyte counts, and each bone metabolism marker as independent variables always showed much more significant regression coefficients of blood Cd than those of other variables. These results indicate that bone Cd would increase blood Pb level in a manner independent of bone metabolism or renal function, although the mechanism is unknown yet.

1578 MYOCARDIAL CONNEXIN-43 ALTERATION AFTER VANADIUM INHALATION.

Air pollution by suspended particles (SP) has been associated to myocardial ischemic events and arrhythmias. One of the components adhered to these particles is Vanadium (V), produced by the combustion of petroleum derivatives. The structural characteristic of the myocardioocyte is presence of special intercellular communications –intercalated discs –rich in gap junctions which are integrated by connexins (Cx), mainly Cx43. Knowing that these Cx are essential for impulse conduction; we decided to explore the changes in Cx43 presence in an inhalation mice model. Male mice were exposing twice a week for 12 weeks to V2O5 (0.02M). Mice were sacrificed at 2, 6, 12 weeks, heart fixed and processed for standard histological evaluation and immunohistochemistry identification of Cx43. Control and exposed mice were analyzed and densitometry for Cx43 was performed. A 19-day course of succimer treatment initially increased the DMAs/MAs ratio. The SNP A-477G was not associated with the risk of arrhythmias and other heart pathologies.

1579 MERCURY TOXICITY FROM MEXICAN COSMETIC SKIN REJUVENATING CREAM.
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Mercury (Hg) toxicity from nonoccupational sources has been a continuing problem in the US. Mercury toxicity has resulted from inhalation of elemental Hg from broken thermometers and Afro-Caribbean religious practices. Some overseas consumer products may contain Hg, eg, herbal medicines, skin lightening creams and other cosmetics and other heart pathologies.
1580 EVALUATION OF A METHODOLOGY FOR CONTROLLING BERYLLIUM EXPOSURE IN LABORATORY SETTING.

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Beryllium (Be) is used in several forms: pure metal, beryllium oxide, and as an alloy with copper, aluminum, or nickel. Beryllium oxide, beryllium metal and beryllium alloys are forms of Be present in the workplace with inhalation being the primary route of exposure. Cases of workers with sensitization (BeS) or chronic beryllium disease (CBD) challenge the scientific community for a better understanding of Be toxicity. Therefore, a toxicological inhalation study using a murine model was performed in our laboratory in order to identify the toxic effects related to different particle sizes and chemical forms of Be. In this paper we describe the approach developed in order to reduce the potential for exposure, of technicians and students involved in the experiments, which includes specific attention to particle migration control through intensive housekeeping and systematic airborne and surface contamination. Results show that the protective measures applied during this research have been effective. The highest airborne Be concentration in the laboratory was more than ten times lower than the Quebec TLV of 0.15 μg/m3. Considering the protection factor of 105 of a complete head cover used in this research, the average exposure level would be 0.03 x 10-4 μg/m3 which is extremely low. Moreover, with the exception of one value, all average Be concentrations on surfaces were below the guideline level of 3 μg/100 cm2 for Be contamination. Finally, all beryllium lymphocyte proliferation tests (BeLPT) on workers were no higher than unexposed controls. Overall, employing a rigorous protective and preventive approach, will allow for future research to be conducted safely in this important field.

1581 COPPER AND MERCURY INDUCED OXIDATIVE STRESS IN AQUATIC SPECIES.

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Metals are known to influence oxidative stress resulting in patho-physiological conditions in aquatic organisms. Copper (Cu) and mercury (Hg) are the most diffused and hazardous organ specific environmental contaminants that exist in a wide variety of physical and chemical states, each endowed with unique characteristics of target organ specificity. We have studied the antioxidant defense levels, lipid peroxidation (LP), and metal accumulation in the liver and hepatic pancreas (hepatic tissue) of three different aquatic species with different life strategies (fish, Tilapia mossambica; snail, Pila globosa and crab, Oziotrephes senex senex). Animals were collected from local fresh water ponds, acclimated to laboratory conditions for one week before the test animals were exposed to sublethal concentrations of Cu (0.6 ppm for fish, 0.04 ppm for snail and 2.8 ppm for crab) and Hg (0.12 ppm for fish; 0.05 ppm for snail and 0.17 ppm for crab). The alterations in the activities of the antioxidant enzymes glutathione-S-transferase (GST), catalase (CAT), LP levels and tissue accumulation of metals by AAS were assessed after 7 and 15 days of metal exposure. The decrease in the specific activities of GST was greater in the hepatopancreas of crab (-63.99 %) and CAT was greater in the liver of fish (-65.97 %). The decrease in the specific activities of GST was greater in the hepatopancreas of crab (-63.99 %) and CAT was greater in the liver of fish (-65.97 %). Among the three species, fish liver exhibited greater accumulation of both Cu and Hg (23.4 μg/g and 8.3 μg/g) than snail (6.3 μg/g and 8.3 μg/g) and crab (4.6 μg/g and 3.5 μg/g). However, the accumulation was significantly decreased during depuration period of 15 days. The data indicate that Cu and Hg induced species-specific adaptive responses in these aquatic animals suggesting that the tissue antioxidants may further serve as surrogate markers of exposure to oxidant pollutants.

1582 A WEIGHT-OF-EVIDENCE EVALUATION OF THE CARCINOGENICITY OF SOLUBLE NICKEL.

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It has been known for decades that inhalation of sulfidic nickel ore refinery dust containing a mixture of soluble and insoluble nickel compounds can increase the risk of lung and nasal cancers in humans. Although a number of studies indicate less clear, however, is the risk of lung and nasal cancers in humans. What is less clear, however, is which species are involved in aquatic organisms. Copper (Cu) and mercury (Hg) are the most diffused and hazardous organ specific environmental contaminants that exist in a wide variety of physical and chemical states, each endowed with unique characteristics of target organ specificity. We have studied the antioxidant defense levels, lipid peroxidation (LP), and metal accumulation in the liver and hepatic pancreas (hepatic tissue) of three different aquatic species with different life strategies (fish, Tilapia mossambica; snail, Pila globosa and crab, Oziotrephes senex senex). Animals were collected from local fresh water ponds, acclimated to laboratory conditions for one week before the test animals were exposed to sublethal concentrations of Cu (0.6 ppm for fish, 0.04 ppm for snail and 2.8 ppm for crab) and Hg (0.12 ppm for fish; 0.05 ppm for snail and 0.17 ppm for crab). The alterations in the activities of the antioxidant enzymes glutathione-S-transferase (GST), catalase (CAT), LP levels and tissue accumulation of metals by AAS were assessed after 7 and 15 days of metal exposure. The decrease in the specific activities of GST was greater in the hepatopancreas of crab (-63.99 %) and CAT was greater in the liver of fish (-65.97 %). Among the three species, fish liver exhibited greater accumulation of both Cu and Hg (23.4 μg/g and 8.3 μg/g). However, the accumulation was significantly decreased during depuration period of 15 days. The data indicate that Cu and Hg induced species-specific adaptive responses in these aquatic animals suggesting that the tissue antioxidants may further serve as surrogate markers of exposure to oxidant pollutants.

1583 ACUTE TOXICITY OF THIN-FILM CADMIUM TELLURIDE PHOTOVOLTAICS.

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In the context of the growing need for energy and the decline in oil and gas reserves, renewable energies, and photovoltaics (PV) in particular, have experienced significant growth. Cadmium-telluride (CdTe) thin-film PV has become one of the leading technologies in the solar market, but concerns have been raised about the usage of cadmium, known for its toxicity. In the absence of specific toxicological data related to CdTe, regulatory agencies usually apply Cd criteria. However, it may be that CdTe has toxicological properties that are different from those of Cd, and it would therefore be useful to determine such data. The goal of this study is to determine if CdTe is a mutagen. In order to establish its acute toxicity in comparison to that of Cd, using an animal model (rats), the method followed essentially that described in the OECD guidelines and in the US Environmental Protection Agency Health Effects Test Guidelines. The 4-hr LC50 of CdTe in rats was established at 2.71 mg/L, showing a very low variability between genders while the LD50 was determined to be greater than 2000 mg/kg. These results clearly show that CdTe is less toxic than Cd. Thus, applying Cd criteria to CdTe by the regulatory agencies does not seem appropriate and further research on CdTe’s other toxicological properties appear to be warranted.

1584 RELEASE OF HEAVY METALS FROM HUMAN BLOOD AND URINE BY CYSTUS-SUD (CISTUS INCANUS SIPPANDALIS).

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Heavy metals in cigarette tobacco may cause serious damage to human health by smoking. Surveys have shown that the body burden of certain metals like cadmium or mercury is the consequence of active smoking habits. In a clinical trial 15 volunteers smoking about 15 cigarettes/day were included; this collective was randomly divided in two groups, blood and urine levels of cadmium, mercury and iron were measured under the influence of Cystus-Sud in a cross-over design. Results: the enhanced levels of cadmium were reduced by 60% in blood and 45% in urine; with mercury the results were more indifferent between both groups. It was concluded that Cystus-Sud intake does influence the iron-status in blood or urine. Our main finding was that Cystus-Sud significantly reduces the cadmium burden in smoking people, this confirms our pilot study in 8 volunteers in 1999.

1585 ARSENIC IN MEXICO: AN UP TO DATE OVERVIEW.

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An extensive part of the Mexican territory has an inorganic arsenic (iAs) rich soil substrata, in addition anthropogenic activities such as mining and smelting process contribute to increase iAs levels in groundwater and soils leading to significant exposure to this metalloid. In Mexico, iAs exposure is mainly through drinking water coming from contaminated sources and affecting to 2 millions people. Therefore, iAs intoxication is a prioritary problem of public health. The aim of this work is to determine the carcinogenic mode-of-action data. Certain epidemiological data, but not all, suggest that soluble nickel exposure leads to increased respiratory cancer risk, but only with co-exposures to high levels of insoluble sulfidic or oxidic nickel. Soluble nickel alone was not carcinogenic in the majority of animal studies, including robust inhalation and oral studies (NTP 1996; Heit et al. 2007). There is limited (although inconsistent) animal evidence that soluble nickel can act as a promoter, although it is unclear whether this mode of action is relevant to humans. Some positive in vitro genotoxicity results have been reported, but these results are not likely to be applicable to humans because they don’t take into account processes that prevent high bioavailability of the nickel ion (the ultimate carcinogen) in the nucleus in vivo. Other in vitro studies have supported plausible non-genotoxic modes of action (cytotoxicity and induction of signal transduction pathways) for soluble nickel acting as a promoter. We considered whether the toxicological and mode-of-action data support the potential for soluble nickel to be either a complete carcinogen or a tumor promoter. We concluded that the weight of evidence does not clearly support a role for soluble nickel alone in carcinogenesis, and there is only limited evidence that it could act as a promoter.
provide an up to date overview of the iAs problem in Mexico. To achieve this objective, we conducted an exhaustive literature mining focused in geological data and its relevance to human health. Arsenic concentrations exceeding the drinking water Mexican norm (0.025 mg/L) have been reported in 17 states, and up to now there are 5 towns with arsenicism reports. iAs chronic exposure to iAs have shown skin lesions, peripheral vascular diseases, hepatotoxicity, immunotoxicity, diabetes mellitus and genotoxicity. In addition, children living close to smelting areas showed neurological disorders. Therefore biomarkers associated with health effects by chronic exposure to iAs have been actively investigated. For example, some biomarkers like frequencies and types of chromosomal aberrations in lymphocytes, urinary COPRO/UCO and COPROIII/COPRO ratios, TGF-alpha level in bladder urothelial cells, as well as micronuclei in buccal and urothelial cells have been investigated. In summary, due to the abundant presence of iAs in soil and water in Mexico, and its reported effects on human health, it is necessary to carry out systematic evaluations of the drinking water sources to identify all the high iAs areas and to develop surveillance program to detect early human effects.

**1586** COMPARATIVE TOXICITY AND TISSUE DISTRIBUTION AFTER REPEATED ADMINISTRATION ORALLY WITH VARIOUS TYPES OF ARSENIC INTO THE MONKEY.

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Arsenic (As) is an ubiquitous element in several forms in food and environment. The main route of As in general population is an oral exposure. However, the toxicity of As varies much by chemical forms. In this study, we compared the toxicity and tissue distribution of the various types of As after repeated administration orally into the animal. The cytosolusus monkey was used in this study. The dosage of repeated administration orally into the monkey was determined based on the previous single administration study. We prepared arsenics, such as sodium arsenite (low: 1.0 mg Au/kg/day, high: 5.0 mg/kg/day), arsenocholine (low: 1.5 mg/kg/day, high: 7.5 mg/kg/day), dimethyl arsenic (DMAs, 0.05 ppm) and arsenic (As), which may play a role in the development of chronic lung disease. It appears that clearance of SS WF is impaired compared to MS WF, which may explain the delayed and persistent inflammatory response. These observations could be related to the presence of insoluble carcinogenic metals in SS WF, such as Cr or Ni, which could play a role in the development of chronic lung disease.

**1587** EFFECTS OF ARSENITE IN DRINKING WATER ON MOUSE LUNG IN A 30 DAY EXPOSURE.

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Humans in Taiwan, Chile and Argentina exposed to inorganic arsenic in their drinking water have demonstrated elevated risks of lung cancer deaths. Since, the carcinogenic mode of action of arsenicals is unknown, we investigated the effects of inorganic arsenic (iAsIII) in female C3H mice, exposed to 0.05, 0.25, 1, 10 and 85 ppm arsenite in drinking water for 30 days. As traditional rodent diets contain arsenic, both inorganic and seafood derived arsicals, we reduced food arsenical exposure by using a purified diet (AIN-93M). Arsenite-treated mice drank less water at all doses (significantly lower at 85, 10 and 1 ppm (66, 49 and 42 % respectively). Only mice in the 85 ppm group weighed considerably lower (12 %) than the control group. Mouse lungs were analyzed for inorganic arsenic, monomethylated and dimethylated arsensicals by hydride generation atomic absorption spectroscopy. The total lung mean arsenic levels were 2.4, 22.5, 30.1, 50.9, 105.3 and 316.4 mg/L lung tissue after 0.05, 0.25, 1, 10 and 85 ppm, respectively. At 85 ppm, the total mean lung arsenic levels increased 14-fold and 131-fold when compared to either the lowest non-toxic control dose, respectively. Gene expression analysis was conducted using Affymetrix Mouse Genome 430A 2.0 GeneChips®. Differentially expressed genes (DEGs) were determined using a one-way ANOVA (p<0.05) by Rosetta Resolver®, a Benjamini-Hochberg FDR multiple testing correction (p<0.05) and a 1.5 fold change cut-off. Surprisingly, we found that arsenic exposure caused minimal numbers of DEGs (57, 9, 3, 22 and 17 DEGs after 0.05, 0.25, 1, 10 and 85 ppm respectively). Thus, from 0.05 to 85 ppm arsenite, we observed monotonic increases in liver arsenic concentrations but no clear dose-related increases in DEGs. In summary, due to the abundant presence of iAs in soil and water in Mexico, and its reported effects on human health, it is necessary to carry out systematic evaluations of the drinking water sources to identify all the high iAs areas and to develop surveillance program to detect early human effects.

**1588** COMPARISON OF THE PERSISTENCE OF DEPOSITED PARTICLES AND THE INFLAMMATORY POTENTIAL OF STAINLESS STEEL VERSUS MILD STEEL WELDING FUME IN RAT LUNGS AFTER INHALATION.


Epidemiological studies have been unable to correlate chronic adverse lung effects associated with exposure to specific welding fume generated from different processes. The objective of this study was to use an animal model to compare the persistence of deposited particles and the inflammatory potential after inhalation of stainless steel welding fume (SS WF) or mild steel welding fume (MS WF), the two most common fumes used in U.S. industry. Male Sprague-Dawley rats were exposed to 40 mg/m³ of SS WF or MS WF for 3 hr/day for 3 days. Controls were exposed to filtered air. Generated fume was collected in the breathing zone of the animals, and particle size, morphology, and composition were determined. Bronchoalveolar lavage was done on days 1, 4, 8, 11, 22, and 43 after the last exposure to assess lung injury/inflammation and to recover lung phagocytes. SS WF and MS WF were similar in particle morphology and size and average aerodynamic diameters of 0.26 and 0.31 μm, respectively. Chemical composition of the fumes was different - SS WF: 57 % Fe, 20 % Cz, 14 % Mn, 9 % Ni; MS WF: 81 % Fe, 15 % Mn. There was no effect of MS WF on lung injury/inflammation at any time point compared to control air. Lung injury was elevated through 11 days after exposure to SS WF; whereas inflammation was delayed and not significantly increased until day 11 compared to control. SS WF also was associated with greater recovery of welding fume-laden cells from the lungs at all time points compared to the MS WF group. Few cells contained MS WF particles at 22 days, whereas 25 % of cells recovered from the SS WF group still contained particles at 43 days. Thus, it appears that clearance of SS WF is impaired compared to MS WF, which may explain the delayed and persistent inflammatory response. These observations could be related to the presence of insoluble carcinogenic metals in SS WF, such as Cr or Ni, which may play a role in the development of chronic lung disease.

**1589** WELL WATER HIGH IN TUNGSTEN, ARSENIC AND POLONIUM 210 FROM CHURCHILL COUNTY, NEVADA INDUCES OXIDATIVE STRESS IN MICE.

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Between 1997 and 2001, 15 children were diagnosed with acute lymphocytic leukemia (ALL), in Churchill County, Nevada. A CDC investigation of the cancer cluster demonstrated elevated levels of arsenic and tungsten in the urine of study participants and tap water samples. Extremely high levels of alpha radioactivity in numerous domestic wells used for drinking water in the Fallon area were also found which could not be accounted for by naturally occurring uranium activity in the area and were subsequently determined to come from polonium-210 contamination. This study was designed to determine if consumption of drinking water from one or more sources high in one or more of these chemicals from Churchill county Nevada induces oxidative stress in mice. C57BL/6 female mice were provided drinking water for up to 10 weeks from selected sources in Churchill County. Several sources of water samples were selected to provide representative ranges of naturally occurring levels of arsenic, tungsten and polonium-210. An in-depth chemical analysis of these water samples was conducted to confirm the concentration and diversity of the contaminants in these tests. The treated mice were assessed for changes in biomarkers of oxidative stress including superoxide dismutase (SOD), glutathione peroxidase (GPOX), total antioxidant capacity (TOAX), lipid peroxidation (LPO) and total DNA damage. Mice provided water high in tungsten but lower levels of polonium 210 and arsenic showed some increases in oxidative stress but not as significant as those high in all three. High tungsten levels are a common feature in the water samples which induce oxidative stress in this mouse model. This study was supported in part by a grant from the U.S. EPA, EM-9696320.
1590 EVALUATION OF THE INHIBITORY EFFECT OF THIMEROSAL TOWARDS THE YEAST PLASMA MEMBRANE H+ATPASE.


Thimerosal (sodium ethylmercurithiosalicylate) is a potent antimicrobial agent extensively used as a preservative in pharmaceutical preparations such as immunogenic, ophthalmic and nasal products. The mechanism of the antimicrobial action of this organomercuric compound has been ascribed to its strong affinity for sulhydryl groups in cysteine residues of key microbial enzymes. The yeast plasma membrane H+ATPase, a vital fungal protein, represents an attractive target for thimerosal by virtue of the nine cysteine residues within its structure. In the present study, the inhibitory action of thimerosal on Pma1p was examined using three types of experiments and compared to that of its structural analog thiosalicylic acid (TSA). In one set of experiments, the antifungal activity of these two compounds was tested using strains of both S. cerevisiae and C. albicans. While thimerosal was a very potent inhibitor of fungal growth (S. cerevisiae IC50 ~0.149 μM, C. albicans IC50 ~0.249 μM, IC100 ~0.826 μM), TSA was devoid of antifungal activity in concentrations up to 10 μM. Time-kill experiments in C. albicans revealed that thimerosal produced its fungicidal activity by 48 h of treatment. In a second set of experiments, thimerosal, but not TSA, was found to inhibit medium acidification by growing yeast. Moreover, thimerosal, but not TSA, reduced the ATPase activity of a sample of Pma1p isolated from S. cerevisiae. Taken together, the present data verifies that thimerosal, but not TSA, can reduce the activity of Pma1p because of its ethylmercurithiosalicylic component. However, since thimerosal is cytotoxic at concentrations ≥1 μM and an inhibitor of Pma1p at concentrations ≥10 μM, its antifungal activity may not be solely dependent on its propensity to inhibit proton pumping function.

1591 REFERENCE VALUES OF 28 ELEMENTS (HEAVY METAL AND OLEIGOELEMENTS) IN HAIR IN A CHILDREN POPULATION IN SPAIN FOR THE USE AS BIOMARKER OF ENVIRONMENTAL EXPOSURE.


The heavy metals in the human body cause damages to the health and the oligoelements are indispensable in small amounts for the life. Non invasive biomarkers are needed for monitoring and for that appropriate reference values are needed to be established. The study has been made in the municipality of Elche, province of Alicante Spain using children local population in the range of 3 to 12 years old that may considered a standard Spanish population from urban, rural, industrial areas. Twenty eight elements (heavy metal and oligoelements) have been measured in hair samples using the ICP-MS technology and to establish the curves of normal distribution for each element of interest in the study population. This is an observational, descriptive cross sectional study. Sampling: 430 samples by simple random method. Methodology for sample treatment and measuring with ICP-MS were improved and validated. The distribution curves are asymmetric. Values of reference for the heavy metal concentrations of the potentially toxic elements were established as the value in μg of the element by gram of dry hair smaller than the 75 percentiles. For the oligoelements the reference value were established as the values between the percentiles 25 and 75. Acknowledgment: Work supported by regional government (D. G. Public health Generalitat Valenciana).

1592 MYOSIN LIGHT CHAIN-1: EXTENDING THE DIAGNOSTIC WINDOW FOR DRUG-INDUCED CARDIAC AND SKELETAL MUSCLE TOXICITY IN VETERINARY SPECIES.


Myosin light-chain-1 (Myl3) has been used as a biomarker of cardiac injury in humans to evaluate the severity and prognosis of patients with acute myocardial infarction and congestive heart failure. Current biomarkers of muscle injury in veterinary species appear quickly in blood and correlate well with injury. However, their rapid elimination from blood decreases their diagnostic value. Myl3 has the added advantage of a wide diagnostic time window, remaining elevated in the blood for hours or days longer than many other markers. We evaluated serumological Myl3 as a biomarker of my injury in veterinary species by studying tissue distribution in rats, serum concentrations after drug-induced muscle injury in rats, dogs and monkeys, and serum pharmacokinetics following IV infusion in monkeys. Myl3 gene expression and protein concentration are most abundant in heart and type-I skeletal muscle. Treatment with cardiac and type I SMK toxins (isoproterenol & PPAR) induced higher and/or more sustained serum concentrations of Myl3 than other biomarkers of muscle toxicity. PK of Myl3 in monkeys revealed rapid elimination of Myl3 from blood, suggesting prolonged treatment-induced increases in serum Myl3 are due to persistent release from injured tissue, not slow elimination from blood. Earlier publications found that a cytosolic pool of "unassembled" myosin light chain is released quickly from injured tissue while ischemia-induced acidosis and proteolytic degradation are possible mechanisms for subsequent dissociation of additional Myl3 from the myofibril enabling persistent release from injured myocytes. These data indicate that Myl3 is a sensitive and predictive biomarker of cardiac and skeletal muscle injury in veterinary species and adds value to a myopathy biomarker panel by extending the diagnostic window.

1593 CHARACTERIZATION OF GLUTATHIONE CONJUGATES OF 1-BROMOPENTANE AND ITS HEPATOTOXICITY.

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Glutathione (GSH) conjugation and hepatotoxicity induced by 1-bromopentane (1-BPT) were investigated in female BALB/c mice. 1-Bromopentyl GSH, 1-bromopentyl cysteine, and mono-hydroxypentyl mercapturic acid were identified in liver isolated from the mice treated orally with 1-BPT by liquid chromatography-electrospray ionization tandem mass spectrometry. Oral treatment of mice with 1-BPT at 1500 mg/kg produced maximum GSH conjugates at 6 h after treatment. For hepatotoxicity tests, the animals were treated orally with 1-BPT at 375, 750, or 1500 mg/kg in corn oil once for a dose response study or at 1500 mg/kg for 6, 12, 24, or 48 h for a time course study. 1-BPT dose-dependently increased serum activity of ALT and AST and decreased hepatic GSH levels, peaking at 6 and 12 h after treatment. 1-BPT (750 and 1500 mg/kg) also significantly increased the hepatic content of malondialdehyde. Thus, 1-BPT could cause hepatotoxicity and depletion of GSH content by forming GSH conjugates, presenting a toxicity mechanism and potential biomarkers for low molecular weight haloalkanes. (Supported by a grant from NITR, Korea).

1594 NOVEL TOXICOLOGY DATABASE ALLOWING FOR CROSS-COMPANY, MULTI-STUDY DATA MINING AND ANALYSIS FOR THE QUALIFICATION OF PREDICTIVE BIOMARKERS.

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The integration of genomics data with traditional toxicology data remains a work in progress despite the positive strides taken by several members of industry. The needs of the Predictive Safety Testing Consortium (PSTC), revolving around the qualification of predictive biomarkers, demand that traditional toxicology data including histopathology be brought together in one database and associated with biomarker data. The PSTC Rosetta Resolver database (PRD) is not only a storehouse of biomarker and toxicity information but also a centralized cross-company resource to which multiple teams can extract data for mining and analysis exercises. The end result is a robust tool with the potential to decrease the time and effort spent qualifying biomarkers and thus accelerating investigative compounds through the early phases of development. As a proof of concept we examined the responsiveness of serum creatine (SCr) to tubular damage as measured by kidney histopathology. Data exported from the PRD represented two companies and 25 studies containing approximately 1600 samples. For tubular damage, samples with a histopathology severity score of one (five point scale) did not correspond to any samples with a SCr level exceeding the ULN. Samples with a score of two (minimum to moderate damage) correlated to only a small number samples with a SCr level above the ULN (2.5%). Samples with a score of three (moderate damage) correlated with the most number of samples with a SCr level above the ULN, but even still, the number of corresponding samples was a modest one (30%). This data confirms the common knowledge that SCr is not responsive to mild kidney damage and underscores the importance of PSTC successful efforts to qualify more sensitive biomarkers of kidney injury. Further samples of data analysis for novel biomarkers of kidney, liver, and vascular injury will be discussed.

1595 DEVELOPMENT AND VALIDATION OF A MULTI-ANALYTE PROFILE (MAP) OF PUTATIVE BIOMARKERS OF HUMAN KIDNEY DAMAGE.


Introduction – In June 2008, the FDA and EMES issued a joint framework allowing submission of a single application for 7 new urinary biomarkers of drug-induced kidney damage in rats. Based on our previous experience in developing both
NGAL EXPRESSION IN KIDNEY OF SPONTANEOUS HYPERTENSIVE RATS (SHR) TREATED WITH DOXORUBICIN ALONE OR WITH DEXRAZOXANE: A COMPARISON WITH KIM-1 EXPRESSION.


Neutrophil gelatinase-associated lipocalin (NGAL) has shown promise as a novel next-generation nephrotoxicity biomarker. The present study was undertaken to examine changes in NGAL expression following acute drug-induced renal injury. Four groups of male SHR (5/group) were treated once with 10 mg/kg doxorubicin (Dox) (iv), 50 mg/kg Dex (ip) given 30 min before Dox, 50 mg/kg dexrazoxane (Dex) (ip) or saline (ip, iv). Rats were euthanized and necropsied 14 days after treatment. Dox-induced acute kidney injury (AKI) which was characterized by glomerular vacuolization, proximal and distal tubular epithelial cell degeneration (cytoplasmic vacuolization and accumulation of hyaline droplet formation in the cytoplasm) and apoptosis identified by TUNEL assay in the proximal tubular epithelium and glomerular cells. Renal damage was attenuated in SHR pretreated with Dex. Immunoperoxidase staining with anti-rat NGAL mAb (BioPorto Diagnostics) and mouse anti-rat kidney injury molecule-1 (Kim-1) ectodomain mAb (Harvard Medical School) showed that (1) NGAL expression was elevated in the proximal tubular cells of Dox-treated SHR compared with that observed in kidneys of saline- or Dex-treated SHR; (2) pretreatment with Dex attenuated increases in renal NGAL expression compared to the intensity of expression observed in SHR given Dox alone; (3) the intensity of NGAL expression correlated with the severity of renal lesion scores; (4) the immunolocalization of renal NGAL in Dox-treated SHR was similar to that observed for Kim-1 as determined in the present and in previous studies; and (5) the immunostaining intensity of NGAL in the kidney of Dox-treated rats was comparable to that of Kim-1. These findings suggest that NGAL can be used as a new biomarker to identify drug-induced AKI in the rat.
detected in parallel. Grating-Coupled Fluorescent Plasmonics (GCFP) may enable us to overcome these limitations. This microarray-based approach builds upon the established Grating-Coupled Surface Plasmon Resonance Imaging (GCSRPI) platform, but incorporates enhanced fluorescent detection. By coupling fluorescent emission from antibody-conjugated fluorophores to surface plasmons generated at the biosensor chip surface, fluorescent detection can be increased by 14-fold. This greatly expands the range of concentrations that can be detected for many analytes including toxicants, cytokines, enzymes, and other biomarkers. By utilizing a microarray format to yield a biosensor chip, we can simultaneously quantify hundreds to thousands of biomarkers characterizing biomolecular sensitivity to generate unique signatures associated with disease states and toxicant exposure. For example, we can detect TNF-α, IL-3, and IL-10 to a limit of less than 28 fm. Our preliminary experiments with GCFP have included quantifying cytokine secretion from differently activated immune cells to generate unique biosignatures of activation. The GCFP technology permits cells to be captured using either antibodies or MHC monomers, separated by phenotype, and then characterized by secretion factors, measures of enzyme specific activity, and other small molecules whose presence or change in concentration might be indicative of a disease state. The ability to define complex biological signatures may lead to earlier diagnosis and more effective treatments of disease and toxicant exposure. Supported by NIH grants ES07408, DK77291, and ES16014.

1601 A NOVEL TOXICOPROTEOMIC/TOXICOGENOMIC METHOD FOR THE IDENTIFICATION OF MOLECULAR BIOMARKERS.

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Chronic low-level lead toxicity takes a silent toll on those it effects, driving cognitive and behavioral alterations that are not apparent for years after exposure. Here genomic and proteomic approaches are used in parallel to identify gene expression and functional protein interaction responses to low-level lead intoxication. The result is a set of candidate molecular biomarkers of chronic low-level lead intoxication. Initial proteomic screens utilized a modified two-hybrid proteomic system to identify a number of potential in vivo protein-protein interactions disrupted by the neurotoxin lead (Pb2+). The proteomic screen consisted of quantifying cytosolic protein SNAP-25h and identified proteins within the SNARE complex including Syntaxin1A and Vamp-2. The neuronal SNARE proteins are the minimal membrane fusion machinery required for vesicle fusion and calcium dependent neurotransmitter release. This method also identified proteins beyond the SNARE complex, including HGS, involved in vesicle transport and sorting pathways and EEF1A2 a protein family that contains a mutant form associated with neuronal wasting in mice. In the second phase of this project the proteomic set of molecular markers was compared with microarray data, global gene expression patterns observed in offspring of time-pregnant Long Evans rats subject to chronic low-level lead exposure vs. control groups. Both genomic and proteomic methods described here are subject to artifacts, but when the data from each method is overlaid, elements present in both data sets identify a subset of molecular biomarkers. To date we know of no reliable biomarkers for low-level lead toxicity in man, nor is there a satisfactory model for how chronic low-level lead toxicity effects the neuronal systems of mammals. This work provides a glimpse at these mechanisms, identifies a candidate model for how chronic low-level lead toxicity effects the neuronal systems of mammals. This work provides a glimpse at these mechanisms, identifies a candidate model for how chronic low-level lead toxicity effects the neuronal systems of mammals.

1602 TRANSCRIPTOMIC CHANGES INDUCED BY LIPOPOLYSACCHARIDE TREATMENT IN RAT WATERY BLOOD: OPTIMIZATION OF A NOVEL COLLECTION PROTOCOL.

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Blood is an easily accessible tissue that can be used to identify biomarkers for a wide range of tissue injuries using transcriptomic profiling. Using blood for transcriptional profiling presents significant technical challenges, particularly because of the presence of large quantities of globin mRNA transcripts that prevent acceptable signal collection. PAXgene™ blood RNA collection tubes have been the traditional method for blood collection. However, they are not ideal for rat toxicity studies because they require large amounts of blood (2.5 mL). We have developed a reproducible method of RNA stabilization and isolation from small quantities of whole blood to be used for transcriptional profiling. To confirm the validity of this technique, male Sprague-Dawley rats (n=3) were treated with vehicle or 5 mg/kg of lipopolysaccharide (LPS, IV) for 2h, 6h and 24h. Two blood collection methods were used: collection of blood in PAXgene™ tubes, or collection of 500μl of blood in 2ml of QiaNorm B. Both techniques yielded abundant, high quality RNA. Total RNA (50 ng) was processed using the NuGent Ovation™ RNA Amplification System, and cDNA was hybridized to Affymetrix RG 230 2.0 microarrays. Similar genes and pathways were disregulated in both techniques following LPS treatment. In particular, genes associated with LPS-induced inflammatory response, LPS and IL-1 mediated inhibition of RXR function and IL-10 signaling were differentially expressed. In conclusion, this novel and cost-effective blood preparation technique yields accurate results, requires less input than the traditional method, and can also be used in preclinical toxicology and pharmacology studies to identify novel biomarkers.

1603 PERFORMANCE OF NOVEL KIDNEY BIOMARKERS IN PRECLINICAL TOXICITY STUDIES.

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The kidney is one of the main targets of drug-induced toxicity, but early detection of renal damage is often difficult. As part of the InnoMed PredTox project, a collaborative effort by 15 pharmaceutical companies, 2 small- and mid-sized enterprises and 5 universities aimed at assessing the value of combining -omics technologies with conventional toxicology methods for improved safety assessment, we evaluated the performance of a panel of novel kidney biomarkers in preclinical toxicity studies. Rats (Han Wistar) were treated with either the reference nephrotoxin gentamicin or one of several proprietary compounds that were dropped from drug development in part due to renal toxicity. Animals were dosed at two dose levels for 1, 3 and 14 days. Putative kidney markers, including Kim-1, lipocalin-2 and clusterin, were analyzed in kidney, serum and urine using qRT-PCR, ELISA and immunohistochemistry. Changes in gene/protein expression generally correlated well with renal histopathological alterations and were frequently detected at earlier time points or at lower doses than traditional clinical parameters. Overexpression of Kim-1 was often one of the earliest responses and might be seen as the most sensitive tissue marker of kidney injury. Urinary Kim-1 and clusterin reflected changes in gene/protein expression and histopathological alterations in the target organ, confirming clusterin and Kim-1 as early and sensitive, non-invasive marker of renal injury. In contrast, urinary lipocalin-2 was found to be less sensitive and not specific for kidney toxicity. The InnoMed Project is supported by partial funding by the 6th Research Framework Program of the European Union (LSHB-CT-2005-518170).

1604 MECHANISM-BASED BIOMARKER GENE SETS FOR GLUTATHIONE-DEPLETION RELATED HEPATOTOXICITY IN RAT LIVER.

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Two types of toxicological mechanisms are reported regarding Chemical-induced glutathione (GSH) depletion, i.e., GSH-conjugation with chemicals such as phorone (PHO) or diethyl maleate (DEM), or inhibition of GSH synthesis by chemicals such as L-buthionine-sulfoximine (BSO). To identify mechanism-based biomarker gene sets for GSH depletion related hepatotoxicity in rat liver, male SD rats were treated with various chemicals including PHO, DEM or BSO, Bromobenzene (BBZ), etc. Liver samples were taken 3, 6, 9, and 24 hr after administration and examined for hepatic GSH content, physiological and pathologic changes, and for gene expression changes by Affymetrix GeneChip. Differentially expressed probe sets responsive to GSH-depletion were extracted according to the following two courses relating to GSH-depletion: gene expression changes associated with large amounts of blood (2.5 mL). We have developed a reproducible method of RNA stabilization and isolation from small quantities of whole blood to be used for transcriptional profiling. To confirm the validity of this technique, male Sprague-Dawley rats (n=3) were treated with vehicle or 5 mg/kg of lipopolysaccharide (LPS, IV) for 2h, 6h and 24h. Two blood collection methods were used: collection of blood in PAXgene™ tubes, or collection of 500μl of blood in 2ml of QiaNorm B. Both techniques yielded abundant, high quality RNA. Total RNA (50 ng) was processed using the NuGent Ovation™ RNA Amplification System, and cDNA was hybridized to Affymetrix RG 230 2.0 microarrays. Similar genes and pathways were disregulated in both techniques following LPS treatment. In particular, genes associated with LPS-induced inflammatory response, LPS and IL-1 mediated inhibition of RXR function and IL-10 signaling were differentially expressed. In conclusion, this novel and cost-effective blood preparation technique yields accurate results, requires less input than the traditional method, and can also be used in preclinical toxicology and pharmacology studies to identify novel biomarkers.
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The purpose of this study was to investigate and establish methods for the identification of proliferating sinusoidal endothelial cells in mouse spleen by flow cytometry. B6C3F1 mouse spleenocytes were isolated and stained with CD31 (PECAM1), CD45, Flk1 (VEGFR-2), CD105 (Endoglin) and CD71 (transferrin receptor) antibodies to characterize the spleen cell populations in normal animals. Extracellular or intracellular Flk1 staining was not observed. The CD105 and CD71 antibodies labeled populations separate from the EC population. Results from these studies demonstrate the spleen cell endothelial cell population to be CD31+CD146+CD45+. To develop appropriate methods to identify proliferating endothelial cells in B6C3F1 mice, proliferation markers were compared in studies designed to induce EC proliferation. Using 2-butoxyethanol (2-BA) as a positive control compound, male B6C3F1 mice were dosed by oral gavage once daily for 7 consecutive days with either 900 mg/kg 2-butoxyethanol (2-BA) or vehicle. Mice were implanted with osmotic pumps containing 5-ethynyl-2′-deoxyuridine (EdU). Proliferating CD45+/EdU and CD31+/CD45+/EdU+ cells were quantitatively measured and absolute counts were calculated by using BD TruCOUNT Tubes. In this study, we observed a 1.2 fold increase of the proliferating LSEC population and a 0.5 fold reduction of the leukocyte (CD45+) population after 2-BA treatment. These results suggest that 2-BA treatment (900mg/kg) may have a proliferating effect on LSEC and a detrimental effect on other cell types in the liver.
measured outside their optimum diagnostic window. In addition, they do not allow the differentiation of type I versus type II muscle fiber damage. Thus, there is a need for new predictive and sensitive markers of myotoxicity, which identify damage early in its progression and allow discrimination of adverse events in type I and type II fibers. In this study, phenotypic anchoring of the expression data from individual animal histopathology or clinical chemistry parameters was applied in order to identify novel genomics biomarkers for skeletal muscle toxicity in rats. In a 14-day rat study, the potent PPAR delta agonist GW501516 was used as a model compound to induce lesions in type I (M solennis) and type II (M quadreadcis) skeletal muscles. Transcripts that correlate with the microscopic findings observed in the respective muscle were identified and used to characterize adverse effects in type I or type II muscles. Among the genes with high correlation with histopathology and significant regulation in M quadreadcis were Scarb1 (scavenger receptor). Antidi (antidiastatic domain 1) and Tpm3 (troponymin 3). Relevant transcripts in M solennis included for example the scavenger receptor Scarb1. Gpmnb (osteovactin), Srfrp4 (secreted frizzled-related protein 4) and Sptip1 (osteopontin) showed high correlation and drug target in both muscles. Several transcripts are suggested as potential genomics biomarkers for myotoxicity. The identified set of transcripts could further provide insights into the potential mechanism of PPAR-induced skeletal muscle damage.

1610 DEVELOPMENT AND VALIDATION OF A GENE EXPRESSION SIGNATURE FOR SKELETAL MUSCLE INJURY IN RAT.


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Skeletal muscle fiber injury is a sporadic but recurrent event that has been seen both preclinically and clinically (e.g., statin-induced rhabdomyolysis) during drug development. Early identification of molecules that may induce muscle injury during preclinical safety studies in the rat will be advantageous. However, established serum biomarkers of skeletal muscle injury in the rat (e.g., creatine kinase, alkaline phosphatase) become elevated only in response to injury, limiting their value as predictive tools. Recently, biomarkers based on gene expression profiles have been shown to have utility as sensitive diagnostic and, in some cases, predictive markers for both pharmacologic and toxicologic endpoints. Consequently, we sought to develop a gene expression signature to detect generalized skeletal muscle injury in short term rat toxicology studies. Seven proprietary compounds directed across several drug target classes were administered by oral gavage to Sprague-Dawley rats at doses that produced microscopic injury at or before day 14. RNA was extracted from muscle tissue at days 1 and 5 and changes in gene expression were determined using Affymetrix RG3 230 2.0 arrays. A sparse linear programming machine learning approach was used to derive a signature comprised of 27 genes that demonstrated excellent performance metrics in cross validation. The signature achieved a sensitivity of 99% and a specificity of 95%. Independent validation testing with 8 compounds demonstrated that the signature accurately diagnosed muscle injury for each of the 6 compounds that caused injury. Furthermore, 4 day tissue samples were sufficient to predict the extent of muscle toxicity resulting in evident histologic changes after 14 days, suggesting the signature has predictive potential. The signature performed well across different compound target classes, in different rat strains, and for injury occurring in different muscle fiber types.

1611 NON-INVASIVE BIOMARKER DEVELOPMENT FOR AIRWAY DISEASE AND EXPOSURE.

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Exhaled breath condensate (EBC) is a biological fluid that contains trace amounts of secreted pulmonary proteins, and is emerging as a potentially valuable and non-invasively obtained source of disease or exposure biomarkers. We developed a protocol for proteome analysis of these samples, which leads to the selection of a biomarker pattern for airway disease or exposure. In short, proteins in 1 ml EBC (about 1 mg/ml) are concentrated on POROS R2 beads and digested with trypsin. Resulting peptides are separated and analyzed by nano-LC/MSMS. Protein patterns are selected by performing Support Vector Machine analysis (Weka software). In a first pilot study, samples from 6 smokers and 6 non-smokers were analyzed and compared. Proteins such as calgranulin A, calgranulin B, hemoglobin, lysozyme and cytokeratins 1, 2, 9 and 10 were identified. Calgranulin B was upregulated in samples from smokers. To investigate the predictiveness of the protein profile towards clinical endpoints, the protocol was also applied on EBC samples from another study including 40 children with asthma, and 30 healthy children (age 6-12 years). A pattern based on 16 peptides was able to differentiate correctly between asthmatic and healthy individuals. Two of these peptides were identified as a part of cytokeratin 1 and 9. The other peptides are still under investigation. The developed procedure led to the identification of new proteins in EBC. Furthermore, it makes it possible to select biomarker patterns for disease or exposure. This study was partly supported by the Belgian Science Policy (Contract number SD/HE/05A/ ANIMO project).

1612 PRELIMINARY FINDINGS OF THE LIVER TOXICITY BIOMARKERS STUDY: ENTACAPONE AND TOLCAPONE.


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Drug-induced Liver Injury (DILI) represents a challenge for new therapeutics despite extensive preclinical efforts to identify hepatotoxic drugs prior to introduction into the clinic. The Liver Toxicity Biomarker Study (LTBS) was conceived to better predict drug candidates likely to produce idiosyncratic DILI. LTBS is a pharmacologically similar drug pair; with one drug being associated with DILI in humans though not predicted by preclinical studies. A broad spectrum of molecular profiling measures in liver, plasma, and serum clinical chemistry provides the foundation for the comparisons. The first drug pair comparison—entacapone (clean) and tolcapone (DILI)—is reported. Ten study cohorts containing 12 male Sprague-Dawley rats per cohort were dosed with vehicle or drug for 3 or 28 days. 3-Day dosing was limited to the high dose while 28-Day dosing included low, medium and high (E 30, 110, 400, 15, 35, 200 mg/kg) doses. Urine was collected predosing through day 4 and on day 27 for the 28 day study. Plasma and liver samples were collected at sacrifice. 1H NMR metabolomic analysis was performed from urine samples. Plasma samples analysis platforms included proteomics (discovery iTRAQ and targeted) and metabolomics (lipid LC/MS, derivatized polar LC/MS and targeted amino acid analysis). Liver sample analysis included transcriptomics, discovery proteomics, and the same metabolic platforms as plasma. The results show high analytical quality data from 240 primary liver and plasma samples, greater than 800 urine samples, with CVs generally less than 20%. Over 2500 significant changes were found. These include differences in drug metabolizing enzymes and transcripts as well as off target effects synchronized between transcriptomics, proteomics, and metabolomic findings.

1613 NATRIURETIC PEPTIDES AS BIOMARKERS OF CARDIAC HYPERTROPHY IN RATS.

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Serological biomarkers of cardiac hypertrophy would be beneficial for drug development, but are not currently available for use in veterinary species or humans. Natriuretic peptides are important in the regulation of plasma volume and associated phenomena that can lead to cardiac hypertrophy. We therefore evaluated the utility of NTproANP and NTproBNP as biomarkers of cardiac hypertrophy caused by PPAR δ/γ dual agonist LS190292 in Sprague-Dawley rats. Heart-to-brain weight ratios were increased 51%, 50%, or 78% after 28 days of treatment with 0.5, 5, or 50 mg/kg LS190292. Transcripts for Nppa (natriuretic peptide precursor A), Nppb (natriuretic peptide precursor B) and Myh7 (myosin heavy chain 7β) were measured in left atria and ventricles by TaqMan. In left atria, Nppa, Nppb, and Myh7 were induced 1.6, 1.7, and 2.0 fold at 0.5 mg/kg, 1.6, 2.0, and 3.0 fold at 5 mg/kg, and 1.6, 1.8, and 5.4 fold at 50 mg/kg, respectively. In left ventricles, transcription of Myh7 was not increased; Nppa and Nppb were induced 4.2, and 4.0 fold at 0.5 mg/kg, 17.0, and 4.4 fold at 5 mg/kg, and 35.0, and 8.4 fold at 50 mg/kg, respectively. Increases in Nppa and Nppb transcripts in left ventricles were correlated with absolute heart weight as well (r=0.73). NTproBNP was measured in serum by mass spectrometry and was 0.18 ± 0.03, 0.30 ± 0.03, 0.27 ± 0.04, and 0.22 ± 0.03 mg/mL, and was not correlated with absolute heart weight (r=0.24). Transcription of Nppa was well-correlated with NTproBNP in plasma (r=0.40). After 1, 3, and 7 days of treatment with 50 mg/kg LS190292, NTproBNP in serum was increased 2-5 fold from control. These results suggested that NTproANP may be a predictive biomarker of cardiac hypertrophy induced by PPAR agonists in rats.
1614 EXPLORATION OF NEW LIVER-SPECIFIC MRNAS DETECTABLE IN PERIPHERAL BLOOD FROM RATS WITH DRUG-INDUCED LIVER DAMAGE FOR HEPATOTOXICITY BIOMARKERS.

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If organ/tissue-specific mRNAs leaking from drug-damaged organs/tissues could be detected in peripheral blood, such mRNAs are expected to be toxicological biomarkers for identification of the target organs for drug toxicity. Recently, we reported that α1-microglobulin/bikunin precursor (Ambp) and albumin mRNAs, which are specifically expressed in the liver, were detectable in peripheral blood from rats with chemical-induced hepatotoxicity by a reverse-transcription polymerase chain reaction (RT-PCR) (Miyamoto et al, Tox Sci, in press). In order to identify the target organs for drug toxicity, evaluation with several marker mRNAs would be valuable to enable the acquisition of more reliable results and to avoid false-positive findings. Therefore, we explored new liver-specific mRNAs other than Ambp and albumin mRNAs. Peripheral blood was collected from rats receiving D-galactosamine HCl and total RNA was extracted. Based on several reference databases for gene expression analysis, we selected 31 candidate genes which were considered to be expressed exclusively and at high levels in normal liver. Of their transcripts, 9 mRNAs were detected by RT-PCR in peripheral blood from the rats with hepatotoxicity but not from normal rats. Their expression levels were examined for a total of 9 major organs/tissues from rats and finally Apoh (apoprotein H), Sko1β2 (solute carrier organic anion transporter family 1b2), Pgl (plasminogen) and GC (group component specific) mRNAs were judged to be liver-specific.

In conclusion, 4 new liver-specific mRNAs were found in the present study. A total of 6 liver-specific mRNAs, including the previously explored Ambp and albumin mRNAs, are expected to allow more accurate detection of hepatotoxicity when they are used in combination.

1615 PORCINE-TO-MURINE TESTICULAR XENOGRAFTS AS NOVEL BIOMARKERS FOR PREPUBERTAL EXPOSURE TO ENDODUCRINOPHOTOXICIS.


There is increasing concern about the potential adverse effects of endocrine disruptors (EDs) on human fertility. Relatively little is known about the effects of EDs on prepubertal reproductive development in non-rodent mammals. Swine share many anatomic and physiologic similarities with humans and are likely to provide an accurate model for human risk assessment involving exposures to EDs. However, there are financial and logistical concerns associated with the use of porcine models in comparative research. The recent development of testicular tissue xenografting techniques using immunodeficient murine recipients addresses some of those concerns. Using the antidiabetic function of vindoline (VCZ) as a model ED, it was hypothesized that oral exposure to VCZ would adversely affect the development of spermatogenically and steroidogenically competent porcine-to-murine testicular xenografts. Two, 8-week-old boars were orally exposed to either 0 or 100 mg VCZ/ kg body weight for 5 weeks. Portions of the testes from each animal were xenografted into castrated and vasectomized immunodeficient mice (n=6 for each group). Sections of the xenografts were evaluated at 10 or 21 weeks post-surgery. Porcine-to-murine testicular xenografts originating from the control boar were larger at both 10 and 21 weeks post-implantation and appeared to have more “functional” seminiferous tubules at 21 weeks, as compared to xenografts of tissue collected from the VCZ-treated animal. In addition, both testosterone and immunosuppressible testosterone were detected in serum samples from recipient mice xenografted with testicular tissue from the control animal, but the serum from mice with xenografted tissue from the VCZ-treated boar contained essentially undetectable concentrations of immunosuppressible estrogens.

The results of this preliminary study demonstrate that altered development of porcine-to-murine testicular xenografts can be used as a novel biomarker for the effects of prepubertal exposures to EDs on porcine spermatogenesis and steroidogenesis.

1616 EARLY METABOLIC BIOMARKERS OF DRUG-INDUCED NEPHROTOXICITY.


Nephrotoxicity is still one of the most frequent reasons for late-stage drug failures but there are no biomarkers monitoring the early pathophysiological events involved in glomerular and tubular dysfunction. In this longitudinal study, a targeted metabolomics approach quantifying several hundred endogenous metabolites in plasma and urine has been applied to study dose- and time-dependence of biochemical alterations associated with puromycin-induced nephropathy in Sprague-Dawley rats. Metabolites such as amino acids, biogenic amines, polyamines, glycophosphop- hospho sphingolipids, reducing sugars, acylarnitines, polyunsaturated fatty acids, eicosanoids, etc. were analyzed by multiple reaction monitoring on tandem mass spectrometers, and concentrations were determined by stable isotope dilution. The quantitative data were then statistically analyzed and mapped onto biochemical pathways for a mechanistic understanding of the observed deviations from healthy homeostasis. By systemically assessing the intra-individual time-courses and the significant differences between the controls and three dose-groups (10, 20, and 40 mg/kg/day) we could identify a set of biomarker candidates for crucial events in nephropathy such as tissue damage, mitochondrial leakage, phospholipase activation, lipoxygenase and cyclooxygenase activation (i.e. the orchestration of several specific metabolic classes, oxidative stress, regulation of immune cell activity, and various other accompanying phenomena). Most interestingly, some of the markers were able to indicate significant biochemical alterations even in the low-dose group which was previously characterized as showing no histopathological changes at all. This clearly demonstrates that targeted metabolomics is ideally suited as a sensitive tool for early detection of nephrotoxicity, and also grants mechanistic insight into the underlying biochemical pathways.

1617 DETECTION OF THE MITOCHONDRIAL AND CATALYTICALLY ACTIVE ALANINE AMINOTRANSFERASE (ALT2) IN HUMAN TISSUES AND PLASMA.

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Serum alanine aminotransferase (ALT) is used as a clinical marker of hepatotoxicity. Two forms of ALT have been identified in the genome of most species studied, ALT1 and ALT2. A shorter form of ALT2 also exists due to alternative splicing (ALT2_2). The standard ALT activity assay does not discriminate between ALT1 and ALT2 from different organs, or which isoform is being measured in plasma. Here, we show that ALT1 and ALT2 possess similar enzymatic activity for alanine and pyruvate but with different Km and kcat values, while recombinant ALT2_2 protein does not possess any enzymatic activity. We developed an assay that is specifically designed to detect catalytic form of ALT2 to distinguish it from ALT2_2. Isolation of organelles from cultured human skeletal muscle cells, showed localisation of ALT2 to the mitochondrial fraction and endoplasmic reticulum (ER), but not to the cytosol. In human hepatocytes, on the other hand ALT1 was only localized to the cytosol and ER, with no detection in mitochondria. ALT2 was not detected in cultured human hepatocytes, liver extract or liver tissue using western blotting or immunohistochemistry. Skeletal muscle, the islet of Langerhans and cardiomyocytes were other examples of cells with high expression of catalytic ALT2. A clinical method for selective measurement of ALT1 and ALT2 in human plasma is described, and both ALT1 and ALT2 were immunoprecipitated from human plasma and structurally detected using western blotting techniques.

1618 IDENTIFICATION OF GLUTATHIONE-DNA ADDUCTS INDUCED BY FORMALDEHYDE.

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Formaldehyde (HCHO) is a known human and animal carcinogen. Previous studies have demonstrated that inhaled HCHO causes nasal cancers in rats and humans. However, determining whether or not distant site effects of HCHO occur remains a critical issue for understanding HCHO toxicity, carcinogenicity and epidemiology. Limited epidemiological data suggest that leukemia may be associated with HCHO exposure, however, there are no experimental data which demonstrate that HCHO can reach distant sites in an active form. This has been attributed to the rapid metabolism of HCHO by glutathione (GSH)-dependent enzyme systems. Among the intermediates in the pathway of HCHO detoxification, S-hydrazinhydrilglutathione is a reactive species that has the potential to further conjugate with DNA bases. To test this hypothesis, a physiologic concentration of GSH (5mM) was incubated with HCHO, followed by the addition of deoxyguanosine or DNA. The coupling product eluted at 11.87 min on a C18 column and gave an UV absorbance maximum at 260nm. The exact mass of the protonated adduct was 587.1896 Da, consistent with the expected elemental composition C10H9N2O2S. The HCHO-derived methylene linkage between the methylene carbon of the Cys residue and exocyclic N of dsG was established by NMR spectroscopy. The amount of adduct rose with increasing HCHO concentration from 0.1mM to 5mM in vitro. At higher HCHO concentrations, adduct formation was limited by available GSH as a result of the conjugation of S-hydrazinhydrilglutathione with a second HCHO molecule. Demonstration of the formation of HCHO-induced 3-[1-(5′-deoxyguanosinyl)-methyl]GSH adduct between GSH and DNA is unique
because of the involvement of S-hydroxymethylglycine, a key player during the detoxification of HCHO. This adduct may serve as a promising biomarker to understand HCHO toxicity and carcinogenesis at contact and distant sites if coupled with the application of isotope-labeled HCHO to differentiate the endogenous and exogenous HCHO.

1619 INTERPRETING TRIHALOMETHANE BIOMONITORING DATA IN A PUBLIC HEALTH RISK CONTEXT USING BIOMONITORING EQUIVALENTS.

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Biomonitoring Equivalents (BE) provide a useful tool for interpreting biomonitoring data in a public health risk context. Interpretation of biomonitoring data for compounds with short half-lives in humans, however, poses challenges for the BEs construct. This paper presents the derivation of BE values for trihalomethane compounds (THMs) and recommendations for evaluation of biomonitoring data for short-lived compounds using BEs for THMs. THMs commonly form as byproducts during drinking water disinfection, leading to episodic exposures to THMs from drinking, showering or bathing in disinfected water. THMs have short half-lives in humans. Variations in population biomonitoring data for THMs will reflect the variabilities associated with: 1) the concentration of THMs in water; 2) the extent of exposures; 3) the rate of metabolism and elimination of THMs in individuals; and 4) the timing between exposures and collection of a blood sample. However, the BE values for THMs correspond to the steady-state concentrations of THMs in blood consistent with the current USEPA RfDs for these compounds. This disconnect influences the interpretation of exceedances of the BE in population-based biomonitoring data sets. The BE values were derived using currently available physiologically based pharmacokinetic models for THM compounds and analysis of the underlying toxicity data used as the basis for the RfD values. BE values corresponding to current USEPA RfDs for chloroform, dichloroacetic acid, and dibromochloromethane, dibromochloromethane, and tribromomethane are 230, 80, and 130 pg/ml in blood, respectively. Interpretation of CDC biomonitoring data for these compounds using the BE values is discussed.

1620 MEASURING 1, 4-DIOXANE IN BLOOD AS A BIOMARKER OF EXPOSURE.


1,4-Dioxane is a relatively polar ether that is a rodent carcinogen and has potential toxicity to the central nervous system, liver and kidneys. 1,4-dioxane has many industrial uses as an aprotic solvent and can also form in consumer products as a byproduct of reactions. Thus, it is of interest to understand 1,4-dioxane toxicity and carcinogenesis at contact and distant sites if coupled with the application of isotope-labeled 1,4-dioxane to differentiate the exogenous and endogenous 1,4-dioxane.

1621 USING BLOOD MEASUREMENTS TO ASSESS EXPOSURE TO DIOXIN/FURANS: POTENTIAL INFLUENCE OF ELEVATED DETECTION LIMITS.

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Exposure to polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) is frequently estimated by measuring congener levels in blood. Total toxic equivalent (TEQ) concentrations are often used to characterize blood levels of the PCDD/F congeners. In this study, blood PCDD/F levels of 11 residents (ages 37–79 years) of a community situated in close proximity to a wood treatment plant were compared to the 2003-2004 National Health and Nutrition Examination Survey (NHANES) serum concentration data for participants 57 years and older (n=661). Average limits of detection (LODs) were 5-27 times higher for the community cohort than the corresponding LODs for the NHANES data. Additionally, on average, only ~3 of 17 congeners were detected for the community cohort, with the percentage of congeners not detected (ND) in the blood of these 11 individuals ranging from 65 to 94%, whereas an average of 8 congeners were detected for NHANES participants. When congeners reported as ND were assumed to have a concentration equal to the LOD/2, higher average total TEQ concentrations (stratified by age) were observed in the blood of the community cohort relative to the NHANES population (~5 to 6 times higher). However, when total TEQ levels were calculated using a value of zero for non-detected congeners, the average total TEQ in blood was, generally, lower in the community cohort than the NHANES population. Therefore, the observed difference in total blood TEQ may simply be the result of 1) the higher percentage of congeners not detected in the blood of the community cohort and 2) the much higher analytical LODs for these 11 individuals compared to the NHANES data. In conclusion, when comparing blood PCDD/F levels of a population to NHANES, it is important to carefully analyze the congener detection frequencies and LODs before drawing conclusions, and in some cases, a comparison of congener concentrations for those congeners with a high detection frequency may be more appropriate than calculating total TEQs.

1622 INFLUENCE OF THE UNIT OF EXPRESSION OF BIOMONITORING DATA ON THE ASSESSMENT OF PYRETHROID EXPOSURE.

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Human exposure to non-persistent pesticides such as pyrethroids is often based on urinary biomarker measurements. These are usually expressed in volume-weighted or creatinine-adjusted concentrations measured in spot urine samples. This research aimed at studying the effect of the unit of expression of biomonitoring results (volume-weighted or creatinine adjusted concentrations or 24-hour amounts) on the assessment of pyrethroid absorbed doses at individual and population levels. Using population data from previous studies, intra-individual (void-to-void) and inter-individual variations in the urinary flow rate and creatinine excretion rate were assessed. Individual daily absorbed doses of permethrin were then reconstructed from volume-weighted or creatinine-adjusted concentrations of urinary biomarkers, according to published approaches, and compared to a benchmark dose estimate obtained from amounts of biomarkers in timed collections. The effect of the units of measurements on results of comparisons of biomarker levels between two populations was also assessed. There were large void-to-void variations in the urinary flow rate and creatinine excretion rate (%CV of up to 101% and 48%, respectively) as well as large between-individual variability (%CV of up to 48% and 45%, respectively). Estimation of individual absorbed doses of permethrin from volume-weighted or creatinine-adjusted concentrations of biomarkers was thus found to potentially lead to substantial under or overestimation when compared to doses reconstructed directly from amounts excreted in urine during a given period of time (~3 to 5 times higher). It was also found that the variability in creatinine excretion rate and urinary flow rate may introduce a bias that limits the validity of between population comparisons. The unit chosen to express biomonitoring data may thus influence the validity of estimated individual absorbed dose as well as the outcome of between population comparisons.
strated good accuracy and precision. Recovery of the three compounds averaged between 97 to 105% of theory with precision [measured as percent relative standard deviation (%RSD)] as high as 8.4% for 4-ketoCP. Linearity of response was verified for concentrations of 0.5 to 25 ng/ml CP and IF in urine, and from 10 to 625 ng/ml 4-ketoCP in urine. Correlation coefficients of 0.99 or greater were obtained for all standard curves. This method offers a valid test for the determination of the urinary levels of CP, 4-ketoCP and IF as demonstrated by the accuracy and precision of the recovery studies. Disclaimers: Mention of company names and/or products does not constitute endorsement by the National Institute for Occupational Safety and Health (NIOSH). The findings and conclusions in this abstract have not been formally disseminated by NIOSH and should not be construed to represent any agency determination or policy.

1624 URINARY PROTEIN/PEPTIDE ADDUCTS AS MARKERS OF REACTIVE NAPHTHALENE METABOLITE FORMATION IN MALE MICE.

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Naphthalene (NA), a ubiquitous polyaromatic hydrocarbon in the environment, has shown dose dependent cytotoxicity in the murine respiratory epithelium and in the rat and murine nasal epithelium. The relevance of these animal studies to human health is not clear due to several factors, including species differences in NA susceptibility and a high background of lung diseases in the human population. Previous work has shown that metabolism of NA in target tissues of rodents to reactive metabolites leads to their covalent binding to potentially critical proteins, which may result in cytotoxicity. Although the formation of covalent adducts with hemoglobin and albumin has been used as surrogates for the generation of reactive metabolites, their measurement has not led to a clearly established relationship between adduct levels and toxicity. The urine is a relatively unexplored site for the measurement of protein adducts generated from the interaction of reactive metabolites with proteins. The current work focuses on methodology development for the separation, detection, and identification of urinary protein/peptide adducts as markers of key processes associated with NA toxicity. Both radiolabeled and label-free methods will be applied, including the development of an antibody specific for the naphthalene-cysteine moiety of adducts. Preliminary data showed that 24-hour urine collected from male mice following ip administration of 14C-NA revealed specific activities of covalent adducts exceeding those observed in critical tissues, like the lung and nose. In addition, we have tentatively identified a few urinary protein adducts similar to ones previously identified in the lung and nasal epithelium following naphthalene exposure. Resolution of urinary proteins by high performance liquid chromatography and 2D electrophoresis and their subsequent identification by mass spectrometry is ongoing. We conclude that adducted proteins eliminated in the urine may be excellent markers of processes critical to target tissue toxicity. Supported by NIEHS 04311 and 04699.

1625 INCREASED MALONDIALDEHYDE LEVELS IN EXHALED BREATH CONDENSATE OF RETIRED COAL MINERS WITH DECREASED LUNG FUNCTION.


Inhaled coal mine dusts can cause inflammation and fibrosis in the lung called coal worker’s pneumoconiosis. Chronic inflammatory process in the lung is associated with reactive oxygen species (ROS) formation. Disturbance of the balance between ROS and antioxidant defenses produces oxidative stress, which leads to tissue damage. The aim of the present study was to compare malondialdehyde in exhaled breath condensate (EBC-MDA) as a biomarker of lipid peroxidation resulted from pulmonary oxidative stress in retired coal miners. In this study, we measured EBC-MDA, pulmonary function test, radiological findings, and urinary cotinine, and analyzed the difference among indices. The study population contained 69 retired coal miners. EBC-MDA level of retired workers with decreased lung function (%FVC<80, %FEV1<80, FEV1/FVC<70) was higher than those of subjects with normal lung function (8.5±3.9 nmol/L vs. 6.5±3.3 nmol/L, p<0.05). EBC-MDA level (7.5±3.1 nmol/L) was increased in patients with medication for pneumonia as compared with subjects with medication for pneumonia (4.0±2.8) (p<0.05). There were no significant correlation between EBC-MDA levels and the progression of radiological findings, age, BMI, urinary cotinine, and work duration. In this study, there was significant difference between the EBC-MDA levels and decreased lung function. It was supposed that increased EMD levels were resulted from pulmonary oxidative stress, but, it was necessary to evaluate reliable variables for the effects of oxidative stress in the lung such as aldehydes, hydrogen peroxide, leukotriene B4, and 8-iso-prostanate in EBC, lung tissue or bronchoalveolar lavage fluid.

1626 A MULTI-ANALYTE PROFILE OF SERUM PROTEINS TO SCREEN FOR TOXICOLOGICAL EFFECTS OF ANTICHLINESTERASE INSECTICIDES IN THE RAT.


The development of high throughput biochemical screens could be useful to assess the broad spectrum of physiological effects of environmental toxicants. To explore the prospect of using a screen in an in vivo exposure scenario, we applied a commercially available multipanlyte profile (MAP) of 58 serum biomarkers to rats exposed acutely to two anticholinesterase insecticides, chlorpyrifos (CHP) and carbaryl (CAR). Male, Long-Evans rats were dosed orally to 30 mg/kg CHP, 75 mg/kg CAR or the corn oil vehicle. Doses were selected based on their equivalent physiologically effects (hypothermia and reduced motor activity). The animals were terminated 24 hours or 7 days after dosing. Serum was collected and analyzed for 58 biomarkers consisting primarily of cytokines, chemokines, and a few hormones. There were changes in six analytes (4 up, 2 down) following CHP and eight analytes (5 up, 3 down) following CAR at 72 hours. There were significant changes in only two biomarkers when measured 7 days after dosing with CHP. Overall, the MAP detected a broad spectrum of unique effects for CHP and CAR. It is concluded that the MAP is a useful tool of screening two environmental toxicants and its use could lead to the discovery of new mechanisms of action. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

1627 MICRORNA EXPRESSION CAN BE SIGNIFICANTLY ALTERED ON DAY 1 AFTER CARCINOGEN TREATMENT.

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Dysregulated expression of microRNA (miRNA) has been extensively detected in human cancer and has shown promise in defining tumor status. It, however, is little known whether expression of miRNAs can be changed in non-cancerous tissues after carcinogen exposure. To explore whether changes of miRNA expression can be utilized as a short-term biomarker for carcinogen exposure, we treated five mice per group with one dose of 120 mg/kg model genotoxic carcinogen N-ethyl-N-nitrosourea (ENU) and vehicle control. The miRNAs were isolated from the liver of ENU-treated and control mice one day after the treatment. The miRNA expression profiles were determined using RT2-mouse miRNA PCR Array. There are significantly different between miRNA expression profiles of control and treated samples. Among the 376 mouse miRNAs, 18 miRNAs were down-regulated and 10 miRNAs were down-regulated (p < 0.05). Primary analysis of the altered miRNAs demonstrates that most of them are involved in cell growth, apoptosis and other functions related to carcinogenesis. These results suggest that miRNAs might have the potential to be exploited as a biomarker for early prediction of chemical carcinogenicity.

1628 A REVIEW OF THE METHODS FOR LIPID CONTENT IN HUMAN SERUM AND THE IMPACT ON SERUM LEVELS OF PERIODIC ORGANIC POLLUTANTS.


Because many persistent organic pollutants (POPs), such as polychlorinated dibenzo-p-dioxins and furans (PCDD/Fs), are adsorbed to fat, serum concentrations are often reported on a lipid basis instead of a whole weight or volume basis. Thus, the method used to estimate the lipid content in a particular serum sample is important because the accuracy of the lipid analysis method can impact the accuracy of the associated serum concentrations. The two methods for determining serum lipid are the gravimetric and the enzymatic methods. We compared these methods to understand the differences in lipid content for split samples using two data sets. Data collected in 2006 and 2008 as part of the Arctic Monitoring and Assessment Program (AMAP) interlaboratory comparison for certain POPs were used along with lipid content data from 10 people from Mills et al. (2007). For these data, the absolute relative difference between the actual lipid content of the sample and the measured lipid content was significantly lower for the samples analyzed using the enzymatic method versus those analyzed using the gravimetric method.
method (p<0.002), indicating that the enzymatic method produced more accurate lipid content estimates than the gravimetric method. Also, the relative differences for the enzymatic data were significantly less than zero while the relative differences for the gravimetric data were significantly greater than zero, which indicates that the enzymatic method consistently overestimates lipid content while the gravimetric consistently underestimates lipid content. For the Mills et al. (2007) data, the gravimetric lipid contents were, on average, 55.5%, and ranged from 39.2% to 64.4% of the enzymatic lipid contents. Applying this range to the 2001/2002 data results, the enzymatic method would be 2.5% more accurate than the gravimetric method would be 2.5% less accurate for serum lipids concentrations that are a factor of 1.55 to 2.55 times those for the enzymatic method. Due to these differences, it is critical that the method used for lipid content be consistent between any potentially exposed population and background population.

1629 EVALUATION OF BIOMARKERS OF EXPOSURE IN CLINICAL SAFETY ASSESSMENTS.


Biomarkers of exposure are an important consideration when designing clinical trials intended to assess product safety or efficacy. Without proper biomarkers of exposure, interpreting study findings and drawing conclusions about safety is difficult because the proportion of the test compound that reaches systemic circulation and/or target tissues is unknown. When selecting biomarkers of exposure, it is essential to consider several factors such as route of administration, for food additives and ingredients, the matrix in which the compound is delivered. Additionally, biomarkers should address not only the absorption of the test compound from the GI tract into the circulation, but also distribution to target tissues, although this may be difficult to achieve in humans. Levels of known metabolites of the test compound should also be measured to avoid underestimating absorption and distribution. Finally, the biomarker must be measured at an appropriate time point, when sufficient time has elapsed after administration to allow for steady-state levels to be achieved. As a case study, clinical trials conducted with phytosterols that included measurements of safety endpoints were examined to determine the prevalence of appropriate biomarker selection in clinical research in this area of study. Phytosterols have recently been reviewed by various regulatory bodies (e.g., FDA, EFSA, and JECFA) with the goal of deriving safe and efficacious levels of intake. Appropriate biomarker selection in clinical research in this area of study is critical for future mechanistic and toxicity studies in the bone marrow of mice.

Optimization of a Protocol to Isolate Genomic Material from Buccal Cells.


Buccal cells have been used as sources for genomic DNA in a variety of settings and are an attractive potential source of RNA since they can be collected in noninvasive manners that cause little to no discomfort to subjects. Recent studies have shown reproducible and quantifiable gene expression signatures in exfoliated buccal cells from human subjects. In the present study, we optimize a protocol for extraction of RNA using samples from a pooled set of buccal cell samples consisting of 770,000 cells per sample to isolate high yield (~1 mg/sample) and high quality (260/280 and 260/230 ratios ~2.0) RNA for gene expression analysis. We compared three different protocols: an RLT mini protocol using Qiagen's RLT buffer in combination with proteinase K incubation, a Fibrous Tissue protocol using the same buffer but with a different incubation and centrifugation parameters, and a Trizol method with column cleanup. We also employed mouse fibroblast cells (500,000 per sample) for comparison. The highest yields of RNA (mean, standard deviation) from buccal cells were obtained using the Fibrous Tissue protocol (1.5 μg ± 0.1), followed by the RLT (0.2 μg ± 0.03) and Trizol (0.1 μg ± 0.01) protocols. The fibroblast cells provided a similar trend in the results between the Fibrous Tissue (28 μg ± 3), RLT (20 μg ± 1.6), and Trizol (16 μg ± 2.5) protocols. For buccal cells, no differences were seen in the 260/280 values, but the 260/230 value were greater for the Fibrous Tissue method compared to the RLT and Trizol methods: 1.1 (0.5), 0.3 (0.1), and 0.4 (0.3), respectively. No significant differences in the absorbance ratios were observed in the fibroblast samples, which were near the optimum value of 2.0. The total amount of RNA and quality isolated using this protocol will enable us to further explore the gene expression differences in field samples obtained from farmworkers and non-farmworkers as part of a longitudinal study during pesticide spray and non-spray seasons. This project was supported by NIHES grant 2P01 ES090901 and EPA grants RD-8370901-0 and RD-83273501-0.

1630 QUANTIFICATION OF BUTADIENE-MONOEPoxide AND DIEPOxIDE N-TERMINAL VALINE ADDUCTS IN RODENTS AT LOW EXPOSURES.

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Butadiene (BD) is an industrial chemical used in the production of synthetic rubber and found in gasoline and combustion products. It is a multisite and multispecies carcinogen in rodents with mice being much more sensitive than rats. BD is metabolized mainly by P450 2E1 to 1,2-epoxy-3-butenyl (EB), 1,2,3,4-diepoxypentane (DEB) and 1,2-epoxy-3,4-butadienyl (EB-diol). For risk assessment, the in vivo dose of each epoxide can inform their relative risks because they show significantly different mutagenicity. The internal formation of EB can be measured indirectly by analysis of the 2-hydroxy-3-butenyl-valine (HB-Val) adduct. In this study, the formation of the HB-Val was evaluated in globin samples (40-50 mg) from female mice and rats exposed to 1, 1.5, 6.25, and 62.5 ppm BD by inhalation for 10 days. An immunofluorescence in-situ UPLC-MS/MS assay was developed for HB-Val analysis. In addition, the cyclic adduct N-N(-2,3-dihydroxy-1,4-butyldiol)valine (pyr-Val), a biomarker for DEB, was simultaneously determined. Utilizing this assay increased the sensitivity and allowed detection of both adducts in mice and rats exposed to BD as low as 1 ppm. The amounts of HB-Val in mice were about 12-fold higher than rats at the same exposure. In both species, the amount of pyr-Val was about 1.5-fold higher than HB-Val. These data show that mice are much more efficient at forming EB and DEB than rats, particularly at low exposures. The data are in accord with carcinogenic effects of BD in mice and rats. Overall, the analyses of adducts derived from 1,3-butaedene metabolites provided valuable insight for cross species comparison at low exposures which will be readily comparable with similar data from molecular epidemiology studies on 1,3-butaedene-exposed workers.

1631 THE ASSESSMENT OF ENDOTHELIAL CELL PROLIFERATION BY FLOW CYTOMETRY WITH PROLIFERATION MARKERS BRDU AND EDU IN MOUSE BONE MARROW AFTER TREATMENT WITH 2-BUTOXYETHANOL.

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The purpose of this study was to identify proliferating endothelial cells (EC) and monocytes in mouse bone marrow by flow cytometry with incorporation of the thymidine analog BrdU and EdU. Bone marrow cell suspensions were evaluated by staining with inclusion markers CD45, CD31, Flk1 and CD105 and exclusion markers CD71, B220, and CD115. To compare proliferation markers BrdU and EdU male B6C3F1 mice were dosed by oral gavage once daily for 7 consecutive days with either 900 mg/kg 2-butoxyethanol (2-BE) or vehicle. Mice were implanted with osmotic pumps containing either 20 mg/mL 5-bromo-2'-deoxyuridine (BrdU) or 5-ethyl-2'-deoxyuridine (EdU). In proliferation experiments, the bone marrow cell suspensions were stained with antibody panels containing the CD45, CD31, Flk1, EdU and BrdU markers. Results indicated the CD31+CD45+ and Flk1+CD45+ phenotypes identified EC's in bone marrow suspensions. In each study, total nucleated cell counts from vehicle control and 2-BE treated animals were comparable. Although absolute cell counts of CD31+CD45+ and Flk1+CD45+ increased approximately 1.5 fold with 2-BE treatment, the total number of proliferating EC's recovered after processing for BrdU was 4 fold less than for EdU. These results also indicated that the harsh treatment required for detection of proliferating EC's with BrdU might result in significant sample cell loss. Our results suggest that EdU is the preferred proliferation marker for identification of proliferating EC's in bone marrow by flow cytometry. Overall, these results provide important and useful information for future mechanistic and toxicity studies in the bone marrow of mice.

1632 COMPARATIVE MICROARRAY STUDY OF GENE EXPRESSION IN CULTURED RAT LUNG CELLS FOLLOWING JET FUELS EXPOSURE.

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Each year over 2 million military and civilian personnel are occupationally exposed to jet fuels including jet propulsion fuel-8 (JP-8) and its civil aviation equivalent Jet A. Jet fuels are among the most common sources of military and nonmilitary occupa-
pational chemical exposures. Standard commercial and military jet fuels consist of kerosene fractions of petroleum with additives. A new synthetic jet fuel, designated synthesized paraffinic kerosene (SPK), is being phased into military use. To assess the effects of exposure to jet fuels and to compare the synthetic SPK to the petroleum based standard fuels, we analyzed gene expression patterns of cultured 1.2 rat pulmonary epithelial cells following exposure in vitro to equitoxic concentrations of Jet A, JP 8, JP 4 and SPK. Total RNA was extracted and mRNA was processed for analysis and placed on the Affymetrix rat genome 230-2 microarray. The main biological function affected by these fuels were similar, and related to gene expression, growth and proliferation, cell cycle, cell development and cell death. Two (2) hr functional changes were related primarily to cell death and lipid metabolism. At 4 hr lipid metabolism, cellular development and small molecule biochemistry were the most changed functions. One signaling pathway related to vitamin D metabolism (VDR/RXR) was significantly changed at both 2 and 4 hr post-exposure. The results suggest that these fuels have significant functional effects in these cells.

1634 GENE-ENVIRONMENT INTERACTIONS: EPIGENETIC PATHWAYS IN CHRONIC DISEASE PROMOTION AND PROGRESSION.
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The study of gene-environment interactions has become increasingly more common as it relates to disease susceptibility and chronic disease development. These studies aid in the characterization of environmental exposures and development of targeted prevention/treatment regimens. Traditional genetic endpoints can be expanded to include epigenetic modifications related to altered DNA methylation patterns, histone modifications, and germ-line reprogramming. Heritable alterations in the expression of particular genes or gene clusters and transgenerational effects that are linked to environmental exposure and tumor development, are of particular interest. Alterations that result in chronic conditions present in early to mid-life stress the importance of ongoing research efforts to characterize molecular mechanisms associated with these conditions. Gene-environment interactions resulting in the promotion of autoimmune and neurodegenerative diseases serve to highlight current public health issues with an epigenetic basis. This is an important platform that will highlight toxicologically relevant epigenetic alterations with accompanying disease states and showcase trainee achievements.

This session is brought to you through the collaborative efforts of the Post-Doctoral Assembly and the Student Advisory Council.

1635 PRECLINICAL EVALUATION OF CANCER HAZARD AND RISK OF BIOPHARMACEUTICALS.
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The carcinogenicity testing of biopharmaceuticals may not always be possible by conventional means due to factors such as species specificity and immunogenicity. However, cause for concern for nontumorigenicity of biopharmaceuticals is heightened based on knowledge and plausibility of particular mechanisms of action. Mitogenicity is a concern for exogenously administered biopharmaceuticals such as hormones and growth factors and may also be a concern for pharmaceuticals designed to stimulate their endogenous production. In an attempt to address the potential risks of these agents, investigators have explored the ability of growth factors to influence the growth of tumor cells expressing their receptors in in vitro and in vivo models. However, the value of these models to adequately address the clinical risk of enhanced tumor growth with therapeutically administered growth factors is not clear. Special issues of concern following chronic treatment of immunomodulatory pharmaceuticals and biopharmaceuticals include the potential for immune impairment leading to opportunistic infections and/or lymphoproliferative disorders. Experimental data will be presented from approaches that have been used in an attempt to answer the central question of the role of exogenous growth factors and immunomodulatory agents in tumor progression in vivo; these approaches include rodent tumor xenografts, and alternative short-term and traditional carcinogenicity models. This material will also provide an overview of the current practices in the assessment of carcinogenic risk of biopharmaceuticals including the challenges in assessing human derived proteins in animals and developing waivers of carcinogenicity assessments and labeling considerations.

1636 KINASE INHIBITORS AS TARGETED THERAPEUTICS IN INFLAMMATION AND ONCOLOGY - APPROACHES TO PREDICT AND MANAGE TOXICITIES.

Targeted therapy with either monoclonal antibodies or small molecules has been a major focus of recent pharmaceutical research efforts in the inflammation and oncology disease areas. In particular, cell surface and cytoplasmic kinases have been targeted with varying degrees of clinical success in human oncologic disease. Although targeted therapy does provide an opportunity for more predictable and manageable toxicity based on anticipated and characterized exaggerated pharmacology of kinase inhibition, there have been considerable safety surprises with the various kinase inhibitor therapies in man. Therefore, it is important to address the pharmacologic rationale of targeted therapeutics in inflammation/oncology and provide an overview of kinase inhibitor toxicity in preclinical and clinical settings to demonstrate the importance of kinase activity profiling. To highlight these various issues participants will be provided with an overview of kinase biology and the use of high-throughput kinase profiling as a drug development tool and the correlation of kinase-inhibitor cardiotoxicity to kinase specificity profiles using in vitro tools to predict toxicological liabilities of kinase inhibitors with an emphasis on cardiac injury. The on and off-target toxicity for currently marketed kinase inhibitors of observed toxicities of currently marked kinase inhibitors will be provided to gain a broader understanding of the complexities of kinase pharmacology. Finally, the tools to be better prepared to assess the possible toxicity of kinase inhibitors through systematic kinase target analysis and will be addressed that will enable us to ultimately devise an early identification of safety and derisking strategies.

1637 THE ROLE OF OXIDANTS IN ETHANOL-INDUCED HEPATIC DNA SYNTHESIS.
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Ethanolic risk increases the risk of hepatocellular cancer in humans and rodents following chronic consumption; however, the mechanism(s) involved are not known. A common finding following ethanol exposure is an increase in reactive oxygen species (ROS) and oxidative stress in both rodents and humans. The present studies examined whether oxidative stress induction participated in ethanol-induced hepatocellular growth. We first performed a dose response study (0-5% ethanol (v/v) in liquid diet (LD), 7 days) in male C57BL mice to characterize the growth response characteristics of ethanol in liver. Ethanol at non-hepatotoxic doses (2% and 3% ethanol) increased hepatic DNA synthesis (-2-fold over control); while ethanol at hepatotoxic doses (4% and 5%, evidenced by increased serum ALT and AST) failed to increase DNA synthesis. To determine whether increased DNA synthesis by ethanol was mediated by oxidative stress, mice were exposed to 3% ethanol (v/v) in the presence or absence of the antioxidant polyphenon (3g/L, in LD). Exposure to ethanol (3%) produced the expected increase in DNA synthesis (-2-fold over control), while co-treatment with polyphenon reduced DNA synthesis to basal levels. CYP2E1 levels were increased (-2-fold) by 3% ethanol. Ethanol also increased the expression of the ROS-sensitive transcription factor, nuclear factor E2-related factor (Nrf2; -2-fold over control), supporting that ethanol increases ROS. Similarly, ethanol (10, 25, or 50mM; 24 h) increased Nrf2 protein expression in a dose dependent manner in primary cultured hepatocytes. Increased ROS following ethanol exposure may arise from CYP2E1 metabolism or Kupffer cell activation. Depletion of Kupffer cells reduced ethanol-induced increases in Nrf2 while CYP2E1 levels remained elevated in Kupffer cell depleted mice. These data suggest that the enhanced growth and survival seen following non-toxic doses of ethanol may arise through mechanisms involving Kupffer cell-mediated ROS production and enhanced Nrf2 expression.

1638 ROLE OF KUPFFER CELL IN TUMOR PROMOTION CAUSED BY NON-GENOTOXIC CARCINOGENS WYETH 14, 643 AND PHENOBARBITAL IN DIETHYLITROSAMINE INITIATED C3H MICE.
T. J. Peat, B. K. Philip and J. E. Klaunig 1Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN.

Kupffer cells (KC), the resident macrophages in the liver, play an important role in normal physiology and homeostasis. Upon activation, KC release inflammatory mediators, growth factors, and reactive oxygen species, which may modulate acute and chronic liver responses including hepatic cancer. Wyeth 14,643 (WY) and
Phenobarbital (PB) are non-genotoxic hepatic carcinogens in rodents. This study examined the role of the KC in tumor promoting effects of WY and PB in initiated C3H mice. Male 21 day old C3H mice were injected with DEN (50 mg/kg, ip, 1X/wk for 4 wks). After focal lesions were apparent, mice were treated with WY (0.1% in diet) or PB (500ppm in drinking water) for 28 days. Liposome-encapsulated (1 mg/kg, ip, 2X/wk), a known activator of the KC, was used as a positive control. Liposome-encapsulated clodronate (10 ml/kg, iv, 3X/wk) was given to animals to deplete the KC. Osmotic pumps containing BrDU (20 mg/ml) were surgically implanted 7 days prior to sacrifice to evaluate DNA synthesis. Treatment increased the relative focal volume of both basophilic and eosinophilic lesions and depletion of the KC decreased the focal volume. The volume of basophilic lesions in WY treated mice increased, and with depletion of the KC, focal volume decreased while eosinophilic lesion volume was unchanged. WY and PB increased DNA synthesis in both basophilic and eosinophilic lesions, similar to LPS. Upon KC depletion, a decrease in DNA synthesis was seen in PB treated mice. In the surrounding normal (non-lesion) tissue, PB produced a marginal increase in DNA synthesis (two-fold over control) and decreased with the depletion of the KC co-enrofloxacin control. DNA synthesis increased greatly (ten-fold over control) when treated with WY. These results show that the KC participates in the tumor promoting effects of PB and WY. 1643 KUPFFER CELL INVOLVEMENT IN PHENOBARBITAL AND WYETH-14, 643 INDUCED CLONAL EXPANSION OF PRENEOPLASTIC HEPATIC LESIONS. B.K. Philip, T.J. Peat, J.M. Kamendulis and J.E. Klumpp, Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN.

Non-genotoxic tumor promoters, phenobarbital (PB) and WY-14,643 selectively increase the growth of preneoplastic focal lesions via increasing cell proliferation and/or decreasing apoptosis. In the present study, we have examined the role of the KC in the clonal expansion of preneoplastic lesions induced by these compounds in diethylstilbestrol (DEN)-initiated F344 rats. In addition, lipopolysaccharide (LPS) a known KC activator was examined as a positive control. After lesions were histoologically apparent (5 months), rats were treated with LPS, PB, WY, 14,643 or control diet, and sampled after 28 days. KC depletion was achieved using liposome-encapsulated liposomes or with dietary glycine respectively. Growth of GSTp-positive focal lesions and various focal lesion phenotypes (H&E-staining) were evaluated using stereological methods. Focal lesion volume was increased by LPS, PB, and WY. However, each chemical exhibited differential progression. LPS increased the volume of GSTp-positive and eosinophilic focal lesions, while WY increased eosinophilic and basophilic lesion, KC depletion/deactivation reduced PB- and LPS-induced increases in lesion volume. Rates of DNA synthesis (labeling index) in focal lesions was increased by PB, WY, and LPS (1.7 to 2.8 fold over control), KC depletion/inactivation decreased PB- and LPS-induced increases in DNA synthesis. The non-focal hepatic labeling index remained elevated in the WY treatment group but was reduced by KC depletion/inactivation. The labeling index in normal liver of PB and LPS groups were similar to control. While each non-genotoxic chemical produced a different phenotypic profile for lesion growth, our data provide evidence supporting a role of the KC in tumor progression (Supported in part by NIH CA109008 [JEK]).

1640 MECHANISMS FOR POLYHYDROXYMETHYLENE BIGUANIDE (PHMB) CARCINOGENICITY. L.M. Kamendulis1, Y. Pu1, S.J. Barber1 and Z. Wang1, 1Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN and 2Arch Chemicals Inc., Chesire, CT.

Chronic dietary exposure to polyhydroxymethylene biguanide (PHMB) resulted in an increased incidence of liver hemangiosarcomas in C57/Bl6 mice (4/55 in control, 20/55 in 4000 ppm group). The mechanism for liver hemangiosarcoma induction was not understood, but since PHMB does not induce mutations or produce genotoxicity, these tumors are produced through non-DNA reactive mechanisms. The present studies evaluated the dose-response profile of PHMB on endothelial cell growth, C57/Bl6 mice were exposed to 0, 100, 200, 400, 1200, and 4000 ppm PHMB for 7, 14, and 28 days. PHMB did not induce hepatotoxicity (evidenced by ALT and AST) at any dose or time evaluated. Rates of DNA synthesis, quantified using BrEtU immunohistochemistry, showed that 4000 ppm PHMB decreased DNA synthesis after 7 and 14 days, a finding that may be related to decreased body weight, food utilization, and thinning of the intestines seen at this dose. At 28 days, PHMB increased DNA synthesis in a dose-responsive manner (-1.5- and 1.6-fold over control at 1200 and 4000 ppm), with no increases in this endpoint at or below 4000 ppm. In vitro, PHMB (0-10 ppm) did not increase endothelial cell DNA synthesis. Concomitant with the increased liver tumors in the 2-year study was an increase in Kupffer cell pigmentation, suggesting that this cell type may be activated and participate in the carcinogenic response. To test this, DNA synthesis was measured in endothelial cells co-cultured with macrophages. PHMB (0-1ppm) did not increase DNA synthesis in cultured endothelial cells or macrophages. Accompanying the gastrointestinal effects caused by PHMB in vivo was an increase in plasma endotoxin, a known activator of macrophages, by 1200 and 4000 ppm PHMB for 28 days. Collectively, these results suggest that PHMB does not produce a direct effect to endothelial cell growth, but rather through an indirect effect, perhaps involving endotoxin-mediated activation of macrophages.

1641 THE AH RECEPTOR PLAYS AN ANTI-APOPTOTIC ROLE IN DEN-INDUCED LIVER CARCINOGENESIS. Y. Fan, E. Knudsen and A. Dugas, Environmental Health, University of Cincinnati, Cincinnati, OH.

In previous work, we have characterized an interaction between the aryl hydrocarbon receptor (AHR) and the transcription factor E2F1 that results in attenuation of E2F1-mediated apoptosis. Whereas loss of AHR expression causes a significant elevation of oxidative stress, DNA damage and E2F1-dependent apoptosis, AHR activation suppresses the transcriptional induction of E2F1-dependent pro-apoptotic genes Apaf1 and p73 and the induction of apoptosis resulting from E2F1 stabilization. These results suggested that the AHR might have an anti-apoptotic function that is likely to play a role in tumor progression. To test this hypothesis, we developed a strain of mice with a double knockout liver genotype resulting from the mating of knockout Ahr-/- mice with mice bearing a liver-specific Rb ablation. Mice of the four homozygous genotypes were challenged with a single IP injection of 20 mg/kg of DEN or with control vehicle. Livers of control Ahr-/- mice showed a higher proliferative index at 3-weeks of age, significantly higher levels of polyplody and higher levels of liver apoptosis at 28 weeks than mice of the other genotypes. Nine months after DEN challenge mice were sacrificed and scored for liver tumors and expression of genetic markers. Regardless of Rb genotype, Ahr-/- mice had highly elevated levels of liver DNA damage and apoptosis, while the incidence of hepatocellular carcinoma was higher in Ahr+/+ mice. A real-time RT-PCR screen of liver tumors and surrounding tissues of these mice for expression of a battery of genes involved in proliferation, apoptosis and differentiation was also consistent with an anti-apoptotic role for the AHR. These results indicate that the Ah receptor plays an important role in tissue and organ homeostasis that is independent of its activation by xenobiotic ligands. (Supported by NIH R01ES06273, NIH R01ES10807, and NIH P50 ES06096).

1642 ESTROGEN RECEPTOR-ALPHA IS PROTECTIVE AGAINST LIVER TUMOR DEVELOPMENT AND IS CRITICAL FOR SEXUAL DIFFERENTIATION OF HEPATIC GENE EXPRESSION. M. H. Feld1, 2 and N. R. Drinkwater1, 1McKee Laboratory for Cancer Research, University of Wisconsin, Madison, WI and 2Department of Biology, Boston University, Boston, MA.

In mice, androgens promote hepatocarcinogenesis while ovarian hormones are known to be protective. Estrogen has been suggested to be responsible for this protection. Following perinatal treatment with N,N-diethylnitrosamine, we found that female Estrogen Receptor-α (ER-α) KO mice had a 5.9-fold increase in tumor multiplicity compared to wild-type and heterozygous litters (p < 0.06). Because sex-hormones affect susceptibility to liver tumor development, we examined the effects of sex-hormones on hepatic gene expression. Gene expression in the ER-α KO females did not resemble the pattern seen in ovariectomized mice but was similar to the pattern seen in male mice. This masculinized hepatic gene expression profile may account for their susceptibility to liver tumor development. Sex-dependent gene expression differences were dramatic, with several genes differing over 100-fold between sexes. For genes that were regulated by ER-α in female mice, and were also sex-dependent, most of these genes differed to a similar degree between sexes and between the wildtype and ER-α KO females. For example, the male predominant cytochrome P450 4a12 and members of the hydroxysteroid dehydrogenases β family varied to a similar degree between sexes and between the wildtype and ER-α KO females. Female-predominant cytochrome P450 3a4 was expressed higher in ER-α KO females, compared to wildtype littersmates, and the degree of this difference was similar to the difference seen between the sexes. Other genes, such as sulfotransferase 3a1 and flavin-containing monooxygenase 5, also differed between ER-α KO females but these changes were much less extreme than seen for the sex-dependent differences in transcription of these genes. These studies implicate the importance of hepatic ER-α in protecting against hepatocarcinogenesis and suggest that regulation of hepatic gene expression in the ER-α knockout females occurs in a multimodal manner.
1643 HISTOLOGIC AND IMMUNOHISTOCHEMICAL CHARACTERIZATION OF CYP1C1 AND CYP1A EXPRESSION IN PAH-INDUCED FUNDULUS HEPATIC LESIONS.

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Mammalian CYP1B1 has been recognized for its role in tumorgenesis not only by its ability to activate poly cyclic aromatic hydrocarbons (PAHs), but also by its increased expression in a variety of human tumors relative to normal tissues. CYP1C1 is the newest member of the cytochrome P450 family identified in teleost. Amino acid analysis suggests CYP1C1 may be structurally and/or functionally more similar to mammalian CYP1B1, rather than CYP1A. However, its physiological role and full significance in tumor development is unclear. We have hypothesized that CYP1C1 genes may be involved in the molecular mechanisms of PAH carcinogenesis. In a previous study, we initiated hepatic lesions in Fundulus heteroclitus by exposing them to 5 mg/L BaP or dimethylbenzanthracene (DMBA). Eight months after the PAH exposure, livers from 20 fish per treatment were sectioned and stained for microscopic evaluation. Histopathologic findings included the presence of nonneoplastic proliferation (nodular hyperplasia), preneoplastic foci of cellular alteration (eosinophilic and basophilic foci) and hepatocellular carcinomas. Inflammatory changes including mild peripheral lymphocytic infiltration and hepatic granulomas were also observed. Immunohistochemistry (IHC) was performed with CYP1A and CYP1C1. Increased CYP1A staining was present diffusely in hepatocyte cytoplasm, both in areas of nodular hyperplasia and foci of cellular alteration. In hepatocellular carcinomas, an intense CYP1A signal was detected at the border of the tumors (invading cancer cells), while a lower amount was seen in the remainder of the neoplasm. In contrast, CYP1C1 was detectable and highly expressed only in granulomas involving cells suggestive of bile duct epithelium. Our results suggest a important role of CYP1C1, as well as CYP1A, in carcino genesis and tumor formation. This project is supported by NIEHS (R01ES012770).

1646 MODULATION OF ACRYLONITRILE ON MITOCHONDRIAL GENE EXPRESSION AND MEMBRANE POTENTIAL.

S. Zhou, Z. Wang, X. Pu and J. E. Klaunig. Pharmacology and Toxicology, Indiana University, Indianapolis, IN.

Acrylonitrile (ACN) causes increased glial cell tumors in rats following chronic exposure. Oxidative stress induced by ACN is proposed to be a mechanism underlying ACN carcinogenesis. Previous studies demonstrated that ACN induces a dose-related increase in oxidative DNA damage in rat astrocytes. This study determined whether ACN treatment in cultured rat astrocytes interferes with mitochondrial genomic expression and membrane potential resulting in oxidative stress. The mitochondrial DNA content was evaluated by mitochondrial gene COXII expression level detected by RT-PCR, and membrane potential determined by flow cytometry using JC-1 as a probe. Treatment of astrocytes with ACN (0 to 0.5 mM) for 2 days suppressed mitochondrial membrane potential. Determination of mitochondrial DNA content revealed a 2-fold increase in cells treated with ACN. To determine if this ACN induced mitochondrial effects are involved in oxidative DNA damage, we determined ROS production in cells treated with ACN. We found 0.25 mM ACN at 2 d induced increase in ROS generation determined by fluorescent probe DHR123. These data indicate that ACN affects mitochondrial biogenesis and bioenergetics, and causes increased ROS generation. Whether this modulation of mitochondrial functions contributes to increased oxidative DNA damage induced by ACN remains further investigated.

1647 ALTERATION OF H4K16 ACETYLATION VIA SAS2 IN YEAST AND MYST1 IN HUMANS IS ASSOCIATED WITH INCREASED SENSITIVITY OF ARSENIC TOXICITY.

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Arsenic is a human carcinogen commonly found in drinking water. An important mechanism of arsenic carcinogenesis is through alterations in gene expression, which has been correlated to epigenetic changes. Recently, we found that genes which encode subunits of the heterotrimeric something about silencing (SAS) complex, are required for the optimal growth of yeast in arsenite (AsIII) -containing medium. Within the SAS complex, the catalytic subunit Sas2 and its human homolog, MYST1, are orthologous acetyltransferases responsible for the acetylation of histone 4 lysine 16 (H4K16). To further explore their roles in arsenic toxicity, we used short hairpin RNA (shRNA) to silence endogenous level of MYST1 in the human uroepithelial cell line (UROtsa cells) and subsequently exposed the cells to AsIII in its toxic metabolite monomethylarsonous acid (MMAIII). Knockdown of MYST1 in UROtsa cells significantly increased their sensitivity to both AsIII and MMAIII treatment. The observed increased sensitivity to arsenic treatment correlated with reduced acetylated H4K16 resulting from silencing of MYST1 in UROtsa cells. Further, we found that the H4K16-lysine 16 mutant strain, which

1645 IMPACT OF LIFE STYLE AND DURATION OF EXPOSURE ON ARSENIC-INDUCED PROLIFERATIVE LESIONS, NEOPLASIA, AND GENE EXPRESSION IN MALE C3H MICE.

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Previous studies have demonstrated increased liver and adrenal tumor incidence in male mice exposed gestationally to 85 ppm inorganic arsenic via the dams’ drinking water. To further characterize age susceptibility to arsenic carcinogenesis we have male mice continuously exposed to arsenic was significantly decreased compared to control and in control only exposed mice. This apparent protective effect of continuously arsenic exposure was accompanied by expression changes for many genes known to be involved in liver cancer including cell growth and proliferation, cell death, and oxidative stress genes. In addition, overlap was noted between genes affected by chronic arsenic exposure and those reported in the literature to be associated with caloric restriction and the metabolic syndrome. In particular, the gene for stearoyl-CoA desaturase (Scd1), which catalyzes the synthesis of monounsaturated fatty acids, was down-regulated by continuous arsenic treatment but up-regulated by in utero only treatment. Because Scd1 is important in the metabolic regulation of body weight, this response may explain the decrease in body weight gain seen in continuously exposed pups despite normal food consumption. Scd1 is implicated in hepatocarcinogenesis due to both environmental and genetic factors in rodents. Therefore, Scd1 and the genes it interacts with may be of key importance in the treatment-dependent tumor responses reported here. (This abstract does not necessarily reflect EPA policy.)

1644 DOSE EFFECTS OF ARSENITE ON RODENT BLADDER UROTHELIUM.

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Inorganic arsenic (arsenate and arsenite) is a known human carcinogen, inducing tumors of the skin, urinary bladder and lung. In short-term experiments in rats, treatment with 100 μg/g arsenite in the diet or drinking water induced cyto toxicity and necrosis of the urothelial superficial layer, with increased cell proliferation and hyperplasia. These phenomena might be involved in the development of arsenic-induced bladder cancer in humans. Therefore, the objectives of this study were to determine if the arsenic-induced urothelial effects are dose responsive and to determine the dietary dose of arsenic at which no urothelial effects are detected. We treated female F344 rats with arsenic (as sodium arsenite) in the diet at doses of 0, 1, 10, 25, 50, and 100 μg/g for 5 weeks. We detected hyperplasia in the urothelium at the high doses by light microscopy with superficial necrosis detected by scanning electron microscopy. No hyperplasia or necrosis was detected at the lower dose. The majority of arsenic present in the urine of arsenite-treated rats was in the form of the organic arsenical dimethylarsinic acid (DMA2). However, arsenite and trimethylarsine oxide (TMAO) were also present. Interestingly, the ratio of DMA2/TMAO in the urine was different between the low dose and high dose treatments. These data suggest that arsenic-induced urothelial cytotoxicity and proliferation are dose responsive and the urothelial effects have a threshold. Further investigation of the dynamic metabolism of arsenicals is needed to fully understand the urothelial effects of arsenic in vivo.
contains a non-acetylatable arginine residue instead of the regular lysine, was sensitive to high doses of AsIII. Together, these data suggest that the alteration of H4K16 acetylation affects arsenic toxicity in both yeast and human cells. Acetylation of H4K16 is known to regulate the transcription of genes and promote DNA repair; therefore, aberrant gene expression induced by changes of H4K16 acetylation may be involved in the resistance of human cells to arsenic. Funding: This work was supported by the National Institute of Environmental Health Sciences [P42 ES04705].

These data suggest that pharmacological inhibition of ALK5 enhances malignant conversion possibly through effects on inflammatory cells and changes in epidermal differentiation.

### SOT 1650 Targeted Disruption of Bcl-xL in Mouse Keratinocytes Inhibits Both UVB- and Chemically-Induced Skin Carcinogenesis.

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Bcl-xL, one of the members of the Bcl-2 family, is an antiapoptotic protein that has been found to play a role in a wide variety of human malignancies including skin cancer. Bcl-xL is one of several antiapoptotic proteins regulated by signal transducer and activator of transcription 3 (Stat5). We have recently shown that Stat5 is required for both chemically-induced as well as UVB-induced skin carcinogenesis. In this study, the functional role of Bcl-xL in skin carcinogenesis was investigated using skin-specific Bcl-xL-deficient mice. In this model, Bcl-xL expression is disrupted in the basal compartment of mouse epidermis using the bovine keratin 5 (K5) promoter to drive expression of Cre recombinase (i.e., K5.Cre × Bcl-xLfl/fl mice). A significant increase in apoptosis induced by either UVB irradiation or 7,12-dimethylbenz[a]anthracene (DMBA) treatment was observed in the epidermis of skin specific Bcl-xL-deficient mice. Furthermore, an increase in apoptotic cells was noted in the bulge region of hair follicles. In contrast, cell proliferation was not affected by Bcl-xL deficiency following exposure to either UVB or 12-O-tetradecanoylphorbol-13-acetate (TPA). In skin carcinogenesis experiments using UVB or two-stage DMBA/TPA protocols, Bcl-xL-deficient mice were more resistant than wild-type controls to skin tumor development with delayed onset and reduced number of tumors. Moreover, Bcl-xL-deficient mice showed a significant reduction in the epidermis of Bcl-xL-deficient mice in the absence of stimuli. Further studies, as well as these antiapoptotic proteins were also high in skin tumors from Bcl-xL-deficient mice that developed in response to either UVB or two-stage carcinogenesis protocols, indicating partial compensation for the loss of Bcl-xL. Collectively, these studies demonstrate that Bcl-xL plays a role early in skin carcinogenesis through its anti-apoptotic functions to enhance survival of keratinocytes, including those found in the bulge region, following DNA damage.
1652 GASTROESOPHAGEAL REFLUX LEADS TO ESOPHAGEAL CANCER IN A SURGICAL MODEL WITH MICE.

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Esophageal-gastroduodenal anastomosis (EGDA) with rats mimics the development of human Barrett’s esophagus (BE) and esophageal adenocarcinoma (EAC) by introducing mixed RNA of gastric and esophageal contents into the duodenum. However, use of this rat model for mechanistic and chemopreventive studies is limited due to lack of genetically modified rat strains. A mouse model of BE and EAC is needed. We performed EGDA on wild-type, p53A135V transgenic, and Ink4a/Arf+/- mice of all strains. Some mice were also treated with omeprazole (1,400 ppm in diet), iron (50 mg/kg/m., i.p.), or gastrectomy plus iron. Mouse esophagi were harvested at 20, 40, 80 weeks after surgery for histopathological analysis. At week 20, we observed metaplasia in wild-type mice (5%, 1/20) and p53A135V mice (5.3%, 1/19). At week 40, metaplasia was found in wild-type mice (16.2%, 6/37), p53A135V mice (4.8%, 2/42), and wild-type mice also receiving gastrectomy and iron (6.7%, 1/15). Esophageal squamous cell carcinoma (ESCC) developed in Ink4a/Arf+/- mice (7.1%, 1/14), and wild-type mice receiving gastrectomy and iron (21.4%, 3/14). Among 13 wild-type mice which were given iron from week 40 to 80, twelve (92.3%) developed ESCC at week 80. None of these mice developed BE or EAC. Surgically induced gastroesophageal reflux produced ESCC, but not EAC, in mice. Dominant negative p53 mutation, heterozygous loss of Ink4a/Arf, antacid treatment, iron supplementation, or gastrectomy failed to promote EAC in these mice. Further studies are needed in order to develop a mouse model of EAC. (This study was supported by NIH grants R01 CA75683, R03 CA107583, R01 CA128504, and U56 CA90277).

1653 TUMOR SUPPRESSOR MEL-18 IS A NOVEL GLOBAL SUMOYLATION INHIBITOR.

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Small Ubiquitin-like Modifier (SUMO) pathway is an important modifier in the carcinogenesis, as well as toxicity in the neurodegenerative diseases. The sumoylation generally requires E3 ligases to specifically recognize substrate, however little study showed the existence of the anti-E3 ligase. Here we show that tumor suppressor Mel-18 can function like a novel anti-E3 ligase to down-regulate cellular sumoylation globally, which may give clues to the further studies in its tumor suppressor activities. In this study, extracts of HEK 293 cells transfected with GFP-Mel-18 or GFP alone, and Myc-SUMO-1 were subjected to Western blotting using anti-Myc and anti-SUMO-1 antibodies, and the results indicate that increased expression of Mel-18 is associated with a detectable decrease in conjugation of SUMO-1 to cellular proteins. Consistent with this hypothesis, we found that Mel-18 can interact with at least two sumo substrates (HSF2 and RanGAP1) and that overexpression and RNA interference-mediated reduction of Mel-18 also result in decreased and increased HSF2/RanGAP1 sumoylation, respectively. In the study of the underlying mechanism, we found that Mel-18 can interact with UBC9 and inhibit UBC9 activity by blocking its ability to transfer the SUMO group from its active site to the target protein. This was tested by transfection of GFP-Mel-18 or GFP alone, and then extracts of the transfected cells were subjected to Western blotting with anti-UBC9 antibodies, the results indicate that expression of GFP-Mel-18 results in increased levels of the SUMO-containing form of UBC9 compared with cells expressing the GFP-alone construct. In the summary, tumor suppressor Mel-18 function like a novel anti-SUMO ubiquitin-protein isopeptide ligase (E3), interacting both with substrates and the SUMO E2 UBC9 but acting to inhibit UBC9 activity to decrease sumoylation of a target protein.

1654 GENETIC AND EPIGENETIC EFFECTS OF FORMALDEHYDE ON HUMAN BLOOD STEM AND PROGENITOR CELLS.

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Formaldehyde, an economically important industrial chemical, is a human carcinogen that causes nasopharyngeal cancer, and is also a suspected leukemogen (IARC, 2006). Formaldehyde can cause leukemia in addition to nasopharyngeal can-
cer, which is relatively rare. Our recent meta-analysis of the epidemiological studies to date on this topic corroborates the association between formaldehyde exposure and leukemia, especially for myeloid leukemia at high levels of exposure (Zhang, et. al., 2008, Mut Res Rev). Leukemia originates in the pluripotent stem and progenitor cells that are located mainly in the bone marrow but also in the peripheral blood where they constitute up to 0.05% of total nucleated cells and can be cultured in vitro. To further examine whether the observed association between formaldehyde and leukemia is biologically plausible, we investigated the effects of formaldehyde on DNA-protein crosslinks (DPCs), chromosome alterations (CAs) and DNA methylation in CD34+ cells and blood myeloid progenitor cells cultured and harvested in colony-forming assays using growth factor enriched semi-solid media. We found that formaldehyde induces DPCs in a dose-dependent manner in CD34+ cells. We also examined metaphase and interphase cells from the cultured CFU-GM colonies and CD34+ cells using classical and molecular cytogenetics, and found that formaldehyde caused an increase in structural and numerical CAs. Further, we demonstrated that formaldehyde altered global DNA methylation and caused hypo- and hyper-methylation of specific cancer genes measured using an Illumina methylation array. These results show that formaldehyde can cause significant genetic and epigenetic changes in human hematopoietic stem and progenitor cells, and add further weight to the biological plausibility of an association between formaldehyde exposure and leukemia.

1655 BENZENE-INHALATION ENHANCED THE INCIDENCE OF HEMATOPOIETIC NEOPLASMS IN P53-DEFICIENT MICE WITH RESPECT TO STRAIN-DIFFERENCES BETWEEN C57BL/6 AND C3H/HE.

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The dose-range of benzene exposure for induction of the hematopoietic neoplasia including leukemia is narrow, and the relevance between benzene-exposure and the hematopoietic neoplasms is not clear but remained to be elucidated, specifically on; a low incidence, like a threshold, of hematopoietic neoplasms at low dose exposure, despite of Ames test-negative, known genotoxicity in its metabolites; a reason of its unlikely linear-dose-response relationship, specifically at high dose; its strain/species differences in disease spectrum, specifically in incidences of myelogenous leukemias among the experimental animals and human. In this study, to resolve such questions in in vivo-studies after benzene exposure, we chose p53-knockout mice because of high sensitivity in tumorogeneity for elucidating underlying background mechanism of the threshold-like low incidence at a low dose and an unlikely linear-dose-response curve at a high dose for experimental hematopoietic neoplasms; and compare C3H/He mice, a myelogenous leukemia prone, with C57BL/6, a prone for lymphoid neoplasms, focusing on the strain differences. As results, an increased number of benzene-exposure induced a high frequency of hematopoietic neoplasms up to 62% including myelogenous leukemias (38%) with plateau forming incidence at the high dose of benzene exposure in p53-heterozygous deficient C3H/He mice. There were nonthreshold hematopoietic neoplasms observed in p53-heterozygous deficient mice, whereas it was not clearly defined in wild-type mice because of only limited incidence of hematopoietic neoplasms in wild-type mice; thus, possible threshold in wild-type mice might be false.

1656 COMPARISON OF HEPG2 AND HEPARG CELL LINES EXPOSED TO DIFFERENT CARCINOGENS BY GENE EXPRESSION ANALYSES.

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The demand for toxicogenomics-based tests assessing the carcinogenicity of chemicals as an alternative to current rodent bioassays has resulted in the investigation of potential in vitro models. Two such models are the human hepatoma cell lines HepG2 and HepaRG. HepG2 cells are metabolically competent with respect to biotransformation of mutagens and carcinogens and carry no p53 mutations. HepaRG is a cell line that under specific conditions can be highly differentiated and quiescent, and in contrast to HepG2 cells expresses various CYP450s enzymes and exhibits other metabolic functions comparable to those in cultured human primary hepatocytes. The purpose of this study was to investigate the effect on gene expression of HepG2 and HepaRG after exposure to different carcinogens. Both cell lines
were exposed to the genotoxic carcinogens BaP and AFB1 and to the non-genotoxic carcinogens TCDD, CaA and Estradiol for 48 h. Gene expression analysis was carried out using Affymetrix Human Genome U133 Plus 2.0 Arrays. PCA showed that the expression data of all exposures were grouped together with the exception of TCDD in HepG2 and CaA in HepaRG. Gene expression was highest for BaP and TCDD in HepG2 and for AFB1 and CaA in HepaRG. The same compounds revealed the most pathways using MetaCore. Furthermore, the largest overlap in pathways between the two cell lines was found for the TCDD treatment. Further analyses were conducted to examine whether HepG2 as well as HepaRG can be used to predict genotoxicity by gene expression profiling. First results indicated that both cell lines perform equally well. The HepaRG cell line was kindly provided by Biopredic International, Rennes, France.

1657 THE INFLUENCE OF DIETARY FAT ON BENZO(A)PYRENE (BAP)-INDUCED DNA ADDUCT CONCENTRATIONS AND COLON TUMORS IN APEC5(+/+) MICE.

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Studies conducted in our laboratory have shown that consumption of chemically contaminated food rich in fat, leads to a greater retention of toxican in the body. These findings have relevance to human health in that in US alone around 60,000 lives is lost every year to colon cancer. Diet and environmental toxicants contribute to the development of sporadic colon tumors. Therefore, the objective of this study was to determine how dietary fat accelerates the development of colon tumors induced by benzo(a)pyrene (BaP), an environmental toxicant. Benzo(a)pyrene was administrated to Apc5(−/−) mice in unsaturated- (peanut oil) and saturated- (coconut oil) fats at doses of 50 and 100 μg/kg via oral gavage over a 60-day period. Blood, jejunum, colon and liver, were collected at 60 days post-BaP exposure; adenomas in colon were counted and subjected to histopathology. Samples were analyzed by reverse phase-HPLC for BaP metabolites. DNA was isolated from the tissues and analyzed for BaP-induced DNA adducts by the 32P-postlabeling method using a four-directional thin-layer chromatography system. BaP exposure through dietary fat induced adenomas and adducts in colon in a dose-dependent manner, with 100 μg/kg dose group registering more adenomas and adducts compared to the 50 μg/kg dose group (p < 0.05) for the fat categories tested. The number of adenomas, concentrations of BaP metabolites and adducts showed a statistically significant increase (p < 0.05) for saturated fat-treatment group compared to unsaturated fat group. These findings clearly demonstrate that the high residence time of BaP when given through saturated fat allows extensive metabolism, resulting in formation of reactive metabolites that bind with DNA and contribute to neoplastic transformation of adenomas in a long-term exposure scenario (supported by the NIH grants S1ES014156, ST32 HL07735-14 and R03CA130112-01).

1658 REQUIREMENT FOR METALLOPROTEINASES-DEPENDENT ERK AND AKT ACTIVATION IN UVB-INDUCED G1-S CELL CYCLE PROGRESSION OF HUMAN KERATINOCYTES.

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Ultraviolet B (UVB, 280-315 nm) in natural sunlight represents a major environmental challenge to the skin and is clearly associated with human skin cancer. Here we demonstrate that low doses of UVB induce keratinocyte proliferation and cell cycle progression of human HaCaT keratinocytes. Different from UVU, UVB irradiation induced ERK and AKT activation and their activation are both required for UVB-induced cell cycle progression. Activation of EGFR was observed after UVB exposure and is upstream of ERK/AKT/cyclin D1 pathway activation and cell cycle progression following UVB radiation. Furthermore, metalloproteinase inhibitor GM6001 blocked UVB-induced ERK and AKT activation, cell cycle progression, and decreased the EGFR phosphorylation, demonstrating that metalloproteinases mediate the EGFR/ERK/AKT/cyclin D1 pathways and cell cycle progression induced by UVB radiation. In addition, ERK or AKT activation is essential for EGFR activation because ERK or AKT inhibitor blocks EGFR activation following UVB irradiation, indicating that EGFR/AKT/ERK pathways form a regulatory loop and converge into cell cycle progression following UVB radiation. Identification of these signaling pathways in UVB-induced cell cycle progression of quiescent keratinocytes as a process mimicking tumor promotion in vivo will facilitate the development of efficient and safe chemopreventive and therapeutic strategies for skin cancer.

1659 CENTROSOMAL AMPLIFICATION IN XPA/P53 TRANSGENIC MICE EXPOSED TO ZIDOVUDINE (AZT).

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In cultured cells, exposure to the highly-effective nucleuside reverse transcriptase inhibitor (NRTI) Zidovudine (AZT) induces genomic instability, causing cell cycle arrest, micronuclei, sister chromatid exchanges, and shortened telomeres. In previous studies, we demonstrated for the first time that AZT induced centrosomal amplification (delineated by > 2 centrosomes/cell), a novel manifestation of genotoxicity. Here, bone marrow cells from transgenic C57BL/6 mice with the genotypes XPA/p53 wild type, XPA null/p53 wild type, and XPA null/p53 heterozygote, were cultured in vitro to obtain homogenous populations of mesenchymal-derived fibroblasts. Cells grown from mice (n=5/group) of the above genotypes were exposed to AZT for 24 h, and centrosomal amplification was examined by immunohistochemical (IHC) staining with an anti-pericentriol antibody. Values for centrosomal amplification (% of cells with ≥2 centrosomes/cell) were 4.6, 11.2, 12.1 and 17.0 % in XPA/p53 wild type cells exposed to 0, 10, 100 and 200 μM AZT, respectively. Values for centrosomal amplification were 20.9, 33.3, 49.2, and 41.1% for XPA null/p53(+/+) cells exposed to 0, 10, 100 and 200 μM AZT. For XPA null/p53(−/−) cells exposed to 0, 10, 100 and 200 μM AZT, centrosomal amplification values were 20.9, 28.1, 40.6 and 41.9 %, respectively. The data suggest that loss of both XPA alleles confers genomic instability, but that additional loss of one p53 allele does not further enhance instability. Whereas nucleotide excision repair is not considered to impact NRTI intracellular processing, these data would argue that lack of intact nucleotide excision repair (XPA null status) enhances genomic instability in AZT-exposed cells.

1660 OGG1 SER326CYC AND P53 ARG72PRO POLYMORPHISMS IN A TURKISH POPULATION WITH GASTRIC CARCINOMA.

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Initiating factors that damage the gastric cell DNA leading to gastric carcinoma has still been poorly understood. Constantly generated reactive oxygen and nitrogen species may contribute to the process of carcinogenesis by interacting with DNA. 8-oxoguanine DNA glycosylase-1 (OGG1) is an enzyme involved in the base excision repair of 8-hydroxyguanine (8-OHG). Deletion or functional loss of the p53 tumor suppressor gene contributes to the oncogenic transformation. Thus, polymorphisms of DNA repair gene OGG1 Ser326Cys and tumor suppressor gene p53 Arg72Pro were assessed in eligible 100 gastric cancer patients. One hundred and ten matched controls were included to the study. Genotypes were determined by Polymerase Chain Reaction Restriction Fragment length Polymorphism (PCR-RFLP) using DNA extracted from peripheral blood cells. Homozygote variant allele Cys of OGG1 at codon 326, Pro of p53 at codon 72 were found to be not associated with increased risk of gastric carcinoma (OR=0.694, 95% confidence interval; (0.291-1.657), 1.282 (0.581-2.831), respectively). (Supported by The Scientific & Technological Research Council of Turkey, SBAG-3820, 107S363)

1661 ATM DEFICIENT MICE DEMONSTRATE AN EXACERBATED RESPONSE TO DEXTRAN SULFATE SODIUM-INDUCED COLITIS CHARACTERIZED BY ELEVATED DNA DAMAGE AND A CRONICALLY ACTIVATED IMMUNE RESPONSE.

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The deficiency of Atm, a DNA damage recognition and repair protein, is recognized to lead to high levels of oxidative stress and increased susceptibility to cancers, however its role in inflammation is largely unknown. To determine the effect of Atm deficiency in inflammation, we induced experimental chronic colitis in Atm deficient and WT mice with dextran sulfate sodium (DSS) treatment. The immune response and systemic DNA damage were then characterized by repeated draws of peripheral blood throughout cycles of treatment. Atm deficient mice had higher levels of DNA single and double strand breaks than WT mice throughout each cycle of treatment, demonstrating lack of repair of damage during remission periods. Micronuclei formation in normochromatic erythrocytes was also markedly increased in Atm deficient mice, illustrating the clastogenicity of treatment on erythrocytes. DNA damage was highest in Atm deficient mice even up to four weeks after end of treatment. Although Atm deficient mice had lower counts of CD4+ and CD8+ T cells in peripheral blood than WT mice, higher percentages were activated at all points of treatment. Finally, both a Th1 and Th2 inflammatory response were seen in both genotypes, with Atm deficient mice showing greater upregulation
of both Th1 and Th2 cytokines in the latter chronic phase of treatment. In conclu-
sion, Atm deficient mice mounted a stronger immune response characterized by
both pro-inflammatory and anti-inflammatory cytokines towards the chronic phase
of treatment, which may have been both a cause and an effect of the larger amount
of DNA damage to peripheral lymphocytes and erythroblasts. Atm therefore may be
a critical factor in dampening the deleterious effects of chronic DSS-induced
inflammation, necessary for genomic stability and homeostasis of the gut epithelial
barrier.

**1662 MESOTHELIOMA DIAGNOSIS: SHOULD GENETIC
SCREENING BE USED TO EVALUATE PRIMARY SITE
AND PLAUSIBILITY OF ASPEROSIS CAUSATION?**

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Some epidemiologic observations suggest that the incidence of mesothelioma
(meso) caused by asbestos exposure may have peaked in the 1990s because the
highly potent amphibole exposures were largely curtailed in the 1960s and the typ-
cal latency period of 20 to 50 years has now transpired. Epidemiologic observa-
tions also suggest a much lower or zero potency for the more prominent chrysotile
exposures from various sources and products in subsequent years. If this is true,
then a larger proportion of future meso cases may be due to causes other than as-
bestos. While some 80% of recent pleural meso cases may be caused by asbestos,
the risk factors for peritoneal meso are far more diverse with less than half of
the cases explained by heavy amphibole exposures. Routine genetic screening of meso
patients may assist in more definitive identification of tissue/cell type of origin, risk
factors, and more effective treatment regimens for the underlying cancer. First, ex-
tragonadal germ cell tumors may present as diverse tumor types, including peri-
neal or pleural meso, that can be distinguished by specific mutations (iso12p) of
chromosome 12 in tumor tissue. Second, apparent meso can sometimes represent
fied candidate biomarkers of AIG.

**1663 THE MOLECULAR BASIS OF REVERSIBLE AND
IRREVERSIBLE ANCHORAGE-INDEPENDENT
GROWTH.**


We have established an experimental model that reflects the reversible phenotypic
characteristics regulated by basic fibroblast growth factor (bFGF) during normal
wound healing and irreversible phenotypic characteristics associated with carci-
ogenesis regulated by phorbol ester (TPA). In this context, bFGF induces 1) persist-
ent activation of the ERK pathway that is characterized by prominent ERK oscilla-
tions, 2) efficient cell migration and 3) reversible AIG in Jb6 mouse epithelial
cells. In contrast, TPA induces 1) persistent activation of the ERK pathway without oscilla-
tions, 2) negligible cell migration and irreversible AIG in side-by-side com-
parisons. Using this newly developed model, we have undertaken a genomics ap-
proach to identify biomarkers of anchorage-independent growth. Distinct gene
expression profiles are associated with anchorage-independent colonies arising from
bFGF-stimulated Jb6 cells, relative to colonies arising from fully tumorigenic Jb6
RT101 cells. 43 differentially expressed genes were common to colonies arising from
bFGF-treated Jb6 cells and TPA-treated Jb6 cells (RT101 genetic variants). The majority of genes exhibited comparable patterns of regulation in terms of in-
creased or decreased expression, while 12 genes exhibited reciprocal regulation pat-
tterns. Hepatic leukemia factor (HLF) and D-site albumin promoter-binding pro-
tein (DBP) expression were increased in both bFGF and RT101 colonies. Ectopic
expression of DBP and HLF dramatically enhanced tumor promoter (12-O-
tetradecanoyl phorbol-13-acetate, TPA: epidermal growth factor, EGF)- and low
dose X-ray radiation (10 cGy)-induced AIG responses. In a preliminary screen, we
observed an increase in HLF and DBP mRNA expression in human basal cell car-
cinoma, relative to paired non-tumor tissue. Collectively, our approach has identi-
fied candidate biomarkers of AIG.

**1664 INCREASED FORMATION OF MDA-DNA ADDUCTS
IN ANILINE-INDUCED SPLENIC TOXICITY.**

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Malondialdehyde (MDA), one of the most abundant lipid peroxidation-derived
aldehydes, is known to be mutagenic and carcinogenic. MDA can transfer meth-
onylpropargyl group to guanine residues of DNA to yield propargylguanine (MG)
adducts. M1G is a biologically important adduct that serves as a useful dosimeter
of MDA-induced DNA modifications. The focus of this study was to evaluate if ani-
line-induced increased lipid peroxidation also leads to increased formation of
MDA-DNA adducts in the spleen. To accomplish that, male SD rats were sub-
chronically exposed to aniline (0.5 mmol/kg/day via drinking water for 30 days),
while controls received drinking water only. Quantitation of M1G in the DNA
samples from control and aniline-treated rats was done using an enzyme-linked im-
munosorbent assay (ELISA) and by using an anti-MDA-DNA monoclonal anti-
body. M1G levels in the spleen of aniline-treated rats showed remarkable increases
which were 4.1 fold or 315% greater than the controls. To further validate our
M1G ELISA results, immunohistochemistry of MDA-DNA adducts was also per-
formed. MDA-DNA adduct-specific immunostaining showed more nuclear stain-
ing in the red pulp areas of the spleens of aniline-treated rats compared to the con-
trols. Our study clearly shows that aniline treatment leads to significant increases
in MDA-DNA adduct formation in the spleen, suggesting greater DNA base modify-
cation by MDA. Formation of MDA-DNA adducts may serve as a biomarker of
DNA damage by MDA, and also presents a potential mechanism in the pathogen-
esis of aniline-induced fibrosarcomas in the spleen.

**1665 PHOSPHORYLATION OF CYCLIN-DEPENDENT
KINASES AND RETINOBLASTOMA PROTEIN IN RAT SPLEEN FOLLOWING ANILINE EXPOSURE.**

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Aniline exposure is associated with toxicity to the spleen leading to splenomegaly,
hyperplasia, and a variety of sarcomas of the spleen on chronic exposure. In earlier
studies, we have shown that aniline exposure leads to iron-overload, oxidative stress
and activation of redox-sensitive transcription factors, which could regulate various
genes leading to a tumorigenic response in the spleen. This study was focused on
assessing the expression of classical cyclins and cyclin-dependent kinases (CDKs)
which were 4.1 fold or 315% greater than the controls. To further validate our
M1G ELISA results, immunohistochemistry of MDA-DNA adducts was also per-
formed. MDA-DNA adduct-specific immunostaining showed more nuclear stain-
ing in the red pulp areas of the spleens of aniline-treated rats compared to the con-
trols. Our study clearly shows that aniline treatment leads to significant increases
in MDA-DNA adduct formation in the spleen, suggesting greater DNA base modify-
cation by MDA. Formation of MDA-DNA adducts may serve as a biomarker of
DNA damage by MDA, and also presents a potential mechanism in the pathogen-
esis of aniline-induced fibrosarcomas in the spleen.
stained cells with fluorescent phallolidin to decorate microfilaments, and fluorescent antibody to α-tubulin/β-tubulin to decorate microtubules, then examined cells by fluorescent confocal microscopy. In non-transformed 10T1/2 cells, microfilaments/microtubules were arranged homogeneously in long fibers. In those Ni3-transformed/over-expressed NIO transformed cell lines, microfilaments and microtubules were over-expressed/aggregated in some areas, absent in other areas, changing shapes of transformed cells, rounding them/altering their contact with extra-cellular matrix. This likely leads to differential expression of eight genes in transformed cell lines, contributing to induction/maintenance of transformed states. Supported by grants R01 ES0-3341/NIEHS (PL JRL) and 5 T32 AI07078/NAIAD, USC Discretionary Funding.

1667 ALTERATIONS OF DIURNAL EXPRESSION RHYTHM OF CIRCADIAN GENES AND HORMONE RECEPTOR GENES BY CHEMOPREVENTIVE DOSE OF SELENIUM IN RAT MAMMARY TISSUE.

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To further define the molecular basis for methyleneoncysteine (MSC)-mediated chemoprevention, we compared global gene expression profiles in normal mammary tissues from rats maintained on a diet supplemented with MSC (3 ppm Se) or a control diet (0.1 ppm Se), with and without exposure to N-nitrosodimethylamine (NDM). The results of this analysis indicated that the most dramatic effect of Se diet was the coordinated change in the expression of genes that regulate circadian rhythm. Our results further demonstrated that a single carcinogenic dose of NMU ablated and that dietary MSC supplementation restored the coordinated diurnal expression of circadian genes Per1, Per2, and RevErba in mammary tissues, suggesting that normal circadian rhythm suppresses mammary carcinogenesis. We also found that neither carcinoma exposure nor dietary MSC altered blood melatonin levels, indicating that neither treatment had an effect on light-mediated regulation of melatonin through the Suprachiasmatic Nucleus. By contrast, MSC induced a dramatic increase in the expression of the melatonin receptor 1α in mammary tissues, suggesting that increased melatonin signaling contributed to or affected by the MSC-mediated restoration of circadian rhythm in NMU treated mammary tissues. More importantly, we demonstrated that MSC induced a 7-fold increase in the circadian expression of Estrogen Receptor beta (ERβ) in mammary tissues. These findings suggest that dietary MSC might prevent mammary carcinogenesis through enhanced rhythmic expressions of circadian genes, induced melatonin signaling, and ERβ-mediated differentiation and growth inhibition in mammary epithelial cells. Further studies are needed to elucidate the mechanistic associations between these tumor-inhibition pathways. Supported by the NIEHS grants, U19ES011387, P01ES007033, and P30ES005022.

1668 A CB1 SPECIFIC AGONIST, JWH-133 ACCELERATES TUMOR GROWTH IN A XENOGRAFT MODEL OF ESTROGEN RECEPTOR NEGATIVE BREAST CANCER.


Natural and synthetic cannabinoids have shown anti-cancer actions in a variety of in vitro and in vivo cancer models. However, there is limited information regarding their actions in breast cancer. Therefore, we investigated two synthetic cannabinoids in an ER- breast cancer model. Athymic nude female mice were implanted subcutaneously with MDA-MB-231 cells (2x10⁶) and left to form palpable tumors. The mice were then treated with JWH-133 (a selective CB1 agonist), WINN 55,212-2 (a mixed CB1 and CB2 agonist) at 50, 25 or 12.5 μg/mouse or vehicle control, ip for 70 days. Tumor volume was measured weekly with electronic calipers. At necropsy, tumors and organs were weighed and blood was collected to determine plasma ALT activity. Tumor extracts were also prepared. The results demonstrated that JWH-133 (50 μg/mouse) significantly increased tumor volume (tumor volume of 662 ± 305 and 832 ± 283 mm³, for vehicle and 50 μg, respectively, p<0.05). This correlated to tumor weights of 415 ± 162 and 281 ± 135 mg for 50 μg and control, respectively. In contrast, WINN 55,212-2 (25 μg/mouse) decreased tumor volume 70% compared to control. Importantly, both cannabinoids were non-toxic to the mice, as ALT activity, weight gain and gross organ weights were all normal. Western blotting of tumor extracts demonstrated that the tumors expressed CB1 and CB2 receptors, but their expression was unchanged following cannabinoid treatment. The angiogenic factor VEGF and its receptor VEGFR-1 were also unchanged following cannabinoid treatment. The results from this study demonstrate that JWH-133 accelerates tumor growth in an MDA-MB-231 xenograft model of ER- breast cancer, but this did not occur following treatment with WINN 55,212-2. The mechanism for this effect is not clear, but it does not involve up-regulation of VEGF or VEGFR-1. Therefore, further research is required on the anti-cancer effects of synthetic cannabinoids, as we are the first to demonstrate that low doses of a selective CB1 agonist accelerates tumor growth in vivo.

1669 INHIBITION OF MAMMARY DNA ADDUCTS AND TUMORS INDUCED BY XENOESTROGENS.

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Breast cancer is a leading cause of cancer-related death among American women. The etiology of majority of breast cancers is multifactorial, of which 95% is environmental and hormonal. Xenoestrogens are present in the environment, includes PAHs, and range from natural plant-derived phytoestrogens to man-made substances, such as pharmacological agents and organochlorine pesticides. Exposure to xenoestrogens leads to adverse health effects such as reproductive dysfunction, developmental disorders, malignancies etc. 3-Methyl-cholanthrene (3-MC) represents a novel subclass of xenoestrogenic compounds that activates both ER-α and AR signaling pathways. The formation of deprotoxinating estrogen-DNA adducts appear to be a critical step in the initiation of cancer by estrogens. CYP19 or aromatase is involved in the biosynthesis of estrogens. Phytochemicals have the advantage of being dietary compounds that are less toxic to animals, abundant, and inexpensive. Many molecular and cellular studies have revealed the ability of dietary phytochemicals in vegetables and fruits to reduce the risk of cancer. The major mechanisms of chemoprevention involve induction of phase II detoxification enzymes and/or inhibition of phase I enzymes that are involved in the activation of certain carcinogens. In the present study, we evaluated the chemopreventive and chemotherapeutic potencies of dietary phytochemicals to suppress xenoestrogen induced mammary DNA adduct and tumor formation in female mice. Our studies show that in mammary tissues of mice exposed to 3-MC, EROD activities, CYP1A1 (phase I enzyme) and NQO1 (phase II enzyme) gene expressions increased when compared to controls. Significant reduction of phase I enzymes along with elevation of several phase II enzymes was observed in female mice treated with dietary phytochemicals. Thus, the dietary phytochemicals could modulate the Phase I/Phase II enzyme systems, a critical mechanism involved in the formation of cancers. These studies will result in improved prevention or treatment methodologies in endocrine therapy for hormonally induced breast cancer.

1670 THE RAT HOMOLOG OF THE DROSOPHILA FRY GENE ENCODES A PUTATIVE MAMMARY CANCER SUSCEPTIBILITY LOCUS.

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Rat strains vary in their susceptibility to mammary carcinogens. Using a genetic backcross between the resistant Copenhagen (Cop) and sensitive Fischer 344 (F344) strain, we mapped a Mammary carcinoma susceptibility (Mc) locus to the centromeric region on the long arm of rat chromosome 12. The locus is contained within a ~9 centimorgan region whose synteny is conserved on human chromosome 13. Rat BRCA2, along with several other candidate genes within this interval, were examined for polymorphisms and differences in expression levels in the mammary tissue of the two rat strains. Our results revealed two non-synonymous mutations within the rat ortholog of the Drosophila furry gene in the Fischer 344. Comparative genomic analysis indicated that these F344 specific mutations occur within a region of the coding sequence that is highly conserved (~90%) in eutherian mammals. One of these mutations results in a serine residue at codon 2170 of the mutant Furry protein in the F344 rat. This amino acid substitution creates a consensus sequence that has a high probability of being a substrate for various cancer related protein kinases, suggesting altered activity or regulation of the mutant Furry protein. Examination of Furry gene expression indicated a reduction in the majority of rat mammary tumors, as well as several human breast carcinoma cell lines. In an effort to further examine Furry protein expression as well as perform functional studies, we have developed a polyclonal anti-peptide antibody against a conserved sequence within the predicted human and rat furry protein. We are also conducting functional studies using shRNA to functionally "knock out" the Furry gene in mammary cells.
1671 ESTROGENIC STATUS MODULATES THE EFFECT OF SOY ON HEPATIC RESPONSES TO 7,12-DIMETHYLBENZ(A)ANTHRACENE (DMBA).

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We examined the influence of estradiol (E2) status and soy protein isolate (SPI) intake on the hepatic responses altered by 7,12-dimethylbenz(a)anthracene (DMBA, a polycyclic aromatic hydrocarbon [PAH]). Sprague-Dawley rats were ovariectomized (OVX) at PND50 and infused with E2 or vehicle for 14d and gavaged with 50mg/kg DMBA or vehicle 24h before sacrifice at PND64. Rats were fed an AIN-93G diet made with SPI or casein as sole protein source throughout the study. Basal AhR protein levels were reduced (P<0.05) by SPI feeding irrespective of the E2 status. However, DMBA increased (P<0.05) AhR-induced CYP1A1 gene expression in OVX, SPI-fed rats, but reduced (P<0.05) CYP1A1 in OVX+E2, SPI-fed rats. Chromatin-immunoprecipitation demonstrated lower (P<0.05) DMBA-mediated recruitment of estrogen receptor alpha to the CYP1A1 promoter by SPI feeding in the presence of E2, suggesting an estrogen-like action of SPI on DMBA-mediated signaling in the absence of E2. Further, microarray analysis (Rat 230-2.0 Affymetrix-GeneChip™) revealed 231 genes common to SPI + DMBA and SPI + E2 + DMBA (normalized to E2 treatments). AhR-activated genes (CYP1A1, CYP1A2, and NQO1) were down-regulated by SPI + E2 + DMBA compared to SPI + DMBA. Unique interactions among SPI, DMBA and E2 altered the expression profile of 316 genes, not observed by either treatment alone. Our data suggest that SPI + DMBA and SPI + E2 + DMBA altered the expression of CYP1A1, CYP1B1, and NQO1.

1672 EFFECT OF CAFFEIC ACID PHENETHYL ESTER AND QUERCETIN ON N-7-ETHYL-1NITROSOAMINE ADDUCT FORMATION INDUCED BY N-DIETHYL-NITROSAMINE IN RAT LIVER DNA.

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Caffeic acid phenethyl ester (CAPE) and quercetin (Q) have shown anti-carcinogenic properties in the modified resistant hepatocyte model when they are administered on initiation stage. In this model, N-diethylnitrosamine (DEN) cytochrome P450 (CYP)-dependent bioactivation, the ion ethyldiazonium and ROS are generated to induce ethylated and oxidized DNA adducts. N-7-ethyl-N-nitrosamine (N7-EtN) is one of the most important adducts formed by DEN treatment. The information about the action mechanism of CAPE and Q in initiation stage is limited. The aim of this study was to determine the effect of CAPE and Q on the formation of N7-EtN in DNA induced in rat liver after DEN administration. F344 male adult rats were administered with a CAPE i.p. dose (20 mg/kg) 2h before DEN i.p. administration (200 mg/kg). Animals were sacrificed at different times after DEN administration and liver was removed and processed to obtain DNA. N7-EtN was determined in hydrolyzed liver DNA in 1 N HCl by HPLC/MS/MS. A maximum of N7-EtN adduct formation was observed at 18h after DEN administration and decreased gradually after CAPE and Q pretreatment decreased the N7-EtN formation occurred at 12 h, in addition, at 6, 12 and 18 h, N7-EtN concentration decreased 50, 43 and 89%, respectively; in comparison to the DEN treated animals. Q treatment produced a decreasing from 35 to 65% at all determined times, showing a similar trend to CAPE. These results suggest that CAPE and Q reduce the formation of ethylated-DNA adducts and support the proposal that their chemopreventive effects may be by altering CYP-dependent DNA bioactivation in chemical carcinogenesis (Project supported by Conacyt-Grant 48,774).

1673 CAFFEIC ACID PHENETHYL ESTER INHIBITS CYP1A1 ACTIVITY IN HEPA 1C1C7 CELLS.

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Caffeic acid phenethyl ester (CAPE), a natural component of propolis, is known to antioxidant, anti-inflammatory, antiviral, and anticancer activity. However, CAPE is unknown to effects of metabolism of xenobiotics and endogenous compounds. The present study, we investigated effect of CAPE on CYP1A1 related to metabolism of xenobiotics and 7,12-dimethylbenzanthracene (DMBA)-DNA adduct in Hepa1c1c7 cells. CYP1A1 catalyzes the metabolic activation of procarcinogenic polyaromatic hydrocarbons (PAHs), such as DMBA. CAPE inhibits DMBA induced DNA adduct formation. Thereupon we examined the effect of CYP1A1 enzyme activity by CAPE using a 7-ethoxyresorufin O-deethylase (EROD) assay. CAPE inhibits DMBA-induced CYP1A1 enzyme activity. However, CAPE was not changed DMBA-induced XRE promoter activity. Moreover, CAPE also was not changed to protein level. Subsequent studies we confirmed by measuring direct EROD the effect of DMBA-induced CYP1A1 activity, and we known to CAPE directly express DMBA induced CYP1A1 enzyme activity. Taken together, these results suggest that CAPE has chemoprevention effect through inhibition of DMBA induced CYP1A1 activity and of DMBA induced DNA adduct formation, as well as a direct inhibitor of CYP1A1 enzymes.

1674 EFFECTS OF DIETARY FISH OIL ON THE DECREASE OF CARCINOGENIC PAH-DNA ADDUCT LEVELS IN THE LIVER OF B6C3F1 MOUSE MALE.

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Genotoxic complex mixtures are released into the environment from a variety of sources. Many carcinogetic PAHs and their metabolites can bind covalently to DNA. The formation of covalent DNA adducts is known to be a key event in the initiation of neoplasia and, also, contributes significantly to the progression of tumour. It has been reported that fish oil (Omega-3, FO), compared to corn oil (FO), may play a role as an apoptosis inducer, and thus be protective against formation of DNA adducts. In this study we tested hypothesis that fish oil could decrease the levels of PAH-DNA adducts by removing damaged cells. B6C3F1 male mice were fed a FO or CO diet for one month. The animals were then treated with several carcinogenic PAHs including BaP with two doses via intraperitoneal injection. Animals were terminated at 7d after treatment. The levels of DNA adducts were analyzed by the nuclear P1-enhanced 32P-postlabeling assay. Our results showed that the levels of total DNA adducts in the livers of mice fed with FO were significantly decreased compared to CO mice. The mean RAL values for total DNA adducts in the liver of mice treated with the high dose PAHs were 52.4+8.07 and 89.11+8.99 in 109 nucleotides (R=0.05), respectively, for the FO and CO groups. The levels of BP-dG adduct were also diminished in FO group (23.35+8.07 and 34.46±8.99, P=0.029). The levels of BP-dG adduct were 7.76±1.74 and 13.27±0.90 in 109 nucleotides, respectively, for FO and CO diet group (P=0.031). Our results suggest a possible mechanistic link between cellular apoptosis and DNA adduct formation. In conclusion, these results help furthering our understanding of the mechanisms of carcinogenesis, especially in relation to the chemopreventive properties of nutritional apoptosis inducers. (Supported by P30 ES09106)

1675 TRANSCONTINENTAL CHEMOPREVENTION BY CHLOROPHYLLIN, PURIFIED CHLOROPHYLLS AND FROZEN-DRIED SPINACH: CONFIRMATION OF THE COMPLEXATION THEORY FOR CHL.

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The carcinogenic potential of dibenz[a]pyrene (DBP) has been well characterized in numerous animal models. We have previously documented that a single dose of 15 mg/Kg DBP to pregnant mice late in gestation (CD 17) produces an aggressive T-cell lymphoma as well as lung and liver cancer in offspring. The current study examines the chemopreventive properties of chlorophyllin (CHL) and chlorophyll (Chl) in this transplacentally carcinogenic model. Pregnant B6129SF1 females, bred to 129SF1/Sv males, received purified diets incorporated with either 0 or 2000 ppm CHL, 2000 ppm CHL, or 10% freeze-dried spinach beginning at gestation day 9. Lymphoma-dependent mortality was not significantly altered by maternal consumption of any of the diet and little effect on lung tumor burden in mice surviving to 10 months of age was observed. However, co-administration of CHL at 380 mg/Kg with DBP by gavage (molar ratio of 10:1, CHL:DBP) provided significant protection against DBP initiated carcinogenesis. Offspring born to dams receiving CHL co-gavaged with DBP exhibited markedly less lymphoma-dependent mortality (p<0.001). The degree of protection by CHL, compared to controls dosed with DBP in tricaprylin (TCP) as the vehicle, was less marked, but still significant. Co-administration of CHL (TCP as vehicle) also reduced lung tumor multiplicity in mice by approximately 50% and this was observed throughout the study (p<0.005). This is the first demonstration that CHL can provide potent
chemoprotection in a transplacental carcinogenesis model and supports a mecha-
nism involving complex-mediated reduction of carcinogen uptake. The work re-
ported here was supported by PHS grants CA90890, ES07060 and ES00210.

1676 SAPONINS DERIVED FROM ROOTS OF PLATYCODON
GRANDIFLORUM INHIBIT 4-(METHYL-
NITROSAMINO)-1-(3-PYRIDYL)-1-
BUTANONE (NNK)-
INDUCED NF-KB TRANSACTIVATION AND CELL
TRANSFORMATION IN BEAS-2B HUMAN BRONCHIAL
EPITHELIAL CELLS.

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Epidemiological data suggest that consumption of fruits and vegetables has been as-
associated with a lower incidence of cancer. Platycodon grandiflorum is a traditional
oriental herbal medicine that is known for its immunostimulatory and antitumor
effects. In this study, we investigated effects of Changkul saponins (CKS) from
roots of Platycodon grandiflorum on 4-(methyl nitrosamino)-1-(3-pyridyl)-1-
butanone (NNK)-induced NF-kB transactivation and cell transformation in BEAS-2B human bronchial epithelial cells. We found that CKS significantly
blocked NNK-induced cell transformation in a concentration-dependent manner.
CKS significantly attenuated NNK-induced NF-kB activation, and transcription
factor. CKS inhibited the NNK-induced phosphorylation of extracellular-signal
regulated protein kinases Akt and p38 kinases, which NF-kB regulates.

Taken together, our results suggest that inhibition by CKS of NNK-induced neo-
plastic transformation in BEAS-2B cells is related to blocking of Akt and p38 acti-
vation, and also attenuation of NF-kB activation. These results indicated that CKS
has potent anticancer-promoting activity.

1677 EFFECT OF CELASTROL ON SPECIFICITY PROTEIN
TRANSCRIPTION FACTORS IN PANCREATIC
CANCER CELLS.

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Specificity protein (Sp) transcription factors Sp1, Sp3 and Sp4 are overexpressed in
multiple tumor types and negative prognostic factors for survival. Since Sp tran-
scription factors regulate genes associated with survival (survivin), angiogenesis
(vascular endothelial growth factor (VEGF) and its receptors) and growth (cyclin
D1), research in this laboratory has focused on development of anticancer drugs
that decrease Sp protein expression. Celastrol, a naturally occurring triterpenoid
acid from an ivy-like vine exhibits anticancer activity and treatment of Panc28 and
L3.6pl cells with 1–5 μM celastrol induced apoptosis and decreased expression of survivin, cyclin
D1, epithelial growth factor (EGFR), and VEGF in Panc28 and L3.6pl cells, sug-
gestig that decreased expression of these genes may be due to downregulation of Sp proteins. Treatment of Panc28 and L3.6pl cells with 1–5 μM celastrol de-
creased Sp1, Sp3 and Sp4 protein expression in both cell lines and corretament with the proteasome inhibitor MG132 did not inhibit downregulation of Sp proteins.

The mechanism of celastrol-induced repression of Sp proteins was associated with
downregulation of miRNA-27a (miR-27a). Decreased miR27a results in the in-
duction of ZBTB10, a zinc finger protein that acts as an Sp-repressor by competi-
tive interaction with Sp proteins bound to GC-rich promoter elements. At a dose of
4 mg/kg every second day, celastrol also inhibited pancreatic tumor growth in
athyric nude mice bearing L3.6pl cells, and Sp proteins were also de-
creased in tumors from treated mice. This study demonstrates that the anticancer
activity of celastrol in pancreatic cancer due to celastrol-miR27a interactions result-
ing in ZBTB10-dependent repression of Sp and Sp-dependent genes.

1678 1, 1-BIS(3-INDOLYL)-1-(P-BROMOPHENYL) METHANE
AND RELATED COMPOUNDS DECREASE
PANCREATIC AND COLON CANCER CELL SURVIVAL
AND EXPRESSION OF SURVIVIN.

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1,1-Bis(3-indolyl) methane (DIM), a metabolite of indole-3-carbinol which is ex-
pressed in brassica vegetables, exhibits anticancer activity in a wide variety of tu-
ors. 1,1-Bis(3-indolyl)-1-(substituted aromatic) methanes (C-DMIs) are struc-
turally related to DIM and are potent anticancer agents. The para-bromo-substi-
tuted analog (DIM-C-pBrH) and a 2-methyl indole ring derivative (2-methyl-
DIM-C-pBrH) were used in this study to investigate their anticancer activity in
Panc28 (pancreatic cancer) and SW480 (colorectal cancer) cell lines. Inhibition of Panc28 and SW480 cell proliferation was observed after treatment with 5–15 μM
concentrations of both compounds. IC50 values for DIM-C-pBrH in Panc28 and
SW480 were 5.29 and 4.9 μM, respectively, and values of 4.75 and 6.6 μM were
observed after treatment with 2-methyl-DIM-C-pBrH. In addition, both com-
ponds induced caspase-dependent cleavage of poly (ADP-ribose) polymerase
(PARP) which is indicative of apoptosis. Since, survivin negatively regulates apop-
tosis, we further investigated the effects of DIM-C-pBrH and 2-methyl-DIM-C-
pBrH on survivin expression in Panc28 and SW480 cell lines. Both compounds
induced a concentration- and time-dependent decrease in survivin protein and
mRNA levels. In addition, we also investigated the effects of DIM-C-pBrH and 2-
methyl-DIM-C-pBrH on survivin promoter activity. SW480 cells were transfected with pSurvivin 269, a construct containing the -269 to +49 GC-rich region of the
tumor suppressor linked to the luciferase gene, and after treatment with 10, 15 and
20 μM DIM-C-pBrH and 2-methyl-DIM-C-pBrH, there was a decrease in lu-
ciferase activity of 30, 80 and 95% and 40, 90 and 95%, respectively. Similar results
were obtained in cells transfected with pSurvivin 150 which contains the -150 to
+49 GC-rich region of the survival suppressor linked to the luciferase gene.

1679 PREGNANE X RECEPTOR SUPPRESSES
PROLIFERATION AND TUMORIGENICITY OF COLON
CANCER CELLS THROUGH REGULATING RB/E2F1
PATHWAY.

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Pregnane X receptor (PXR) is nuclear receptor that coordinately regulates the
metabolism and disposition of various xenobiotics and endobiotics. In the present
study, we report a novel tumor suppressor function of PXR in cell culture and
xenograft models. We demonstrated that expression of PXR through stable trans-
fection of human PXR in human colon epithelial cancer cell line (HT29) signifi-
cantly inhibited cell proliferation as determined by MTT assay (p<0.01). Colony
growth of the PXR-transfected HT29 cells was suppressed in soft agar assay. In
 xenograft assay, the tumor size formed in nude mice was significantly suppressed
with PXR gene stable-transfection (310 mg±6.2 v.s. 120 mg±6, p<0.01). The Ki-
67 positive cells were significantly decreased in PXR-transfected HT29 xenograft
tumor tissue comparing to the vector-transfected HT29 controls (p<0.01) as deter-
mined by immunohistochemistry suggesting that PXR inhibits the proliferation of
colon cancer cells. Results of flow cytometry analysis indicated that PXR-transfec-
tion in HT29 cells caused G0/G1 arrest in PXR-HT29 cells. The cell growth in-
hibitory effects of PXR are likely mediated through the E2F2/Elk-regulated check
point as the E2F1 nuclear expression was significantly inhibited by PXR overex-
pression. Taken together, these results suggest that PXR directly inhibits the prolif-
eration and tumorigenicity of colon cancer cells in an antigen response. Our results in-
dicate that the evolutionarily conserved PXR protects organisms from
carcinogenesis by inhibiting tumor growth as well as eliminating carcinogenic sub-
cstances. (The research is supported by ES09859 to VT).

1680 INHIBITION OF EPIDERMAL GROWTH FACTOR
RECEPTOR (EGFR) IN BLADDER CANCER CELLS BY
CURCUMIN AND BETA-LINOLENIC ACID.

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The epidermal growth factor receptor 1 (EGFR1) is overexpressed in multiple
tumor types and is an important drug target for cancer chemotherapy using either
neutralizing antibodies or tyrosine kinase inhibitors. Research in this laboratory
characterized betulinic acid (BA) and curcumin as anticancer drugs that act by re-
pressing specificity factor (Sp) transcription factors and Sp-dependent genes asso-
ciated with cancer cell growth, survival and angiogenesis. Treatment of 235B1-B and
KU7 bladder cancer cells with curcumin and BA decreased cell survival, whereas
the tyrosine kinase inhibitor gefitinib differentially inhibited growth of these cells
since KU7 cells are gefitinib-resistant. Curcumin and BA also decreased expres-
sion of Sp1, Sp3 and Sp4 proteins and the Sp-dependent genes survivin and vascular en-
dothelial growth factor (VEGF) and induced caspase-dependent PARP cleavage in
both cell lines. We hypothesized that curcumin and BA would also decrease EGFR1
in bladder cancer cells since EGFR1 expression is Sp-dependent in some cancer cell
lines. Curcumin and BA decreased EGFR1 protein and mRNA levels and also in-
hibited EGFR1-dependent downstream kinase activity including phosphatidyli-

nositol 3-kinase (PI3K)-dependent phosphorylation of Akt in both cell lines. In addition, BA and curcumin also decreased expression of sodium/glucose co-transporter 1 (SGLT1) which is also regulated by EGFR1 (kinase-dependent). Conformation that EGFR1 is an Sp-dependent gene was determined in RNA interference studies where knockdown of Sp1, Sp3 and Sp4 decreased EGFR1 protein. Thus, BA and curcumin specifically target down-regulation of EGFR1 in bladder cancer cells and also in tumors (xenografts), demonstrating that these agents and other drugs that decrease Sp protein expression can be used in cancer chemotherapies that specifically target EGFR1.

**1682** TCDODA- the selective ARYL HYDROCARBON RECEPTOR (AhR) MODULATORS INDOLE-3-CARBINOL (13C) AND 3, 3-DIINDOLYMETHANE (DIM) REGULATE PROSTATE TUMORIGENESIS IN TRAMP MICE.

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In vitro and lactational (IUL) TCDODA exposure increased preneurobic cibrofoliferation lesion incidence in dorsolateral prostates (DLP) of senescent wild-type mice, while AhR acts as a prostate tumor suppressor in TRAMP mice. We therefore hypothesized that perinatal AhR signaling pathway activation would increase susceptibility to prostate cancer while AhR activation during adulthood would be protective. Pregnant TRAMP mice were given 1 μg TCDODA/kg or vehicle on embryonic day 13.5; macroscopic prostate tumor incidence in male offspring was determined at 140 days of age. IUL TCDODA exposure significantly elevated prostate tumor incidence (30% versus 10% in controls). All tumors had neuroendocrine (NE) characteristics. In adult AhR activation studies, TRAMP mice were gavaged 3 times/week with vehicle, 10 or 100 mg/kg DCM or IUL starting at 56 days of age. Other TRAMP mice were given 5 μg TCDODA/kg and weekly 1.8 μg TCDODA/kg maintenance doses. Tumor development was examined weekly by palpation. TCDODA and 5 mg DCM/kg significantly increased the median age for prostate tumor-free survival, and the other three treatments also appeared to be protective. All vehicle-dosed TRAMP mice developed NE-characteristic prostate tumors in the ventral prostate (VP). Papillary adenomas were also found in the DLP and anterior prostate (AP) in H&E, DIM, and TCDODA-treated TRAMP mice. Tumors in younger mice were predominantly NE tumors located in the VP, while older mice typically developed papillary adenomas in DLP and to a lesser extent in AP. These results suggest that adult exposure of TRAMP mice to 13C, DIM, and TCDODA protects against NE tumor development, increasing tumor-free survival and allowing prostate adenomas to be manifested. In summary, perinatal AhR activation enhances prostate tumor development in TRAMP mice while adult AhR activation is protective. 

**1683** A NOVEL NUTRIENT MIXTURE INHIBITS MMP EXPRESSION, INVASION AND APOPTOSIS IN CHONDROSARCOMA CELL LINE SW 1353.

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Introduction: Chondrosarcoma, a malignant tumor of cartilaginous origin, is the second most frequent primary malignant tumor of bone, representing approximately 25% of primary osseous neoplasms. Incidence of tumors in children is low. Most tumors occur in patients older than 40 years. Cancer mortality usually results from tumor invasion of local tissues and metastases to vital organs. A novel nutrient mixture (NM) containing ascorbic acid, lysine, proline and green tea extract has exhibited significant anticancer activity against a number of cancer cell lines, including inhibition of invasive parameters.

Objective: We investigated the effect of NM on human chondrosarcoma cell line SW 1353 for viability, MMP expression, invasion, apoptosis and morphology.

Methods: Human chondrosarcoma cell line SW1353 (ATCC) was grown in DEM media with 10% FBS and antibiotics and treated with NM at 0, 10, 50, 100, 500 and 1000 μg/ml in triplicate at each dose. Cells were also treated with phorbol 12-myristate 13-acetate (PMA) 100 ng/ml for MMP-9 induction. Cell proliferation was measured by MTT assay. MMP-9 activity was determined using Matrigel invasion assay. Apoptosis was examined by light microscopy, TUNEL, and annexin V-FITC staining. Results: NM was not toxic to chondrosarcoma cell line at 100 μg/ml, but exhibited 10% and 40% toxicity at 500 and 1000 μg/ml. Zymography demonstrated two bands corresponding to MMP-2 and MMP-9. PMA treatment enhanced MMP-9 secretion. NM inhibited the secretion of both MMP-2 and MMP-9 by 50% at 100 μg/ml and by 100% at 500 μg/ml. Invasion through Matrigel was inhibited by 40%, 50%, 70% and 100% at 50, 100, 500 and 1000 μg/ml, respectively. NM induced slight apoptosis at 250 μg/ml, moderate at 500 μg/ml and significant at 1000 μg/ml. H&E showed slight changes at the highest concentration.

Conclusions: NM significantly inhibited MMP secretion, invasion through Matrigel and apoptosis, important parameters for cancer prevention, suggesting NM has the potential for therapeutic use in treatment of chondrosarcoma.

**1684** ROLE OF NF-KB IN THE CHEMOPREVENTIVE ACTIVITIES OF CITRUS AND GINGER COMPOUNDS AGAINST SKIN CANCER DEVELOPMENT.

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NF-kB is a survival signaling transcription factor involved in the malignant phenotype of many cancers, including Squamous cell carcinomas. The citrus coumarin, auraptene (AUR), and the ethnomedicinal ginger (Alpinia galanga) phenylpropanoid, 1-acetoxychavicol acetate (ACA), were previously shown to suppress 12-O-tetradecanoylphorbol-13-acetate (TPA) induced mouse skin tumor promotion. The goal of the present study was to determine whether AUR and ACA could suppress NF-kB activation and advanced stages of skin carcinogenesis in mouse models. First, we found that orally administered AUR (100 mg/kg bw) and ACA (200 mg/kg bw) suppressed lipopolysaccharide-induced NF-kB activation in NF-kB-RE-luc (Oslo) luciferase reporter mice by 65% and 76%, respectively. Next, di- etary administration of AUR (1000 ppm) and ACA (500 ppm) was evaluated in a xenograft model. Female SCID-bg mice were fed the test diets, injected with 1 million human squamous cell carcinoma cells (SB12) s.c., palpated and weighed twice a week for 28 days following tumor initiation. AUR and ACA modestly suppressed tumor volume by 30% and 50%, respectively. In a third study, AUR (340 nmol, topical) suppressed TPA-induced skin tumor promotion by 43% in K5.Stat3C transgenic mice. These mice, which possess a constitutively active Stat3, are highly susceptible to skin carcinogenesis. TPA increased the phosphorylation of the p65 transcription factor.
1687 STRUCTURAL AND FUNCTIONAL DIFFERENCES IN RAT LUNGS DUE TO POST-NATA! OZONE OR PARTICLE EXPOSURE.

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Inhaled pollutants cause various lung diseases such as asthma, COPD, and emphysema, but chronic effects of gas and particle pollutants are not nearly as well explored as the acute effects, due to the longer-term nature of chronic effects and the lack of reliable physiological indicators. We measured alterations in airway architecture and lung function due to ozone or particle exposure during development. To analyze alteration of all conducting airways, we developed a computer technique that can automatically extract geometric information of each airway from CT-images that allows quantification of airway structure; for example, diameter, length, branching angle, asymmetry. Male Sprague Dawley rats were exposed to 0.2, 0.5 ppm ozone or 80 μg/m3 flame-generated soot particles from 7 days to 28 days postnatal, 8 hrs/day, 5 days/week. Lung function tests and lung casting for CT-scan were conducted 56 days post-exposure. While there was no significant difference in lung architecture between control and exposed groups, TLC and dynamic compliance changed.

1685 BHAS42 CELL TRANSFORMATION ASSAY FOR INVESTIGATION OF ANTI-TUMOURIGENIC EFFECTS OF CHEMICAL COMPOUNDS.

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The rodent bioassay is used to detect complete genotoxic carcinogens and tumour promoters and co-carcinogens. Cell transformation assays detect morphological changes that provide the earliest phenotypic sign in carcinogenicity. For the improvement of the experimental conditions Saak et al. (1998) developed a cell line, Bhas42, established from Balb/c-C3H cells transfected with v-Ha-ras oncogene for detection of carcinogens and tumour promoters. The purpose is to identify the potential use of the Bhas42 system for the detection of tumour protective effects of chemical compounds. The Bhas-system was established and validated by using the reference carcinogen 3-methylcholanthrene as an initiator and TPA and insulin as promoters. Increasing concentrations led to a dose-dependent increase in transformed foci. To investigate whether the Bhas42 system can detect tumour-protective compounds, 4 substances, a-Tocopherolacetate, b-Naphtoflavone, Astaxanthine and Ellagic acid, known as potentially anti-tumourigenic, were tested. Both, Astaxanthine and Ellagic acid showed tumour protective effects by reducing the number of foci induced by Methyl-Cholanthrene (MCA). Astaxanthine showed the highest tumour-protective effect after a simultaneous 24 hour treatment with MCA. The protective mechanism is assumed to be its antioxidative properties and the increased intercellular gap junctional communication. The treatment of Ellagic acid 24 hours before treatment showed the highest tumour-reductive response. The supposed mechanism is the covalent binding of the compound to DNA which may lead to protection of DNA to initiating compounds like MCA. No tumour-protective effects were observed with the remaining two compounds, a-Tocopherolacetate and b-Naphtoflavone. Overall, our results indicate the Bhas-system is not only useful for the investigation of carcinogens and tumour promoters but can also be used for determining tumour-protective effects of compounds.

1686 EARLY POSTNATAL KETAMINE ANESTHESIA CAUSES PERSISTENT BEHAVIORAL EFFECTS IN RHESUS MONKEYS.

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Our earlier studies in developing monkeys showed that ketamine exposure (24 hours of clinically relevant anesthetia) causes significant increase in neonatal cell death. Sensitivity to the neurotoxicity of ketamine in monkeys was observed on gestational days 122-124 (in utero exposure via maternal anesthesia) and on PNDs 5-6, but not on PND 35. In the present study, subjects were exposed on PND 5 or 6 to 24 hrs of ketamine anesthesia, intravenously (n = 6); controls were unexposed (n = 6). At 7 months of age all animals were weaned and began training via successive approximation to perform a series of cognitive function tasks as part of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Rodent Test Battery. The OTB tasks used here included those for assessing aspects of learning, motivation, color discrimination, and short-term memory. Subjects responded for banana-flavored food pellets by pressing response levers and press plates during daily (M-F) test sessions (50 min) and were assigned training scores based upon task performance. After approximately 60 training sessions (5 months of testing), control animals began to outperform the ketamine exposed animals as evidenced by higher training scores; one month later (80 test sessions) control animals were outperforming the ketamine exposed group by more than 10% and this difference was maintained for another 4 months (animals at about 15 months of age). These observations suggest that, in primates, a single 24 hr episode of ketamine anesthesia occurring during a sensitive period of brain development results in long-lasting deficits in brain function. Whether this effect represents a developmental delay or a permanent alteration remains to be determined.

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1689 REPROGRAMMING OF MATERNAL DIET IN RAT AFFECTS BODY WEIGHT AND AUDITORY STARTLE RESPONSE IN THE OFFSPRING.


People from Dutch Famine studies have been found more obese and at higher risk for chronic diseases like diabetes, but also neurodegenerative diseases. Obesity is thought to be linked to reprogramming of (neuro)physiological set points during early development of the nervous system. In the present study it is hypothesized that maternal diet determines bodyweight (BW) and (neuro)development in the offspring and, with that, the vulnerability for environmental exposure to toxic substances. Three groups of female Wistar rats (n=24) were kept on a semi-synthetic control diet (n=24), a high-caloric diet (15% butter oil, n=48) or a caloric restricted (deficient) diet (75% of control diet, n=38) during 6 weeks premating, mating (1 week) and gestation (3 weeks) period. Pups were cross-fostered immediately after birth.
birth to obtain 5 diet groups, i.e. (gestation/LACTATION): control; def/CONT; high/High; def/DEF; def/High and high/DEF. The programmed diet of high diet group mimics the situation of the Dutch Famine. A selection of male (n=100) and female (n=100) pups was additionally dosed sc. with saline (control) or MeHg (3 mg/kg BW) from PN2 to 21. In time, BW was measured and an auditory startle response (ASR) was performed. The ASR measures anxiety over-time; disability to habituate in the ASR test is considered indicative of neurodegeneration. Results: 1) BWs of the offspring differed significantly between the diet groups; effects were slightly exacerbated by exposure to MeHg and maintained during adulthood, especially for the def/High group. 2) Dietary effects on ASR appeared causally related to the BW changes. Additional effects of MeHg, i.e. a disability to habituate to the ASR, were also seen especially in the female def/High diet group suggesting similar neural deficits as found in the Dutch Famine casualties. The findings support the hypothesis that maternal diet may increase the vulnerability of the developing offspring to (subtle) environmental exposure like MeHg, ultimately resulting in neurodegenerative diseases.

1690 MOTHER’S DIET IN UTERO ALTERS THE HEMATOPOIETIC SYSTEM OF OFFSPRING.

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Chronic exposure to benzene is associated with an increased risk of developing leukemia in adults. Furthermore, epidemiological studies show that in utero exposure to benzene is correlated with an increased incidence of childhood leukemia. Our overall objective is to determine if maternal benzene exposure in mice causes prolonged changes in the hematopoietic system of the offspring. We have previously shown that C57Bl/6j gestational day (GD)16 fetuses exposed in utero to benzene (200 mg/kg) had significantly increased BFU-E and CFU-G (male and female), CFU-E (male only), and CFU-GM (female only) colonies. A higher dose of benzene (400 mg/kg) elicited a significant increase in male CFU-E colonies only. Conversely, the only effect observed in CD-1 GD16 fetuses was in females exposed in utero to 200 mg/kg of benzene, showing a significant increase in CFU-E colonies. This current study evaluated the hematopoietic system of mice exposed in utero to benzene 6 months prior to determination if strain and gender differences were still present. Pregnant C57Bl/6j and CD-1 dams were injected i.p. on GD 8, 10, 12, and 14 with either corn oil (vehicle) or benzene (200 mg/kg, or 400 mg/kg). Six months postpartum, colony formation assays on bone marrow cells and flow cytometric analysis of blood cell parameters in the spleen and bone marrow were performed. C57Bl/6j male mice exposed to 400 mg/kg had significantly increased bone marrow BFU-E and CFU-M colony numbers. Blood cell parameters in C57Bl/6j females revealed a significant decrease in bone marrow myeloid cells and splenic T-helper cells in both the 200 and 400 mg/kg groups. CD-1 mice had no significant changes in colony number; however, females in the 200 mg/kg group had significantly lower numbers of bone marrow myeloid cells. No malignancies were detected. Together, these results support the hypothesis that in utero benzene exposure significantly alters the hematopoietic system of mice and these alterations are strain and gender specific. [Support: CIHR]

1691 GESTATIONAL EXPOSURE TO THE TYPE II PYRETHROID DELTAMETHRIN RESULTS IN DECREASED EXPRESSION OF NEUROTROPHINS 3 AND 4 IN THE DEVELOPING HIPPOCAMPUS AND CORTEX.

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Due to concerns over the neurotoxicity of organophosphate and organochlorine pesticides in children, use of pyrethroids as well as one of the most neurotoxic. DM has been shown to have lasting toxic effects in rats, such as learning deficits and inappropriate induction of apoptosis and Brain Derived Neurotrophic Factor (BDNF) expression. However, there is little information on the consequences of low level, in utero DM exposure on brain development. To examine the neurotoxic effects of DM exposure, adult female Sprague Dawley rats were dosed by oral gavage with DM (1.0 or 0.5 mg/kg) or corn oil vehicle beginning on gestational day 1 (as evidenced by a vaginal plug) and continuing until parturition. Offspring of treated rats were sacrificed on postnatal days 14 and 21 and brain tissue was analyzed by histology and ICC. Preliminary studies revealed that rats treated with 1 mg/kg DM exhibited increased immunoreactivity for active caspase 3 in purkinje cells, as well as increased expression of neurotrophins 3 and 4 in the hippocampus and cortex. Decreased Purkinje cell number was observed in the posterior lobe of the cerebellum (control

1692 PBPK MODELING OF ALKYL BENZENE MIXTURES IN NEONATES: EVALUATION OF THE RELATIVE CONTRIBUTION OF INHALATION AND LACTATIONAL TRANSFER TO THE INTERNAL DOSE.

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Lactational transfer of environmental contaminants is of prime importance in consideration of internal dose to neonates. The objective of this study was to simulate the internal dose of alkylbenzenes (toluene (T), m-xylene (X) and ethylbenzene (E)) in neonates exposed via inhalation alone or by both inhalation and mother’s milk. A validated mixture PBPK model for alkylbenzenes previously validated in adult humans was adopted for pregnant women and neonates, by accounting for physiological differences. The lactational transfer was described as a function of the frequency of lactation, suckling rate and volume of milk. The simulated scenarios consisted of (i) 24 hr-exposure of mother and child to the ambient air containing alkylbenzenes (T and X) and (ii) caloric mixtures in infants via lactational transfer when the mother is exposed for 8 hr to a 3rd of the current limit value limits (TVLs) of the alkylbenzenes. When considering inhalation as well as lactational exposures (associated with maternal occupational exposures) in the PBPK model, the perinatal venous blood concentration (Cmax) of T in infants are simulated to be 16.5 fold higher than when the infant is exposed by inhalation route to TEx mixture at the R level. When area-under-curve of venous concentrations over a 24 hr-period was used as the dose surrogate, this factor, however, was 2.9. Similar values were obtained when considering single chemical exposures as well. This study demonstrates the feasibility of using PBPK models to simulate multiroute exposures of infants to chemical mixtures as well as to evaluate the relative contribution of occupational maternal exposure to the internal dose of chemical mixtures in infants.

1693 PERINATAL EXPOSURE TO A MIXTURE OF ENVIRONMENTAL CONTAMINANTS WHEN ASSOCIATED WITH POSTNATAL STRESS ALTERS THE GLUCOCORTICOID STRESS RESPONSE DURING ADULTHOOD IN THE RAT.


Rat studies suggest that perinatal events could reprogram the expression of the glucocorticoid receptor for the entire lifespan, creating abnormal hormone levels and predispositions to diseases. As part of a larger study, we tested the hypothesis of a link between perinatal exposure to environmental contaminants and abnormal corticosterone (CS) stress response (CSR) in rats. The experiment included 9 treatment groups. From gestation-day 0 (day of vaginal plug) and until postnatal day (PND) 20, dams were fed daily cookies laced with corn oil (control) or Mixture-A (MA): polychlorinated biphenyls, organochlorine pesticides, and methylmercury at 0.5 or 1.0 mg/kg/day (0.5MA, and 1MA). At birth, some control (C) and 1MA littermates were crossfostered to create 4 groups of pups with the following in utero/postnatal exposure: C/C, C/1MA, 1MA/C, 1MA/1MA. Other dams received cookies with a dose of 1.7 ng/kg/day of a mixture of aryl hydrocarbon receptor (AhR) agonists (AhR: non-ortho PCBs, dibenzodiazepins and furans) without or with 0.5MA. A CSR was induced in male offspring at PND85 (10 min exposure to a heating lamp to induce vasodilation and 5 min bag-restraint to facilitate the collection of 500 microliter of blood from the tail vein). The CS decay was assessed from the trunk blood collected at decapitation 30 min later (T30). The concentrations of CS returned to normal at T30 in the group C, 0.5MA, 1MA, C/C and 1MA/C, but it remained elevated in the group AhR, AhR+0.5MA, C/1MA, and 1MA/1MA. The most important finding is that 1MA had no effect on its own but it prevented the CS drop in animals exposed to AhR mixture at the RfC level. When area-under-curve of venous concentrations over a 24 hr-period was used as the dose surrogate, this factor, however, was 2.9. Similar values were obtained when considering single chemical exposures as well. This study demonstrates the feasibility of using PBPK models to simulate multiroute exposures of infants to chemical mixtures as well as to evaluate the relative contribution of occupational maternal exposure to the internal dose of chemical mixtures in infants.
1694 THE EFFECTS OF THE PESTICIDES MANEB AND PARQUAT ON THE DEVELOPING DOPAMINERGIC SYSTEM.

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Parkinson’s disease (PD) is a neurodegenerative disorder characterized pathologically by the loss of nigral dopaminergic neurons, and environmental factors have been widely implicated in the etiology of sporadic PD. Previous studies have shown that exposure to maneb (MB) and parquat (PQ) (two agrochemicals used on various crops) in mice induces a PD phenotype. Additionally, recent data suggests that exposure to environmental factors early in life can predispose or cause nigral dopaminergic neurodegeneration. The mechanism(s) responsible for this increased risk remains unknown and thus, was the focus of this study. Pregnant dams were treated with either saline, 0.6 mg/kg PQ, 2 mg/kg MB or PQ+MB from gestational day (GD) 10 - 15. The effects of these pesticides on the developing dopaminergic system where determined on GD16 and GD18 mouse embryos, 24hrs and 72hrs after the last exposure. The endpoints that were analyzed include PQ toxinokinetics, prostateal function, and catecholamine levels. Observable effects were measured in brain of embryos from dams treated with either PQ or PQ+MB, both at GD16 and GD 18, with higher levels observed at the earlier age. Embryos from dams treated with MB showed an increase in prostateal activity at GD18. A decrease in serotonin occurred in embryos from MB and PQ+MB treated dams at GD16 and GD 18, while a decrease in dopamine, DopAC, and HIAA was observed only in GD18 embryos. It was also determined whether there were gender-related differences due to exposure. These studies will give us additional mechanistic insight into how exposure to environmental factors can alter neurodevelopment, and subsequently increase the risk of PD onset later in life. Furthermore, future studies may identify specific targets, which can eventually be used or manipulated with specific interventions that can alter disease progression.

1695 AKT1-DEPENDENT GENE CHANGES ARE ASSOCIATED WITH BLOOD-TESTIS-BARRIER FOLLOWING EXPOSURE TO A POSTNATAL GOTOFFEN.

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Transient postnatal hypothyroidism induced by exposure to the goitrogen, 6-N-propyl-2-thiouracil, extends cell proliferation and differentiation leading to a subsequent increase in adult testis size. Akt1 has been implicated in the regulation of apoptosis in the testis. We sought to determine if Akt1 modulates germ and/or Sertoli cell proliferation and/or apoptosis following exposure to PTU. We exposed wild type, heterozygous, and Akt1-deficient mice to PTU (0.01%) w/v) via the dams milk. At postnatal day 90, exposed wild type and heterozygous male mice exhibited a 23% and a 15% increase in testis weight, respectively. A trend of increased testis size was also observed in Akt1-deficient males. Sperm production was increased in exposed wild type and heterozygous males by 20 and 16%, respectively. No increase in DSP was observed in Akt1-deficient males. Consistent with the hypothesis that Akt kinase activity is important to circumvent apoptosis, TUNEL analyses revealed a significant increase in apoptosis in the testes of exposed Akt1-deficient males. To elucidate Akt1-dependent gene changes mediating these phenotypic effects, we monitored changes in testicular gene expression in wild type, heterozygous, and Akt1-deficient testes following exposure. Postnatal exposure significantly altered 25,930 transcripts in Akt1-deficient males relative to exposed wild type and heterozygous males. Prominent functional categories were integrin signaling, germ cell maturation, and junction dynamics. Elucidation of regulatory networks was conducted using Ingenuity Pathways Analysis software. Analysis revealed that PTU targets proteins that make up the testicular tight junctions, including several members of the claudin gene family. In summary, the altered developmental pattern of male germ cell cancer. Previous studies demonstrate that exposure to dibutyl phthalate (DBP) during a critical window of gestation results in TDS-like effects. The present study investigated in utero exposure to DBP in the apoptotic resistant p53-null mouse model, evaluating both acute and long-term effects. Dams were treated with 250 or 500 mg/kg DBP by oral gavage from gestational day (gd) 12 until birth. Acute effects were assessed on gd 19 and postnatal days (PND) 1, 4, 7, and 10; long-term time-points ranged from 85 – 200 days. As expected, DBP exposure induced multinucleated gonocytes in the seminiferous cords. In wildtype and heterozygous mice these abnormal gonocytes were eliminated soon after birth, however, in p53-null pups the multinucleated gonocytes persisted as large multinucleated cells adjacent to the seminiferous tubule basement membrane. Immunohistochemical staining of these abnormal cells was negative for a gonocyte marker (GAL-3/4/6) and for a marker characteristic of testicular germ cell tumors (PLAP). This unique model identifies a role for p53 in the perinatal apoptosis of DBP-induced multinucleated gonocytes, and gives insight into the behavior and appearance of these abnormal cells in the p53-null environment.

1698 DEVELOPMENTAL SEX DIFFERENCES IN MYELINATION, ASTROCYTIC AND DOPAMINERGIC DENSITY: IMPLICATIONS FOR INCREASED RISK OF ADHD IN MALES.

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Developmental neurological diseases such as Attention Deficit Hyperactivity Disorder (ADHD) are four-six times more likely to affect males than females. ADHD symptoms are present by 2years of age, involve cerebellar and dopaminergic dysfunction and are associated with abnormal inflammatory responses. Exposure to toxicants and infections are implicated in ADHD suggesting sensitivity of the developing male brain immunogens and toxicants. We hypothesized latency in inflammatory cerebellar and dopaminergic protein synthesis in developing male brains compared with female brains. We obtained brain tissue from 7, 14, 21, and 42 postnatal day (PND) old Long Evans rats. Using a combination of ELISAs, western blotting, and digital immunohistochemistry, we measured markers for astrocytes (GFAP), microglia (Iba-1), myelin (MBP), dopamine (Tyrosine Hydroxylase:TH) and oligodendrocyte precursor cells (T.Lectin) in the cerebellum and caudate. We found increased astrocytic density in the cerebellum of PND14, PND21 and PND24 females (p<0.05) compared with males. PND27 males had increased TH in the caudate compared with females. We also found increased cerebellar MBP in the PND 14 and PND21 females compared with males. We suggest that increased astrocytic density may protect the female brain against infiltration of immunogenic agents through the blood brain barrier. Increased dopamine in...
PND7 males suggests the developing male brain may be particularly sensitive to dopamine-associated toxics. Dopamine differences may be associated with sexual differentiation and the conversion of estrogen to testosterone in the basal ganglia. In the future we aim to characterize the response of developing male and female brains exposed in utero to dopamine-associated toxics; PCBs and PDN. This will help us better understand the functional relationship between development gender differences in dopaminergic and gial cells, and an increased prevalence of ADHD in males. NIH grant #ES0168803

1699 AKTI MEDIATES EPIGENETIC REGULATION VIA HISTONE MODIFICATIONS IN THE POSTNATAL TESTIS.

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Chromatin structure plays an important role in the regulation of gene expression in the testis. Recently, the PISK/Akt signaling pathway has been implicated in the regulation of chromatin remodeling. The methylation of lysine residues on histone tails is an epigenetic mark that influences chromatin repression when specifically imparted on lysines 9 and 27 of histone H3, and on lysine 20 of histone H4. Our recent work indicates that Akt1 plays a role in a stress-related stress response to mono-2-ethylhexyl-phthalate-induced injury. To gain further insight into the regulation of gene expression during postnatal development of the testis, we examined global gene expression changes in postnatal day 28 Akt1 wild type and postnatal day 28 Akt1-deficient testes using Affymetrix oligonucleotide microarrays (mouse 430 2.1). A total of 2,619 genes were significantly different in Akt1-deficient testes compared to Akt1 wild type testes (adj p-value < 0.05). Among these differentially expressed genes, 45% were down regulated and 55% were up regulated in the Akt1-deficient testes. Functional classification based on selected gene ontology terms indicated that over represented terms in genes up regulated in Akt1-deficient testes grouped to Akt1 wild type testes (adj p-value < 0.05). These results suggest a role for Akt1 in the epigenetic control of spermatogenesis and infer chromatin state as an important component in the response to injury.

1700 DEVELOPMENTAL EFFECTS OF PERFLUORONONANOIC ACID ARE DEPENDENT ON PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-ALPHA.


Perfluoronoanoic acid (PFNA) is one of the predominant perfluoralkyl acids in the environment and in tissues of humans and wildlife. PFNA strongly activates the mouse and human peroxisome proliferator-activated receptor-alpha (PPARα) in vitro and negatively impacts development and survival in offspring of mice exposed during pregnancy. In the current study, we use PPARα-knockout mice (KO) and 129S1/SvlmJ wild type mice (WT) to investigate the role of PPARα in mediating the in vivo effects. KO and WT females were mated with males of the same strain overnight and plug positive mice were dissected orally with water (vehicle control; 0.01 ml/g), 0.83, 1.1, 1.5, or 2 mg/kg PFNA on gestational day (GD) 1-18 (day of plug = GD 0). Dams and pups were monitored daily. Dam weight gain was unaffected in either strain. The number of uterine implantation sites was unaffected in either strain. The number of pups at birth was unaffected although pup weight at weaning (postnatal day 21) was reduced in WT females at 2 mg/kg. Eye opening was delayed (mean delay = 2.1 days) in WT pups at 2 mg/kg. Survival of offspring to weaning was reduced to 28% and 21% of control in the 1.1 and 2 mg/kg groups in WT. Liver-body weight ratio at weaning was increased in a dose-dependent manner in WT dams and pups, but only slightly in the highest dose group in KO dams and pups. In dams, this was a 1.1 fold increase over controls at 2 mg/kg in KO compared to a 2 fold increase over controls at 2 mg/kg in WT. In summary, the only response to PFNA found in KO mice was a slight increase in relative liver weight of dams and pups at 2 mg/kg, in contrast to the effects on liver weight, pup eye opening and pup survival in the WT. These results suggest that PPARα is a primary mediator of PFNA effects on pup development and survival to weaning. This abstract does not necessarily reflect UNEPs policy.

1701 NEONATAL AMPHETAMINE EXPOSURE AND CEREBELLAR GRANULE CELL NUMBER IN RATS: A STEREOLOGY STUDY.

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The use of amphetamine (AMPH) is rising globally. Among AMPH abusers, women of reproductive age are of particular concern due to the potentially harmful effects of AMPH on their fetuses. In this study, we examined the long-term effects of AMPH exposure during the brain growth spurt on cerebellar cytoarchitecture by estimating the granule cells in cerebellar lobules I and VII. On postnatal day (PD) 4, Sprague-Dawley rat pups were assigned to four groups: AMPH 5, 15, 25 (5, 15, and 25 mg/kg/day AMPH) and IC (injection control; 0 mg/kg/day). Animals received intragastric intubation twice a day with a 2-hr inter-intubation interval from PD 4-9. A normal control was also introduced. The cerebella of the animals were removed on PD 68 and processed for stereological cell counting. Cerebellar granule cells of lobules I and VII were counted to examine the effects of early postnatal AMPH and to determine whether the detrimental effects of AMPH were lobule-specific. The data showed that the estimated granule cell number and the area (three-dimensional) where the granule cells were located were not affected significantly by the AMPH treatment, and there were no lobule specific effects, indicating that amphetamine exposure during the brain growth spurt did not exert long term effects on the granule cell numbers in both anterior (lobe 1) and posterior (lobe VII) portions of the cerebellum. Although the cell number and size of the granule cell body of these adult cerebells did not result in significant changes regionally, the density of the granule cells in the AMPH 5 showed a marginally significant increase compared with those of other groups. Taken together with findings of other studies evaluating the effects of neonatal AMPH exposure, it suggests that neonatal AMPH treatment has limited effects on cell loss and the ultimate fate of a cell, and that other measures, such as molecular or electrophysiological approaches, are needed to reveal the presence of detrimental AMPH-induced neurotoxicity on developing brains. (Supported by NIH grant DA018809)

1702 EMBRYO-FETAL DEVELOPMENT IN CYCLOMOLGUS MACAQUE: EVALUATION FOR TERATOLOGY BY ULTRASONOGRAPHY.

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Assessment for teratology (Segment II) and F1 (Segment III) preclinical studies in cynomolagus macaque (Macaca fascicularis) is presented, with fetal and infant data obtained using a single maternal animal (N = 36). The end-points for Segment II are measured without fetal evaluations at Caesarean section. Embryo-fetal ultrasonic imaging measurements included crown-rump length (CRL), at gestation day (GD) 25, 31, 37, 45; femur length (FL, at GD 37, 45, 53, 65, 93, 121, 123, 135); biparietal diameter (BPD, at GD 8, 15, 31, 45, 90, 110, 130); and heart rate (HR, at GD 44, GD 51, GD 65, GD 93, GD 121, GD 150). The newborn infant was examined for external morphology, at 30 days for skull and face development by radiography, and at six months for organ development by necropsy. Embryos between GD 25 and 31 were measured for the greatest length (GL). In embryos greater than GD 31, CRL excluded tail measurement. The mean GL at GD 25 was 4.5 mm and 8.5 mm at GD 31. The mean CRL was 14.3 mm at GD 25, 21.3 mm at GD 70, 30.2 mm at GD 90, 36.9 mm at GD 110, and 41.2 mm at GD 130. Mean heart rate ranged from 177 to 181 bpm between GD 53 and GD 150. The longest view of the ossified portion of the skull line on one side to the inner margin of the far one (Tanantal and Hendricks, 1988). Mean BPD was 12.9 mm by GD 53, 21.3 mm by GD 70, 30.2 mm by GD 90, 36.9 mm by GD 110, and 41.2 mm by GD 130. Mean heart rate ranged from 177 to 181 bpm between GD 53 and GD 150. The longest view of the ossified portion of the femur was measured with the linear internal clipper cursors. Mean GL was 9.2 mm by GD 70, 17.4 mm by GD 90, 25 mm by GD 110, and 30 mm by GD 130. Infant weight mean was 431.1 grams. In conclusion, data from the measurements combined with adequate infant examination adequately assesses teratology without resorting to fetal evaluations thus negating the need for Caesarean section and minimizing the number of non-human primates required for stand-alone Segment II and III studies.

1703 THE DEVELOPMENTAL TOXICITY OF ALCOHOL OR NICOTINE: A ZEBRAFISH (DANIO RERIO) MODEL.

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One major challenge for researchers in the field of Development Toxicology is to identify the mechanisms for perinatal drug-induced toxicity on the developing brain. It has been hypothesized that microRNA (miRNA) may play a role in mediating drug-induced developmental toxicity. miRNAs are noncoding transcripts that
are thought to be involved in patterning and differentiation of the central nervous system, post-transcriptionally modulate the expression of mRNAs, and the maintenance of cell fate identity. In this study, a zebrafish (Danio rerio) model system was used to explore the morphological changes of the zebrafish in response to alcohol, nicotine or the combination of the two. Zebrafish embryos were exposed to 2% (w/v) alcohol, 40 μM nicotine, the combination of alcohol/nicotine or 2% artificial salt water (control) beginning at 4 hours post-fertilization (hpf). The treatment lasted for 3.5 hours during gastrulation. The developing embryos were evaluated for morphological changes at 72 hpf and sacrificed for miRNA analysis. The results showed that the zebrafish assigned to the alcohol and the alcohol/nicotine groups exhibited significant morphological changes, such as cardiac edema and a curved vertebral column. However, the zebrafish in the nicotine group did not show significant changes compared with the control group. The miRNA expression ratio between the alcohol and the control group identified a number of miRNAs that were sensitive to alcohol with a significant down-regulation in the alcohol group compared with the controls. These miRNAs include let-7h, let-7i and miR-456, which are involved in axonal guidance, cell migration and the development of the nervous and the cardiovascular systems, and miR-129 which is involved in the cell cycle and nervous system development. These findings provide direction for future studies to evaluate the role of these targeted miRNAs as the underlying mechanisms for alcohol-induced developmental toxicity. (Supported in part by Texas A&M HSC Summer Research Program)

**1704 SPONTANEOUS ABORTIONS, PREMATURE BIRTHS AND STILLBIRTHS IN EMBRYO-FETAL AND POST-NATAL REPRODUCTIVE TOXICITY STUDIES IN CYNOGMOLUS MONKEY.**


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The cynomolgus monkey (Macaca fascicularis) is a valuable animal model for pre-clinical embryo-fetal and post-natal reproductive toxicity studies. For accurate study data interpretation, it is essential to continually update, as a reference, background incidences of spontaneous abortions (AB), premature births (PB) and stillbirths (SB). Loss of embryos or fetuses on or before gestation day 130 (GD130) i.e. AB, delivery (live or dead neonate) between GD131 and GD145, i.e. PB, and delivery of live or dead neonates on or after GD146, i.e. SB data were compared between two CDI facilties reporting incidences at SNBL USA and 12.9% at SNBL Japan. Incidences at SNBL USA / SNBL Japan were 14.8% / 9.6% for AB, 4.5% / 2.0% for PB and 7.1% / 9.0% for SB. Approximately 50% of the abortions occurred on or before GD30. In the case of early embryo losses (on or before GD30), there were no notable differences in maternal age (all 3 to 10 years old), maternal body weight (aborted / normal ~ 3.40 ± 0.96 kg / 3.56 ± 0.84 kg) and menstrual cyclicity (28.8 ± 2.1 days / 30.9 ± 4.5 days) at mating among aborted animals and maternal animals which maintained pregnancy. For stillbirths, there were no notable differences in maternal age (all 3 to 10 years old) and maternal body weight (stillbirth / normal birth ~ 5.12 ± 0.91 kg / 4.92 ± 0.74 kg) at delivery between maternal animals with stillbirth birth and normal birth. In addition, F1 neonate body weight at birth were comparable between stillborn neonates (380.5 ± 38.1 g) and live born neonates (348.7 ± 41.7 g). The stillbirth ratios in the two facilities were lower than that of an outdoor breeding colony reported in the literature. In conclusion, study procedures, such as frequent restriction for dosing and blood collection or sedation, do not affect the rate of spontaneous embryo-fetal losses or natural birth.

**1705 TERATOGENIC EFFECT AND TESTICULAR CHANGES INDUCED BY DI-N-BUTYL PHOSPHATE ADMINISTERED ORALLY TO RATS.**


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Di-n-butyl phosphate (DBP) is one of the commonly used plasticizers. It can enter the environment through various routes and has been shown to affect reproductive and developmental processes of the organism. Therefore, the present study was carried out to explore the teratogenic and testicular effects of DBP in rats after gavage administration. In the first of two experiments, thirty adult pregnant female rats were divided into three groups including control, receiving corn oil, DBP at a dose of 1.10 LD50 (800 mg/kg), and DBP at a dose of 1.20 LD50 (400 mg/kg) from day 6th-15th of gestation. Incidence of resorptions and dead fetuses were significantly increased in both DBP treated groups. In addition, there was a significant decrease in fetal body weight and length and a significant increase in the incidence of fetuses with visceral and skeletal malformations, especially incomplete ossification of cranial bones, open wide fontanelle, absence of ossification center of vertebrae, absence of sternbrae & xiphistrenbrae, absence of phalanges of both limbs and absence of coccygeal vertebrae. In the second experiment, twenty pregnant female rats were divided into three groups including control, receiving corn oil, and DBP at a dose of 1.20 LD50 (400 mg/kg,b.w) DBP daily for 65 days via gastric intubation. DBP induced a significant decrease of sperm motility and sperm cell count and a significant increase in sperm abnormalities including detached head, bent tail, looped sperm, and stained sperm. As the level of testosterone remarkably decreased with increase of LH hormone was observed in treated groups compared with controls. Thus, DBP may affect fertility and epididymal spermatozoal characteristics in males. In addition, teratogenic data demonstrate that DBP also appears to have an adverse effect on the developing fetus.

**1706 ULTRASOUND-BASED DETECTION AND MONITORING OF THALIDOMIDE-INDUCED FETAL MALFORMATIONS IN THE CYNOGMOLUS MONKEY (MACACA FASCICULARIS) MODEL.**

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Recent developments including the increased demand for nonhuman primates in biomedical research have led to considerations of new de- signs for embryofetal and pre-postnatal studies, e.g. advanced imaging (i.e. Doppler-ultrasound-based) of embryonic and fetal development as a surrogate for Caesarean section followed by sophisticated evaluation of the infant. It is crucial to evaluate the sensitivity of ultrasound for detection of abnormalities during embryofetal development. This study evaluates the feasibility of ultrasound-based embryofetal development monitoring. Thalidomide (thal), a well established teratogen, was used as a positive control. Five female cynomolgus monkeys received 15 mg/kg thal on gestational days (GD) 26 to 28. Five controls received vehicle only. Animals were housed individually and studies were conducted in accordance with the German Animal Welfare Law and were approved by IACUC. Ultrasound evaluations were performed on GD 20, 30, 44, 58, 72, and 86. Caesarean section was performed on GD 100 in both groups. In the control group, no prenatal loss and malformations were observed. In the thal-exposed group, in two females early caesarean sections were performed on GD 41 and 49 due to intra-uterine death. Three live fetuses were available as Caesarean-section. For one fetus, polydactyly was recorded by ultrasound on GD 58. For the second fetus, abnormalities of the upper extremities were detected from GD 72 onwards. The ultrasound-based findings were confirmed at Caesarean-section for both fetuses. In the third fetus, no malformations were detected by ultrasound and at external examination the only finding was a shift of prepuvium to the left. In conclusion, advanced ultrasound enables early detection (prior to Caesarean-section) and precise monitoring of embryofetal development and abnormalities in this relevant nonhuman primate model.

**1707 THE CHICKEN EMBRYOTOXICITY SCREENING TEST (CHEST) AS PUTATIVE METABOLICALLY ACTIVE IN VITRO TEST SYSTEM FOR TERATOGENICITY/ EMBRYOTOXICITY.**


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The chick embryo has been described to provide a useful test system for the determination of teratogenic/ embryotoxic effects. Within this study, the chicken embryotoxicity screening test (CHEST) was evaluated for its predictive value for teratogenicity/ embryotoxicity of exogenous chemicals. The CHEST is performed using fertilized hen eggs during early developmental stages which by legislation are not considered to be an animal. Therefore, the CHEST can be considered as a replacement method. In contrast to other embryotoxicity assays (e.g. whole embryo culture), the CHEST is easier to conduct and does not require the use of embryonic stages from mammalian species. Additionally, it has been demonstrated that xenobiotic metabolizing enzyme activities are present in hen eggs and that testing and evaluating twenty of the most potent teratogens should be possible. We have tested teratogens (e.g. retinoic acid, hydroxyurea, valproic acid, thalidomide), non-teratogens and proteratogens in this in vitro assay. The results show a good correlation with the teratogenic potential of these substances in vivo. Specifically, we confirm that proteratogens are detected as being “positive” in this assay. Investigations on the metabolic capacity of the CHEST indicate the presence of metabolic capacity. Therefore, the CHEST assay has confirmed its potential to serve as a replacement study and may significantly reduce the numbers of animals needed for teratogenicity/ embryotoxicity testing.
Low activities of antioxidant enzymes including catalase, which detoxifies hydrogen peroxide (H2O2), leave the embryo highly susceptible to pathogenic reactive oxygen species (ROS). Although treatment with exogenous catalase is embryoprotective, little is known about endogenous embryonic catalase expression, inter-individual variability or protective importance, which we investigated in wild-type (C3H/HeFeJ) and acatalasemic (C3H/Gc-Catf/J) mice. Acatalasemic mice exhibited substantially lower catalase activity in all organs (p < 0.05), with the largest 82% decrease in brain. Conversely, the activity of glutathione peroxidase (GPx), which also detoxifies H2O2, was modestly elevated in all organs but one in acatalasemic mice (p < 0.05), with an increase of only 14% in brain. Untreated acatalasemic mice were more susceptible than wild-type controls to both in utero and postnatal death, with a 3-fold greater incidence of resorptions and preweaning lethality (p < 0.05). On the other hand, when treated with the ROS-initiating anticonvulsant drug phenytoin, acatalasemic dams did not differ in embryonic DNA oxidation or fetal death. These results provide direct evidence that the relatively low level of endogenous embryonic and fetal catalase provides important protection against physiological but perhaps not drug-enhanced oxidative stress. Catalase deficiencies constitute a risk factor notwithstanding the small compensatory increase in GPx activity. This protective importance against endogenous oxidative stress is similar to that observed for a more highly expressed antioxidant enzyme, glucose-6-phosphate dehydrogenase, except the latter also protects against drug-enhanced oxidative stress. (Support: Canadian Institutes of Health Research)

1709 EMBRYO-FETAL DEVELOPMENT STUDY (ICH-3) DATA FOR CD-IGS RATS TREATED BY INHALATION.


Inhalation is becoming a more common clinical route of treatment for a variety of pharmaceuticals and biopharmaceuticals. For embryofetal development studies (ICH-3), nose-only inhalation exposure can be utilized to mimic the clinical route. Data from studies treated by oral gavage, subcutaneous and intravenous injection as well as room controls were compared to the vehicle controls from a number of inhalation studies. Time-mated rats Crf(CD)SD, 10 to 12 weeks of age at the start of treatment, were housed individually and were provided a standard certified pelleted commercial laboratory diet and water ad libitum. The animal room environment was acclimated to the restraint tubes for increasing periods of time for a minimum of 3 consecutive days prior to the initiation of treatment. Animals were exposed to atmospheres (aerosols, typically of dry powders, generated by EDPDS or metered dose inhalers) on a nose-only flow-through chamber for up to 2 hours daily from Days 6 to 17 of gestation, inclusive. Comparison of in-life data, showed no effect from the acclimation or dosing procedures for the inhalation studies when compared to other routes. Maternal, ovarian and uterine findings also showed no difference between inhalation and other routes of administration. No differences were observed from the acclimation or dosing procedures for the inhalation studies when compared to other routes of administration.

1710 ASSESSING THE POTENTIAL DEVELOPMENTAL TOXICITY OF THE RED TIDE TOXIN, BREVETOXIN, IN CD-1 MICE.

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Brevetoxins are produced by Karenia brevis, the algae causing Florida Red Tide. Previously, brevetoxin 3, or its metabolites were detected in fetuses of dams administered the compound via the lung, or systemically. The purpose of this study was to begin evaluation of the potential developmental toxicity of brevetoxin. Pregnant CD-1 mice were administered brevetoxin (50 ng/kg/hr) via osmotic minipump beginning on gestational day (GD) 6. The total deposited dose in these mice is estimated to be 200 times higher than that currently allowed by federal regulations. Dams were treated for a single 2 hour visit to a beach where brevetoxin concentrations were 50 ng/l air. Mice were euthanized on GD19. Numbers of implantsations, resorption sites, live and dead fetuses, and fetal sex were recorded. Fetuses were weighed, crown to rump length measured and external malformations recorded. Pups were fixed for examination of soft tissue and skeletal abnormalities. Brevetoxin-exposed dams exhibited no clinical signs of toxicity. To date there were 16.2 ± 0.5 fetuses/dam (n = 4). Fetal viability was 98%; 49.5 ± 8.7% were females. Pup weights were similar for females (1.44 ± 0.11 g; n = 53) and males (1.51 ± 0.11 g; n = 32), as were crown to rump lengths (1.1 ± 0.15 mm for females, 1.46 ± 0.10 mm for males). By comparison, male and pup weights from saline control dams were 1.35 ± 0.19 g and 1.38 ± 0.18 g, respectively. There were no incidences of exencephaly, eye, limb, digit, or tail abnormalities in pups from either brevetoxin exposed or control dams. These preliminary data suggest that brevetoxin, administered throughout the period of organogenesis, does not adversely affect implantation, fetal viability, fetal growth, or cause obvious external malformations. Further studies, including examination of visceral and skeletal changes, are needed. Progress Research funded by Florida Fish and Wildlife Conservation Control and Mitigation Grant Program Number: 68A-2.015.
1713 EVALUATION OF PHYSICAL, SENSORY AND SEXUAL LANDMARKS IN AN EXTENDED ONE GENERATION REPRODUCTION STUDY: A COMPARISON BETWEEN A CLASSICAL NOAEL AND A BENCHMARK APPROACH.

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The study was designed to investigate in a rat model the changes in developmental landmarks associated with prenatal exposure to methylmercury (MeHg). The landmarks comprised a series of tests establishing the phase of development from postnatal day (PN) 1 to PN 46. A comparison was made between a statistical analysis using the classical NOAEL approach and the benchmark approach. Female F0-animals were dosed with MeHg (GD6-LD10: 0 (corn oil vehicle), 0.1, 0.4, 0.7, 1.0, 1.5, or 2.0 mg/kg/day; oral gavage). F1-animals were used for evaluation of physical, sensory and sexual landmarks from PN 1 to PN 46. In addition to the classical approach, data were analyzed by the benchmark approach using PROAST 16.4 running on Splus. For each landmark, a dose-response model was fitted to the data. From this fitted model, a critical effect dose (CED or benchmark dose (mg MeHg/kg BW)) was derived, for a predefined critical effect size (CES) of 10%. The CED's found for the different landmarks on a specific postnatal day all showed a dose response relationship, except for pinna unfolding and hair growth which could not be fitted to a model. Tooth eruption showed a maximal response of <10% and could not be analyzed with a CES of 10%. Body length showed a CED>2 which is outside the dose range. No dose-response relationship was observed with the classical NOAEL approach and, except for pinna unfolding, hair growth and tooth eruption; not any significance was observed compared to the vehicle control group. So, using identical group sizes, the statistical power of the benchmark approach is stronger than that of the NOAEL approach since a dose response relationship is demonstrated for most parameters. More research with lower numbers/group and reliable CES descriptions is needed and e.g. introduction of corrections for litter size, mortality, etc. to exclude false positive and false negative findings.

1714 INFLUENCE OF AGE ON SUB-CHRONIC TOXICITY OF AQUEOUS LEAVES EXTRACT OF CALOTROPIS PROCERA IN RABBITS.

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Calotropis procera is a major browse toxic plant for grazing animals. Animals un-dergo different changes throughout their developmental stages and this affects their susceptibility to drugs inducing injury. The aim of this study was to investigate the influence of age on sub-chronic toxicity of aqueous leaves extract of Calotropis procera in rabbits. The twenty five rabbits used during the study were classified in five subsets with a 6-color analytical flow cytometry using hematology samples from cynomolgus monkeys. The term dioxin is used for members of the polyhalogenated aromatic hydrocarbons that are structurally related and have a common spectrum of biologic responses mediated via binding to a specific intracellular receptor. The prototype chemical for this class is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). TCDD is a potent suppressant of adaptive immune responses. The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor involved in the altered gene expression and toxicity production by TCDD. To characterize the molecular mechanism by which TCDD alters human B cell differentiation and function, a major obstacle to doing so is a convenient renewable source of human B cells and genomic material from donors. One way to overcome this obstacle is to utilize human cell lines such as the SKW6.4, which are immortalized human B cells. However, these cell lines lack the AHR, the receptor to which dioxin and dioxin like compounds bind to produce their biological and toxic effects. A way to circumvent this problem is to stably introduce the gene that codes for the AHR into the genome of SKW6.4 cells. To assess the influence of a test substance administration on the immune system, especially producing specific antibodies against the T-cell dependent antigen, Keyhole Limpet Haemocyanin (KLH), we developed and validated a method for detecting anti-KLH antibodies in KLH-treated rat sera using indirect ELISA. The parameters taken into consideration in assay development and validation were precision, sensitivity, immunodepletion, drug interference, and stability. Rat blank sera from 20 individual lots were analyzed for determination of negative cut off and cut point factor. The % CV for intra-assay and inter-assay precision were analyzed with positive control samples and ranged from 0.7 to 8.5 and 15.3 to 21.4, respectively. The sensitivity of this assay was 100 ng/ml at which the OD value was greater than the normalized negative cut off value. Immunodepletion assay was developed for further confirmation of the positive samples during the in-study analysis. Drug interference was observed from 1 ng/ml of KLH at low positive control. Results from stability assessment indicated that anti-KLH antibodies were stable for at least 6 hours at room temperature, for 4 weeks in a deep freezer, and during three freeze and thaw cycles. In conclusion, we have validated a method for analyzing anti-KLH antibodies in rat serum. All the results met the preset acceptance criteria suggesting that this method is suitable to assess the immunomodulating potential of a test article through detecting the antibodies elicited by KLH-treatment in rats using ELISA method.
based vectors. In addition, we found that polybione at a concentration of 4ug/mL improved gene transduction by the virus into the SKW6.4 cells. Infection by centrifugation in comparison to mere incubation with virus at 37°C significantly increased the viral transduction. In summary, using polybione and infection by centrifugation resulted in maximal viral transduction of SKW6.4 cells. (Supported by McNair/SDRO Program and NIH ES04911)

**1718 ESTABLISHMENT OF AN IGm ANTIBODY FORMING CELL RESPONSE MODEL FOR EVALUATING IMMUNOTOXICITY IN HUMAN PRIMARY B LYMPHOCYTES.**

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Historically, identification and characterization of immunotoxins has been conducted in rodents, most often using mice. The IgM antibody forming cell (AFC) response developed in rodents, in combination with other immune function assays, has been effective for identifying rodent immunotoxins. In spite of the similarities between the mouse and human immune systems, uncertainties still arise concerning whether results derived from rodent models are predictive of human toxicity. Therefore, the objective of this study was to establish an in vitro IgM AFC response model employing human peripheral blood B lymphocytes. CD40 ligand (CD40L) mimics the cognate T cell-B cell interaction occurring in vivo in lymphoid germinal centers. Thus, a mouse fibroblast line stably transfected to express human CD40L (CD40L-L cell) was utilized to provide the CD40L stimulus for B cells. Human B cells were isolated from leukocyte packs obtained commercially. Initial experiments were conducted to optimize various aspects of the model including, but not limited to, cell culture conditions, magnitude of CD40L stimulation, and identification of critical T cell cytokines. Co-culture of CD19+ human B cells with CD40L-L cells in the presence of interleukin-2 (IL-2), IL-6, and IL-10 induced a robust IgM AFC response as measured by both ELISPOT and ELISA.

Of the donors assayed thus far, the magnitude of response has been remarkably consistent, ranging between 4,000 to 6,000 IgM AFC/106 B cells. CD19+CD27 Naive B cells, more closely reflective of B cells obtained from laboratory animals, could also be driven into AFC. We have also adapted the human IgM AFC response into a 96 well format, which allows the assessment of multiple agents at multiple concentrations using B cells from one single donor. Collectively, a novel human IgM AFC response model has been established for evaluating immunotoxicity in human primary B lymphocytes. (Supported in part by the Dow Chemical Company/NIH ES04911)

**1719 DEVELOPMENT AND VALIDATION OF A HUMAN LYMPHOCYTE ACTIVATION (HULA) ASSAY.**


In vitro assays to assess human antigen-specific responses are needed to evaluate potential immunosuppressive effects and rank order compounds during the drug development process. To address these needs, a human lymphocyte activation (Hula) assay was optimized and validated. Peripheral blood mononuclear cells (freshly isolated or frozen) from donors immunized with influenza vaccine are first incubated for 1 hour with test compound, after which influenza antigen (Fluzone®) is added. Seventy two hours later, cultures are pulsed with BrdU. After 18-20 hours, cell viability is determined with trypan blue exclusion and BrdU incorporation is measured by a 96-well plate-based method. Assay conditions were optimized by evaluating different incubation times, donor cells, influenza antigen concentrations and BrdU staining procedures. Assay sensitivity and performance was evaluated by testing known immunosuppressive drugs. Cyclosporine A (Neoral®) was tested at dose ranges from 3-3000 ng/mL and found to have an IC50 of approximately 100 ng/mL or 85 nM (n=7 donors). Methotrexate was tested at 0.15-20 nM and found to have an IC50 of 4.5 nM (n=7 donors). At the concentrations of cyclosporine or methotrexate tested, decreases in proliferative responses were not associated with cytotoxicity. The recommended clinical therapeutic range for cyclosporine is 100-300 ng/mL or 85-250 uM. Methotrexate blood concentrations of approximately 20 nM is recommended for low dose therapy. Based on the initial findings with this assay, IC50’s appear to correlate well with the desired therapeutic ranges of cyclosporine or methotrexate. In addition, there is only moderate variability in IC50 differences between donors and little variability between assays for individual donors. An advantage of this assay is that a frozen cells can be used such that multiple donors could be driven into AFC. We have also adapted the human AFC response into a 96 well format, which allows the assessment of multiple agents at multiple concentrations using B cells from one single donor. Collectively, a novel human IgM AFC response model has been established for evaluating immunotoxicity in human primary B lymphocytes. (Supported in part by the Dow Chemical Company/NIH ES04911)

**1720 COMPARISON OF BOVINE VERSUS HUMAN PRIMARY EPITHELIAL CELLS IN AN IN VITRO MODEL FOR TESTING PARTICLE TOXICITY ON INNATE IMMUNE RESPONSES OF AIRWAY EPITHELIUM.**

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Primary human lung cells are often hard to obtain or they are expensive for utilization for high volume toxicity testing in vitro. Primary bovine tracheal epithelial cells (BTE) are easy and inexpensive to obtain and were hypothesized to be a suitable model for determining toxicity of particulate matter targeting innate immune responses in normal human bronchial and tracheal epithelium (NBHE). The purpose of this study was to compare the ability of stimulators to induce innate immune mediators in BTE and NBHE and to examine the ability of an air pollutant particle component, vanadium, to modulate these responses in each species. Cells were sham-exposed or exposed to 0.145μg/cm2·2.32 μg/cm3 VOSO4, for 6 hr, washed twice, and then stimulated with LPS or IL-1β for 24 hr. Total RNA was extracted, treated with DNase, and both semi-quantitative and quantitative RT-PCR was performed using primers for either human or bovine β-defensin-2, lactoferrin, lysozyme, IL-8, IL-6, iNOS, ICAM-1, and matrilysin. The innate immune response that vanadium affected similarly in these two species was LPS or IL-1β-induced β-defensin-2, where the induction of mRNA was inhibited by even the lowest [VOSO4]. IL-6, IL-8, lactoferrin, endotheiln-1, ICAM-1 and iNOS responses were differentially affected by vanadium in BTE and NBHE, suggesting that the pathways for IL-1β and LPS induction of these responses may diverge in humans and cattle. Use of BTE for modeling in vitro responses in human trachea is ideal for some specific innate immune responses—in this case, β-defensin-2. This may be an important biomarker for immunotoxicity of air pollutants, for evidence is growing that β-defensins are responsible for early innate immune pulmonary defense against both viruses and bacteria.

**1721 COMPARISONS OF THE IN VITRO IMMUNOMODULATORY EFFECTS OF ELASTIN AND COLLAGEN NANO-FIBROUS BIOMATERIALS BLENDED WITH POLYDIOXANONE.**

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Nanofibrous biomaterials, which can be produced utilizing electrospinning, are of particular interest in tissue engineering for many potential applications including vascular replacement. While electrospun blends of polylactideco-DL-lactideco-trimethylene carbonate (PDLA-ELA) with soluble elastin (PDO-ELAS) and collagen type I (PDO-CI) have each demonstrated significant potential uses, minimal evaluations of the potential effects of these materials on the immune system have been completed previously. This study investigated the immunomodulatory effects of electrospun PDO-ELAS and PDO-CI on human immunity following culture with splenocytes from female B6C3F1 mice. Endpoints examined included: lipopolysaccharide (LPS)-stimulated B-cell proliferation, F(ab’2), antibody fragment-mediated B-cell proliferation, and the Mischell-Dutton T-dependent antibody-forming cell (AFC) response to sheep erythrocytes. Results indicated that PDO-ELAS blends are more immunosuppressive than blends of PDO-CI. Specifically, PDO-CI blends were not suppressive of LPS-stimulated B-cell proliferation, while F(ab’2),-mediated proliferation was suppressed by no more than 50% and the in vitro AFC response by as much as 80%. In contrast, PDO-ELAS blends suppressed B-cell proliferation in both proliferation assays as much as 99.8%, while the AFC response was reduced by up to 70% of control. Based on these results, electrospun elastin may not be suitable for use in regenerative medicine applications, however it is necessary that additional testing of these materials be completed in order to determine if these results correlate with any potential in vivo effects. Funded in part by the American Heart Association Mid-Atlantic Affiliate (0554607U) and NIHES Contract ES 05454.

**1722 ANIMAL EXPOSURE IN EARLY LIFE IS ASSOCIATED WITH DECREASED TNF-α RESPONSES IN INFANCY.**

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Growing bodies of evidence suggests that the immunological development in humans is affected by animal exposure in early life. Because exposure to furred domestic and production animals is very common, the focus of our study was to investigate if animal contact during pregnancy and during the first year of life is SOT 2009 ANNUAL MEETING 357
associated with the cytokine responses of children at birth and at the age 1 year. Cord blood (n=228) and peripheral venous blood (n=200) samples at the age 1 year were collected. Ethical permission was granted by the Research Ethics Committee, Hospital District of Northern Savo. Whole blood samples were stimulated with Staphylococcal enterotoxin B (SEB), lipopolysaccharide (LPS) and the combination of phorbol 12-myristate 13-acetate and ionomycin (P/I) for 48h. The production of TNF-α, IFN-γ, IL-5, IL-8 and IL-10 were measured using sandwich ELISA. Information about animal exposure was collected with prospective questionnaires starting already from pregnancy and the obtained data was compared with stimulated cytokine patterns. We found that especially LPS and P/I-stimulated production of TNF-α was decreased in cord blood and/or blood collected at the age 1 year if mothers had animal contact during gestation. This effect was seen in infants whose mothers either lived at a farm or were in contact with dogs or horses during the pregnancy. In conclusion, maternal contact to pets and domestic animals during pregnancy may be associated with reduced immune responses of their offspring. This attenuation of cytokine production may aid in preventing exaggerated immune responses to harmless antigens later in life. Thus, the exposure to furred animals during pregnancy and during the first year of life may be an important factor in immunological development.

**1723 INDEPENDENT EVALUATION OF CRITICAL WINDOWS IN IMMUNE SYSTEM DEVELOPMENT MAY BE NECESSARY TO ACCURATELY PREDICT DEVELOPMENTAL IMMUNOTOXICITY (DIT).**

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DIT is a major area of investigation and several DIT testing paradigms have been proposed. One approach begins dosing on gestation day 7 (GD7), continuous dosing of the dams through weaning postnatal day (PND) 21. Unless it can be determined that the test compound or its metabolites are present in milk, dosing of the pups begins on PND3 and continues until PND42. This approach encompasses the critical periods during which immune cells and tissues develop and mature. The objective of these studies was to determine the effect of dibenzanthracene (DBA) on the developing immune system and to ensure effects on specific periods of immune development were captured. A study in adult female B6C3F1 mice assessed effects of DBA on antibody (Ab) responses to establish doses for the DIT studies. A dose-related decrease in Ab production was observed following 28 days of treatment with 250, 793, or 2500 μg/kg DBA. Suppression occurred at all doses. Following the DIT paradigm, mice were exposed in utero at 0.25, 2.5 and 25 μg/kg DBA starting on GD7 and continuing through PND42. As DBA metabolites were detected in milk, pups were not directly treated until weaning. Ab suppression was observed at the 25 μg/kg dose, suggesting the developing immune system was approximately 10 times more sensitive than adults. When pups were exposed in utero, through lactation and evaluated on PND42, no significant effects were observed at doses as high as 2500 μg/kg. In contrast, juvenile mice treated from PND21 - PND49 demonstrated decreases in Ab production with a LOEL of 2.5 μg/kg. Thus during the juvenile period, PND21-PND49, mice were more sensitive when evaluated throughout development and 100 time more sensitive than adult animals treated with DBA. These data suggest the developing immune system is a sensitive target following toxicant exposure, and independent assessment of specific windows of immune system development may be necessary to establish accurate safety levels. Supported in part by NIEHS Contract ES 054554.

**1724 PRENATAL EXPOSURE TO CIGARETTE SMOKE SUPPRESSES ANTITUMOR CYTOTOXIC T-LYMPHOCYTE ACTIVITY POSSIBLY VIA CHANGES IN T-REGULATORY CELL NUMBER AND TGF-β LEVEL.**

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Epidemiological studies suggest that prenatal cigarette smoke (CS) exposure increases the incidence of some childhood cancers. Our previous study has demonstrated that male mice, exposed prenatally to mainstream CS (MCS), had a higher incidence of transplanted tumors and reduced cytotoxic T-lymphocyte (CTL) activity. In the current study, mouse dams were exposed to MCS (4h/d, 5d/wk) during gestation at a particulate concentration of 15 μg/m3. Pups were subsequently injected with EL4 lymphoma cells and the spleen and thymus recovered before injection (d 0) and at 1, 8, and 15 d post-injection to determine effects of prenatal smoke exposure on CTL activity, lymphoid organ subpopulations, and TGF-β levels. Whole splenocyte CTL activity against EL4 cells were significantly suppressed by prenatal CS exposure prior to, and at 8 and 15 d post-tumor cell injection; isolated CD8+ T-lymphocytes demonstrated similar suppression. Percentages of thymic T-regulatory (T-reg, CD4+CD25+FoxP3+) cells, which regulate/suppress effector T-cells, were increased in pups exposed prenatally to CS at 0, 1, and 8 d post-injection; significant decreases in splenic T-reg cells were observed, but only on d 8. Prenatal CS exposure increased levels of TGF-β released from both phorbol myristateacetate- and tumor cell-stimulated splenocyte cultures. Given that T-reg cells and TGF-β can suppress anti-tumor CTL activity, these findings suggest a possible mechanism by which fetal insult by CS could result in reduced CTL activity in the offspring during tumor development. These data suggest that children of smoking mothers may be unable to mount an appropriate immune response to tumors, thus, increasing their risk for developing cancer later in life.

**1725 IMMUNOTOXIC EFFECT OF PRENATAL CADMIUM EXPOSURE ON MURINE OFFSPRING.**

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Cadmium (Cd) is an environmental pollutant due to its widespread and continual use. Cd is generally found in low concentrations in food, air, and water, while its concentration in cigarette smoke is high. Although there is evidence demonstrating that adult exposure to Cd causes changes in the immune system, there are no reports of immunomodulatory effects of prenatal exposure to Cd. The thymus, which is the site of T cell development, is a target organ of Cd-induced toxicity. T cell development is essential for the establishment and maintenance of the adaptive immune system. Thymocytes mature through a series of stages defined by expression of cell surface markers CD4 and CD8. The most immature thymocytes are CD4+CD8+ double-negative (DN). In this study, pregnant C57Bl/6 mice were given free access to 10 ppm Cd water during pregnancy. At day 0 of birth, three pups from each litter were euthanized and thymus were removed. Thymocytes were analyzed by flow cytometry for specific cell surface markers. There was a significantly higher incidence of CD4+ and CD8+ cells among Cd-treated mice as compared to untreated controls. In addition, the CD4+/CD8+ SP ratio was significantly higher in Cd-treated mice. At 14 and 49 days of age, thymi and spleens were analyzed from the remaining litters to demonstrate that prenatal Cd exposure leads to immunomodulatory effects throughout adulthood. There was a significantly lower total number of splenocytes in Cd-treated than controls at both ages. Preliminary results suggest splenocyte phenotype changes that may be sex-specific. Following splenocyte stimulation with concanavalin A, cytokine release was analyzed and the IFN-γ and TNF-α levels were significantly decreased in prenatal Cd exposed mice. These results demonstrate that prenatal exposure to Cd results in phenotypic alterations in thymocyte development ultimately leading to immunomodulatory effects evident at adulthood. Supported by NIH grant ES015539.

**1726 CHLORPYRIFOS INDUCES APOPTOSIS IN HUMAN T CELLS.**

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We found previously that organophosphorus pesticides significantly inhibited cytotoxic T lymphocyte (CTL) activity. To explore the mechanism of organophosphorus pesticide-induced inhibition of CTL activity, we investigated whether organophosphorus pesticides can induce cell death/apoptosis in T cells in the present study. Jurkat human T cells were treated with chlorpyrifos at 0-1000 ppm for 2 and 4 hrs at 37°C in 5% CO2 in vitro. We first found that chlorpyrifos induced cell death of human Jurkat T cells in a dose- and time-dependent manner, as shown by LDH and MTT assays. Then, we investigated if chlorpyrifos -induced cell death consisted of apoptosis, as determined by analysis of Annexin-V staining and the intracellular level of active caspase-3 by flow cytometry, and DNA fragmentation analysis. We found that chlorpyrifos induces apoptosis in Jurkat T cells in a dose- and time-dependent manner, as determined by analysis of Annexin-V staining. DNA fragmentation was detected when cells were treated with 50 and 100 ppm chlorpyrifos for 4 and 6 hrs. Chlorpyrifos also induces an increase in intracellular active caspase-3 in Jurkat T cells in a dose- and time-dependent manner, and a caspase-3 inhibitor, Z-DEVD-FMK, significantly inhibited the chlorpyrifos -induced apoptosis. These findings indicate that chlorpyrifos can induce apoptosis in human Jurkat T cell, and this effect is partially mediated by activation of intracellular caspase-3. This work was supported by a grant from the Ministry of Education, Culture, Sports, Science, and Technology (No. 19590602).
1727 GENE EXPRESSION PROFILING OF MOUSE THYMOCYTES UPON IV ZITRO EXPOSURE TO BIS(TRI-N-BUTYL)OXIDE (TBO).
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Bis(tri-n-butyl)oxide (TBO) has been widely used as biocide and belongs to the class of organotins. Immunosuppression is the most important endpoint of TBO and it causes a reduction of thymus weight by depletion of cortical thymocytes. To get a better insight in the mechanism of action of TBO, in this study primary thymocytes of C57BL6 mice were exposed in vitro to different concentrations of TBO for 3, 6 and 11 hours. The applied concentrations were non-cytotoxic as deduced from WST-1 experiments. Subsequently, gene expression profiles were determined using Agilent 4x44K whole mouse genome microarrays and subjected to gene set enrichment analysis (GSEA). Using Gene Ontology gene sets representing various physiological processes, GSEA showed a clear upregulation of cell cycle genes after exposure to 0.5 μM TBO for 3 and 6 hours. Longer exposure time and higher TBO concentrations showed a down regulation of cell cycle related gene sets. Also genes involved in apoptosis and the unfolded protein response appeared to be affected after exposure to TBO. Comparison of the data from this study with various lymphocyte-related gene sets obtained from literature provided evidence for the involvement of different transcription factors like NFκB, NFAT and FoxP3. Particularly, gene sets derived from studies on activated T cells showed an overlap with the gene expression data from our TBO experiment. On the basis of these results it can be hypothesized that one of the effects of TBO on primary mouse thymocytes is the release of Ca2+ from the endoplasmatic reticulum which leads to activation of transcription factors, a Ca2+ influx into the cell and subsequently to activation and nuclear translocation of NFAT. Current experiments are directed toward testing this hypothesis.

1728 STAPHYLOCOCCAL ENTEROTOXIN B INDUCES VASCULAR LEAK AND ACUTE LUNG INJURY THROUGH DIRECT ACTIVATION OF NATURAL KILLER T CELLS.
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Staphylococcal enterotoxin B (SEB) is a highly toxic superantigen that can activate 5 to 30% of the T cell population and unlike conventional antigens; it directly binds to the MHC Class II molecules and activates T cells expressing variable TCR beta chains such as Vβ19. Such activation triggers massive cytokine release that leads to rash, fever, and can often lead to acute respiratory distress, multi-organ failure, coma and death. This study focuses on the precise role of NKT cells in SEB-induced acute lung injury. We demonstrate that the intranasal administration of SEB triggers Vascular Leak Syndrome (VLS) in the lungs of wild-type (WT) mice while VLS was significantly decreased in Cd1d-/- and Jalpha18-/- mice lacking NKT cells. In addition, mice deficient in NKT cells had significantly less amounts of IL-2, IFN-γ and TNF-alpha in the serum and bronchial alveolar fluid when compared to WT mice. Decreased infiltration of lymphocytes was observed in the lungs of Cd1d-/- and Jalpha18-/- mice, correlating with decreased cytokine production. When purified NKT cells were co-cultured with bone marrow-derived dendritic cells (DC), SEB was shown to directly activate NKT cells and lead to IL-2 production. This study, for the first time, demonstrates that NKT cells can be activated by SEB and FoxP3. Particularly, gene sets derived from studies on activated T cells showed an overlap with the gene expression data from our TBO experiment. On the basis of these results it can be hypothesized that one of the effects of TBO on primary mouse thymocytes is the release of Ca2+ from the endoplasmatic reticulum which leads to activation of transcription factors, a Ca2+ influx into the cell and subsequently to activation and nuclear translocation of NFAT. Current experiments are directed toward testing this hypothesis.

1729 TRIBUTYLtin: A DUAL BONE MARROW STROMAL CELL AND LYMPHOCYTE TOXICANT?
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TrIBUTYLtin (TBT) has been the subject of concern in the marine environment due to its use as an anti-fouling agent; however, measurement of organotins in stored seawater on long-term basis is rather difficult due to the tendency of these compounds to bind with biological substances. Recent studies have shown that TBT, uniquely, is a highly potent PPARγ/PGS2dual agonist. We have shown that bone marrow cells are susceptible to apoptosis induced by structurally diverse PPARγ agonists. We show here that TBT suppresses thymidine incorporation in primary pro-B cells (EC50 150 nM), a non-transformed pro-B/pro-B cell model (BU-11; EC50 15 nM), and an immature B cell lymphoma line (WEHI-231; EC50 185 nM) at levels comparable to those measured in humans (0.1-500 nM). Reduced thymidine incorporation largely results from an increase in apoptosis, indicated by the formation of a population of cells with a sub-G1/DNA content in BU-11 cultures treated with TBT (at 40 nM). The concentrations of TBT required to induce apoptosis correspond with those required to stimulate PPARγ/PGS2-mediated gene transcription in a Cos7 transfection assay. Studies are underway to determine if the TBT-induced apoptosis pathway requires PPARγ/PGS2-dependent gene transcription and if it differs from those that we have mapped for other PPARγ agonists. Interestingly, TBT (10-50 nM) induces adipsocyte differentiation, reduces granulocytes/macrophage colony formation, and alters cytokine profiles in human long term bone marrow cultures. We hypothesize that TBT may suppress B lymphopoiesis by two mechanisms, by inducing apoptosis in early B cells directly and by altering the bone marrow microenvironment that supports lymphopoiesis in general. Current studies are addressing the latter question using the B cell-supportive bone marrow stromal cell line BM52.

1730 IMMUNOTOXICOLOGICAL EFFECTS OF ASBESTOS ON HUMAN T CELLS.
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To analyze the possibility that immunotoxicological alteration in asbestos-related diseases (ARDs) such as asbestosis (ASB) and malignant mesothelioma (MM) may affect the progression of cancers, a human adult T cell leukemia virus-immortalized T cell line (MT-2Rst) was continuously exposed to 10 μg/ml of chrysotile-B (CB), an asbestos. After at least 8 months of exposure, the rate of apoptosis in the cells became very low and the resultant subline was designated MT-2Rst. The MT-2Rst cells were characterized by (i) enhanced expression of bcl-2, with regain of apoptosis-sensitivity by reduction of bcl-2 by siRNA, (ii) excess IL-10 secretion and expression, and (iii) activation of STAT3 that was inhibited by PP2, a specific inhibitor of Src family kinases. These results suggested that the contact between cells and asbestos may affect the human immune system and trigger a cascade of biological events such as activation of Src family kinases, enhancement of IL-10 expression, STAT3 activation and Bcl-2 overexpression. This speculation was partially confirmed by the detection of elevated bcl-2 expression levels in CD4+ peripheral blood T cells from patients with MM compared with those from patients with ASB or healthy donors. Further studies will be required to verify the role of T cells with enhanced bcl-2 expression in tumor progression induced by asbestos exposure. In addition, high plasma concentrations of interleukin (IL)-10 and transforming growth factor (TGF)-β, and multiple over-representation of T cell receptor (TCR)-β diversity in peripheral CD3+ T cells found in MM patients. We also detail an experimental long-term exposure T-cell model. Analysis of the immunotoxicological effects of asbestos may alter the occurrence of asbestos-related malignant tumors.

1731 LOW-DOSE MERCURY TARGETS MACROPHAGE ACTIVATION IN HUMAN CELLS IN VITRO.
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There is growing recognition that exposure to both inorganic and organic mercury (Hg) compounds can affect the human immune system, in addition to the nervous system, with potential consequences for host resistance to infection and the risks of autoimmune disease. We have investigated the human response to inorganic Hg using an in vitro system composed of LPS-stimulated human peripheral blood mononuclear cells (PBMCs), a cell population which includes T cells, B cells, monocytes, and macrophages. We have previously reported that we saw no changes in markers of activation or cell populations in both T and B cells, but that we did observe a consistent increase in pro-inflammatory cytokines in response to Hg. These inflammatory signals prompted us to examine the responses of monocytes and macrophages more closely. We recruited six volunteers (3 males, 3 females) to donate blood. PBMCs were isolated and cultured with LPS and low levels of HgCl2 (up to 200 nM). After 48 hours in culture, the cells were stained and analyzed by flow cytometry. While we did not observe a change in the number of CD11b+ cells over the concentration of Hg used, we did observe subtle but consistent changes in markers of macrophage maturation and activation. The percentage of CD11b+ cells that were CD80+/CD86- cells significantly increased, as did the percentage of CD11b+ cells that were CD80+/TLR4-. The percentage of...
CD86+ / TLR4+ significantly decreased. These results indicate that Hg can exert significant effects on the human immune system at very low levels, and that macrophages may be the cell population that is primarily affected by Hg exposure. The observed increases in pro-inflammatory cytokines also support this hypothesis. Hg is also known to disrupt immune signaling pathways and cytokine release by microglia, affecting neuronal migration. These results provide evidence that the immune response to Hg is related to the neurotoxic effects of Hg.

1732 POTENTIAL IMMUNOTOXICITY BY 2,3 BUTANEDIONE IN BALB/C MICE.


Occupational exposure to high concentrations of 2, 3-butanedione (diacetly)-containing butter flavor seasoning has been associated with an increased prevalence of airway obstruction. Previous studies in our laboratory to investigate potential immune-mediated mechanisms of diacetly-induced toxicity have demonstrated that BALB/c mice exposed to diacetly in a modified local lymph node (LN) assay exhibited an increase in cervical lymph node cell proliferation at ≥3 mg/kg/d, and in absolute number of CD3+, CD8+, CD4+, CD8+, and Mac-1+ cells in LN and spleen at 25 mg/kg/d. CXCL10 protein expression was increased at ≥25 mg/kg/d in LN, spleen, and serum, and CXCL9 was upregulated at ≥10 mg/kg/d in LN. As CXCL9 and CXCL10 and their common receptor, CXCR3, have been associated with inflammation and obstructive pulmonary disease in humans and animal models, we examined expression of these inflammatory markers in immune tissues from BALB/c mice following diacetly exposure. Enhanced histopathological evaluation revealed limited adverse pathology; 2-5 out of 5 mice in the 25 mg/kg/d group exhibited lymphoid hyperplasia in lymph nodes. Spleen and thymus of all mice were histologically normal and there was no evidence of apoptosis. Immunohistochemical staining confirmed an increase in CXCL9+ cells within the interfollicular, paracortical, and medullary sinus regions of the cervical LN at ≥25 mg/kg/d. Similar CXCL10+ cells were found in the thymic medulla and splenic white pulp, but there were no treatment related differences in positive cell numbers in either organ. Collectively these data suggest that exposure to diacetly induces changes in leukocyte populations and inflammatory mediators that may contribute to observed pathology in diacetly-exposed individuals.

1733 COVALENT BINDING OF NEVIRAPINE IN VIVO AND IN VITRO.

Y. Li and J. P. Uetrecht. Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada.

Background: Nevirapine (NVP) is associated with a high incidence of idiosyncratic skin rash and liver toxicity. We have developed a Brown Norway rat model in which nevirapine causes a skin rash that has characteristics similar to the reaction that occurs in humans. We demonstrated that oxidation of the methyl group was required in order for nevirapine to cause rash. Most idiosyncratic drug reactions are due to reactive metabolites. Method: We produced an anti-nevirapine antibody by injection of a nevirapine-modified Keyhole Limpet Hemocyanin into New Zealand White Rabbits. The affinity of this antibody was characterized by ELISA. With this antibody the covalent binding of the nevirapine reactive metabolite to liver and skin was studied using immunohistochemistry and immunoblotting. Results: The anti-NVP serum recognized NVP and some metabolites/derivatives, specifically 12-OH-NVP and 12-Cl-NVP. Binding could still be detected at an antiserum dilution of 1:100. Hepatic covalent binding was greatest in the centrilobular area. Co-treatment with albenzimidazole changed this pattern. Several hepatic protein bands were observed at 83, 65, 48, and 40 kD. Using expressed rat CYP2C11 and CYP3A1, covalent binding to P450 was observed. Replacing the methyl hydrogens with deuterium decreased covalent binding to hepatic microsomes and the major protein that was modified had a molecular mass of 48 kD. The rat skin homogenate was incubated with 12-OH-NVP sulfate; the major protein that was modified had a molecular mass of 25 kD. When aryl sulfatase was incubated with 12-OH-NVP sulfate; the major protein that was modified had a molecular mass of 37 kD. Conclusion: Oxidation of the nevirapine methyl group by P450 lead to direct formation of a reactive metabolite, presumably a quinone methide. In addition, sulfation of the benzyl alcohol formed by oxidation of this methyl group also forms a reactive metabolite. Combined with previous data that demonstrated that oxidation of the NVP methyl group is required to cause a skin rash establishes the pathway that is responsible for the idiosyncratic reactions associated with NVP.

This work was supported by grants from CIHR.

1734 DANGER SIGNALS IN NEVIRAPINE-INDUCED SKIN RASH.

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Background: Nevirapine (NVP) is used to treat HIV infections. However, its use is associated with a high incidence of hypersensitivity reactions, specifically, skin rash and liver toxicity. We have developed an animal model in which Brown Norway (BN) rats develop a NVP-induced skin rash with characteristics very similar to the skin rash in humans. The rash is clearly immune-mediated. We have shown that the NVP-induced skin rash is dependent on oxidation of NVP to the 12-hydroxyl metabolite (12-OH-NVP). It is unknown how this pathway leads to a skin rash. The objective of this study was to test the ‘danger hypothesis’ in which cell damage leads to a danger signal that initiates the immune response. Methods and Results: BN rats were treated with NVP (150 mg/kg, ip) and 6 or 12 hours later the animals were sacrificed, skin was removed from the ears, and mRNA was isolated. Changes in gene expression were determined with an Affymetrix gene chip. Already at these early time points 122 and 156 genes were upregulated at 6 and 12 hr, respectively. Potential danger signals such as S100A15 mRNA increased more than 4 fold. Western blot analysis also demonstrated that S100A15 expression level was increased in skin 12, 48 and 72 hr after NVP treatment. 12-OH-NVP treatment results in a rash at a lower dose than NVP. The number of genes upregulated after 12 hours was similar for both treatments. Conclusions: These results are consistent with the danger hypothesis. It now remains to further test the involvement of these genes by finding methods to modulate their expression and determine the effects of this modification on the incidence/severity of the rash. This research was supported by grants from CIHR.

1735 A STUDY OF LYMPHOCYTES SPECIFICITY IN NEVIRAPINE-INDUCED SKIN RASH.

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Background: The mechanisms by which drugs cause idiosyncratic drug reactions (IDRs) are unknown. A novel animal model for studying IDRs developed by us is nevirapine (NVP)-induced skin rash in the Brown Norway (BN) rats. NVP is used to treat AIDS but is associated with a high incidence of skin rash and liver toxicity. The characteristics of the animal model are very similar to those of the rash that occurs in humans. For example, a low CD4+ T cell count decreases the risk of rash in both rats and humans. Furthermore, CD4+ T cells can transfer the sensitivity from sensitized animals to naïve animals. The NVP-induced skin rash in BN rats is clearly immune-mediated and we believe the mechanism is similar to that in humans. Methods and Results: To study the specificity of lymphocytes in this model we used a modified lymphocyte transformation test. Female BN rats were treated with NVP (150 mg/kg/day) in food for 21 days and challenged for 5 days after 4 weeks off drug. Cells from auricular lymph nodes were isolated and cultured with various concentrations of NVP and NVP metabolites for 3 days at 5°C in 5% CO2. The production of cytokines and cell proliferation were measured by Luminex and Alamar Blue assays, respectively. With the stimulation by NVP the proliferation rate of lymphocytes was increased and cytokines including IFN-γ and IL10 were produced. The response to NVP was greater than to its 12-hydroxy metabolite even though we know that 12-hydroxylation is required to induce a rash. In order to determine which cells respond to NVP we depleted CD4+ and/or CD8+ T cells; ELISA showed that both T cells are involved in producing IFN-γ, which is consistent with real-time PCR results. Conclusions: Lymphocytes from sensitized animals respond to NVP with both proliferation and cytokine production. There is a disconnect between what induces the rash and what the T cells respond to. With these studies we have developed a better understanding of which cells are involved in the mechanism of NVP-induced skin rash and, in turn, this may provide possible ways to prevent-and/or treat IDRs. This work was supported by grants from CIHR.

1736 SODIUM METHYLDITHIOCARBAMATE AS A PROBE FOR MECHANISMS OF LETHALITY IN SEPSIS.

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As the third most abundantly used agricultural pesticide in the U.S., Sodium Methyldithiocarbamate (SMD) has been reported that can cause adverse health effects in humans. SMD oral administration decreases IL-12 production and in-
creases IL-10 production induced by lipopolysaccharide (LPS) in mice, indicating suppression of innate immunity. However, the mechanism of this effect and the scope of effects in infections are not known. Studies conducted using SMD treatment in B6C3F1 mice challenged with non-pathogenic E.coli in a mouse model for sepsis show that bacterial toxicity was monitored and immunological parameters, including IL-1B, IL-6, IL-10, IL-12, MIP-1β, were evaluated at different time points (2, 4, 12 and 24 hr). A decrease in IL-12 and increase in IL-10 were observed in SMD treated mice. Other cytokines were decreased at early time points and increased at 24 hr, compared with the group treated with E.coli only. This suggests inhibition of pro-inflammatory cytokines early by SMD leads to outgrowth of bacteria, which then induce higher than normal levels of cytokines after the SMD is cleared. Microarray analysis of peritoneal macrophages also indicates that IL-10 was upregulated in SMD treated mice challenged with LPS (as a non-replicating surrogate of E. coli). Numerous other changes consistent with suppressed innate immunity were also observed. Correlation analysis indicates body temperature was highly related to survival and the levels of cytokines. The result of differential counts on cells from peritoneal fluid shows that phagocytosis by macrophages and neutrophils and trafficking of neutrophils to the peritoneal cavity were not affected by SMD. However, clearance of bacteria was adversely affected. Microarray analysis indicated that cathespin and NADPH oxidase were upregulated, as expected due to increased number of neutrophils, so it is unlikely that decreased expression of these key effectors proteins is a mechanism of SMD action. This work was supported by NIH grant R01ES013708.

PS 1737 TARGETING CANNABINOID RECEPTORS AS A NOVEL APPROACH TO PREVENT DONOR T-CELL-MEDIATED INFLAMMATION DURING GRAFT-VERSUS-HOST DISEASE.

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Inflammation plays a critical role in the pathogenesis of a wide range of diseases. Marijuana cannabinoids activate cannabinoid receptors on immune cells and mediate immunomodulation including anti-inflammatory properties. Graft-versus-host disease (GVHD) is a major challenge to transplantation owing to associated morbidity and mortality. It is caused by activation of the donor-derived mature T cells that recognize the recipient’s alloantigens and mount an strong inflammatory and immunotoxic reaction. In the current study, we tested the hypothesis that activation of cannabinoid receptors on donor-derived T cells may help prevent GVHD. To test this hypothesis, we tested an acute model of GVHD by transferring parental B6 (B6) spleen cells to C57B1/6 X DBA/2 F1 (BDF1) mice. Transfer of B6 cells into BDF1 mice produces lymphoid hyperplasia followed by immunosuppression, weight loss, a T cell pattern of cytokines and mortality. In this model, we tested the efficacy of natural cannabinoid (Δ9-tetrahydrocannabinol, THCV) on the development of acute GVHD. THCV treatment suppressed the expansion of donor-derived T cells and blocked the decrease in the number of host cells. THCV administration led to early recovery from body weight loss, reduced tissue injury in the liver and increased survival. Impaired haematopoiesis seen during GVHD was rescued by treatment with THC. Moreover, the donor derived T cells from THCV-treated GVHD mice failed to proliferate and mediate cytotoxicity against the recipient’s cells. The ability of THC to reduce the clinical GVHD was reversed by administration of CB1 and CB2 antagonists thereby demonstrating that THCV-mediated suppression of GVHD was cannabinoid receptor-dependent. Our results demonstrate for the first time that targeting cannabinoid receptors may constitute a novel treatment modality against acute GVHD (Supported in part by NIH R01-DA016545, R01-ES09998, R01-AI53703, R01-AI058300, R01-HL58641, and P01-AT003961).

PS 1738 Δ9-THC SUPPRESSION OF CD40 LIGAND (CD40L) EXPRESSION IN ACTIVATED CD4+ T CELLS.

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Δ9-THC, a plant-derived cannabinoid, possesses immunomodulatory properties. As demonstrated by this laboratory, tretrahymol (THCV) selectively inhibits T cell function including T cell-dependent humoral immune responses, T cell proliferation, and production of T cell-derived cytokines. The mechanism of immune suppression involves at least in part, the impairment of the nuclear factor of activated T cells (NFAT). Even though Δ9-THC binds both cannabinoid receptors, CB1 and CB2, the role of these receptors in immune regulation remains poorly understood. CD40L, a member of the tumor necrosis factor ligand superfamily, plays a major role in the generation of appropriate T cell-dependent humoral immune responses. Furthermore, optimum expression of CD40L on activated T cells requires the activation of the transcription factors NFAT and early growth response-1 (Egr-1). In light of the above, the objective of this study was to investigate whether Δ9-THC impairs the up-regulation of CD40L expression in T cells activated with different stimuli. Time course studies demonstrated that peak cell surface CD40L expression after anti-CD3/CD28 or phorbol ester plus calcium ionophore (PMA/IO) was at 8 h post stimulation on activated CD4+ T cells, as determined by flow cytometry. Pretreatment with Δ9-THC significantly suppressed the anti-CD3/CD28-induced, but not the PMA/IO-induced up-regulation of cell surface CD40L expression on CD4+ T cells. Furthermore, pretreatment with Δ9-THC also attenuated anti-CD3/CD28-induced CD40L expression on CD4+ T cells derived from CB1−/CB2− mice, but to a lesser extent. Studies are currently underway to further characterize the role of CB1 and CB2 on Δ9-THC-mediated impairment of anti-CD3/CD28-induced CD40L and the involvement of NF-AT and Egr-1 signaling pathways. (Supported in part by DA07908 and Royal Thai Government Scholarships)

PS 1739 IMMUNOLOGICAL CONSEQUENCES OF SUSTAINED VERUS TRANIENT AHR ACTIVATION DURING INFLUENZA VIRUS INFECTION.

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The immunomodulatory effects of aryl hydrocarbon receptor (AhR) activation have been demonstrated in a variety of in vivo models of disease. The majority of these studies have used the experimental model of 2,3,7,8-tetrachlorodibenzop-dioxin (TCDD) to activate the AhR because it is poorly metabolized in vivo and has high affinity for the receptor. Our laboratory has demonstrated that TCDD-mediated AhR activation during influenza virus infection causes significant modifications in both the innate and adaptive immune responses, however the mechanism by which AhR activation causes these alterations remains unclear. An important factor that may influence the underlying mechanism of AhR immunomodulation is the duration in which the receptor is activated. Due to the long half-life in vivo, AhR activation is sustained over the course of influenza virus infection. To directly test whether sustained AhR activation is required for immune modulation, we compared the impact of TCDD with that of the tryptophan photoproduct 6-formylindolo[3,2-b]carbazole (FICZ). FICZ has a higher affinity for the AhR than TCDD and is thought to cause transient activation of the AhR. Our laboratory has demonstrated that TCDD treatment causes significant suppression of influenza specific CD8+ T-cell clonal expansion and differentiation; however FICZ treatment had no effect on these endpoints. Moreover, while TCDD treatment increases neutrophil recruitment and iNOS expression in the lungs of infected mice, treatment with FICZ failed to mimic these effects. The immunological endpoints we have studied require AhR activation in different tissues. Specifically, AhR is required in hematopoietic cells to see effects on CD8+ T-cell response but is not required in these cells for excessive neutrophil recruitment and enhanced iNOS levels. Therefore, failure of FICZ to modulate these endpoints suggests that differences in the duration of AhR activation may play a broad role in AhR-mediated immunomodulation.

PS 1740 DEVELOPMENT OF A HUMAN MODEL FOR 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) DISRUPTION OF LPS-INDUCED B CELL DIFFERENTIATION.

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Rodent models have established TCDD as suppressing the primary IgM antibody response. To better understand potential human health risks posed by dioxins, it would be useful to verify data from rodents in human models; however, donor-to-donor variation in human primary cell models has posed problems for establishing human immunotoxicology models. An attractive alternative has been human cell line models with homogeneity in genotype and stimulus response. To that end, the human lymphoblast cell line SK 6.4 was established as a model to study dioxin immunotoxicity. LPS (100 ng/ml) increased SK 6.4 IgM secretion, but was not impaired by TCDD concentrations up to 310 nM. Western Blot and rt-qPCR demonstrated that SK 6.4 cells do not express aryl hydrocarbon receptor (AHR). To establish a cell line model for mechanistic studies inAHR was cloned into a plasmid containing both native and C-terminal GFP fused forms of AHR for establishing AHR-expressing SK 6.4 cells. Both AHR forms were validated by TCDD-induced CYPIA1 expression in transiently transduced HEK 293 cells. Cells stably expressing AHR were detected by rt-qPCR in G418-resistant SK 6.4 cells. AHR-GFP was subcloned into the plasmid pTRED and

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packaged into a lentiviral vector, then transduced into SWK 6.4 cells to generate stable AHR-GFP expression. GFP fluorescence was verified by FACS. AHR expression was detected by r-qPCR, and AHR-GFP function established by TCDD-induced CYP1A1 gene expression. Future studies will assess TCDD effects on LPS-activated IgM secretion in AHR′ SWK 6.4 cells in comparison to murine models to validate cross-species mechanism conservation. This work was supported in part by the Dow Chemical Company, Superfund Basic Research Program (P42 ES040911), and NIH (RO1 ES052250, T32 ES072755). This is an abstract of a proposed presentation and does not necessarily reflect U.S. EPA policy.

1741 DECREASED IN VITRO CYTOTOXIC T LYMPHOCYTE (CTL) EFFECTOR RESPONSES IN MICE LACKING CANNABINOID RECEPTORS 1 AND 2.

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Cannabinoid compounds have been established as modulators of host immune responses, and exhibit affinity for cannabinoid receptors 1 (CB1) and 2 (CB2). CB1 is highly expressed in the central nervous system, while CB2 is highly expressed in the periphery including the immune system. From studies using agonists or CB1/ CB2 (KO) mice, immune modulation by the marijuana derived compound, ∆9-tetrahydrocannabinol (Δ9-THC), has been found to be mediated only in part through CB1 and CB2. In addition, it has been demonstrated that CB1 and CB2 play a critical role in generation of the in vitro antibody response. Thus, the objective of the present study was to investigate the role of CB1 and CB2 in the generation of functional CTL effectors in vitro. In an allogenic model, the kinetics of CTL activity resulting from co-culture of splenocytes (SPLC) with major histocompatibility complex (MHC)-mismatched P815 (DBA/2) cells showed a peak response on day 5 after elicitation using a standard 51Cr-release assay. The ability to lyse P815 target cells was reduced in CTLs derived from KO mice compared to WT mice. In a syngenic model, CTLs were elicited in response to an immunodominant peptide from influenza (PB176-77) presented in the context of MHC I. The PBl peptide was loaded onto antigen presenting RMA-S cells and co-cultured with SPLC. Five days after elicitation, the resulting population was assayed for CTL activity using UL18 and UL19 reporter constructs to enumerate peptide specific CTLs. Lower numbers of IFN-γ producing cells were observed in peptide-specific CTL populations elicited from SPLC of KO mice compared to WT mice. Collectively, these results suggest that CB1 and CB2 are involved in the regulation of CTL responses (supported by NIH DA07908 and MSU Respiratory Research Initiative).

1742 TCDD INHIBITS TRANSCRIPTIONAL ACTIVATION OF THE 3′IgHRR AND HS3A/HS1, 2 ENHANCERS IN A TRANSGENIC B-CELL LINE.

D. Ellis, T. Fernando and C. Suleri. Wright State University, Dayton, OH.

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a ubiquitous environmental toxin and potent prototype for aryl hydrocarbon receptor (AhR) ligands. The ligand-activated AhR can bind dioxin responsive elements (DRE) and act as transcription factor. Immunoglobulin (Ig) gene expression involves several regulatory elements including the 3′Ig regulatory region (3′IgHRR) and its enhancers (i.e., hs3; hs1,2; and hs4), which contain DNA binding sites for several transcription factors, including NF-κB/Rel and the aryl hydrocarbon receptor (AhR). TCDD induces binding of the AhR nuclear complex to a dioxin responsive element (DRE) in both the hs1,2 and hs4 enhancers and inhibits murine 3′IgHRR activation in a well characterized mouse B-cell line (CH12.LX). In humans, a polymeric imbalance of the hs1,2 enhancer, resulting in varying numbers of a 53 bp sequence tandemly repeated, has been correlated with autoimmune diseases like IgA nephropathy and Celiac disease. The repeated sequence contains KB and DRE binding sites. The objective of this study was to comparatively evaluate the effect of TCDD and LPS on human and mouse hs1,2 enhancers in the CH12.LX model. In transient luciferase studies, an increased number of repeats in the human hs1,2 enhancer increased the sensitivity to LPS. Interestingly, TCDD also markedly enhanced human hs1,2 activity and even augmented LPS-induced activation. TCDD-induced activation positively correlated with the number of repeats. This study contrasted with TCDD-induced inhibition of the mouse hs1,2 and 3′IgHRR in LPS-stimulated CH12.LX cells. Through sequence analyses, we verified that the human hs1,2 enhancer retains the KB and DRE binding sites but lacks binding sites for B-cell specific activator protein and NF-κB which are both important to mouse hs1,2 regulation. Mutational analyses are underway to evaluate the significance of these binding sites in TCDD-induced modulation of both the human and mouse hs1,2 enhancer. Since TCDD represents a large class of chemicals found in the environment, diet, and pharmaceuticals, understanding chemical-induced modulation of the 3′IgHRR enhancers may provide a clue to the etiology of certain autoimmune diseases. (Supported by NIEHS R01ES014676)

1743 TCDD MODULATES THE TRANSCRIPTIONAL ACTIVITY OF THE HS1, 2 ENHANCER IN THE 3′IgH REGULATORY REGION.

T. Fernando and C. Suleri. Wright State University, Dayton, OH.

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

1744 INVESTIGATION OF THE IMMUNOMODULATORY EFFECTS OF PERFLUORINATED FATTY ACIDS (PFFA) IN VITRO.

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Perfluorinated fatty acids (PFFAs) are commonly used as plasticizers, corrosion inhibitors, and fire extinguishers. We studied the immunomodulatory potential of various PFCAs, ammonium perfluorooctanoate (APFO), perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexanoic acid (PFHA), perfluorononanoic acid (PFNA) and perfluoroheptanoic acid (PFDA) in vitro models. Firstly, lymphocytes isolated from spleens of BALB/c mice were incubated with PFOS, APFO, PFHA, PFNA and PFDA at 0.1, 1, 10 and 100 μM concentrations for 24, 48, and 72 hr, and changes in percentages of lymphocytes (CD4+, CD8+, CD45+) were monitored by FACS. We found significant modulation in expression of all the studied lymphocyte markers at a concentration of 0.1 μM for PFNA after 24 hr of incubation. At higher concentrations, effects were more pronounced and were also present with the other PFCAs studied. In another set up, the effects of APFO on dendritic cells (DCs) were studied. DCs were prepared from PBMCs isolated from buffy coats of healthy blood donors by density gradient centrifugation, followed by LPS stimulation. The percentage of all lymphocyte markers at a concentration of 0.1 μM was monitored at 24, 48, and 72 hr. In addition, DCs were cultured with different PFCAs, such as APFO, PFOA, PFHA, PFNA and PFDA at 0.1, 1, 10 and 100 μM concentrations for 24 hr. After 24 hr of incubation, the capacity to stimulate allogeneic T-cell proliferation was not affected. Our results support the hypothesis that PFCAs may be involved in the etiology of certain autoimmune diseases.

1745 IMMUNOSUPPRESSIVE EFFECTS OF 1, 2, 5- DIBENZANTHRACENE ARE MEDIATED PRIMARILY BY CYP1B1 AND NOT CYP1A1 OR AHR.

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The mechanism of polycyclic aromatic hydrocarbon (PAH) immunotoxicity is not completely understood. Because PAHs are agonists of the aryl hydrocarbon receptor (AhR) and they are metabolized by cytochrome P450 enzymes, there are two
competing yet complimentary hypotheses on the mechanism. In these studies, a pharmacological approach was devised to determine if the mechanism of action of 1,2,5,6-Dibenzanthracene (DBA) immunotoxicity is mediated by direct action of the AhR or by metabolism of DBA to metabolites that may subsequently interco-
late into DNA and exert their effects. Utilizing the Mitchell-Duttro in vitro anti-
body forming cell assay, it was possible to show that resveratrol (Rsv; an AhR an-
tagosn) had no effect on the immunosuppressive potency of DBA and that α-naphthoflavone (ANF; an inhibitor of both CYP1A1 and CYP1B1) and 2,3,4,5-tetramethylbenzene (TMB; a CYP1B1-specific inhibitor) both signifi-
cantly reduced the immunosuppressive effects of DBA in Mitchell-Duttro culture.
Whereas ANF may have effects on the AhR and CYP1A1 as well as on CYP1B1, the results suggest that TMS is a competitive inhibitor of CYP1B1 with greater speci-
cificity for CYP1B1 than ANF. These results suggest that the immunosuppressive ef-
cfects of DBA are not receptor-mediated, but instead are mediated by the metabo-
lism of the parent compound to more toxic metabolites by the P450 system. Funded in part by NIEHS Contract ES 05454.

1746 DIFFERENTIAL EFFECTS OF ACUTE ETHANOL ADMINISTRATION ON THE POLY IC-INDUCED IMMUNE RESPONSE: IS ALL POLY IC CREATED EQUAL?
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Acute ethanol exposure inhibits the proinflammatory cytokine response to many pathogens. Double-stranded RNA (dsRNA) and its synthetic analog, polyinosinic-polycytidylic acid (poly IC) are molecular patterns associated with viral infection which are recognized by the pattern recognition receptor, TLR3. This recognition sets off a signaling cascade culminating in the production of proinflammatory cy-
tokines. Poly IC has been used extensively both in vivo and in vitro to mimic viral effects on the immune response. Unfortunately, literature searches to shed light on the pric t
for this variability is endotoxin contamination. Endotoxin is a potent stimula-
tor of the TLR4 pathway, a pathway which also culminates in the production of proinflammatory cytokines. Like many agents used in research laboratories, poly IC is available from a variety of sources. The purpose of this study was to compare and contrast results from experiments utilizing poly IC from two of these sources, Sigma and Invivogen. The effects of these agents were assessed in female B6C3F1 mice treated first oral gavage with ethanol (32%) in water at 6 kg adult weight.
The Invivogen poly IC is considered to be endotoxin-free (< 0.125 EU/mg), whereas Sigma poly IC has been shown to have some level of contamination. Most parameters tested yielded comparable results with the two poly ICs, but a few of the parameters, including IL-10 secretion and NF-kappAB activation showed marked differences. This work was supported by NIH grant AA09595.

1747 A GENOMIC APPROACH TO AN INNATE IMMUNO-
REGULATORY MECHANISM MODULATED BY
ETHANOL: MICROARRAY AND PATHWAY ANALYSIS.
T. Sebastian, M. Glover and S. Pruet 1, Department of Basic Sciences, Mississippi State University, Mississippi State, MS and 3Cellular Biology & Anatomy, LSU Health Sci. Center, Shreveport, MS.
Ethyl alcohol diversely affects intracellular signaling events and gene expression of innate immune system leading to altered inflammatory responses. Our previous studies clearly demonstrated that some of the effects of EtOH on the innate im-
mune system mediated through TLR signaling pathways, and this could suppress cytokine expression depending on which transcription factor inhibited. Through microarray and pathway analysis we made an attempt to analyze the transcriptional profile to study the mechanism of action of EtOH on the innate immune response. MA analysis showed that EtOH can affect cellular signaling events such as activa-
tion of MAPK, NF-kappAB mediated by toll like receptors leading to altered in-
flammatory responses. A number of genes are affected by treating B6C3F1 mice with ethanol. We analyzed 45101 genes, of which 3525 candidate genes showed 2 fold changes in expression level with untreated naive. Among the genes that medi-
ate inflammation, IFN-α, IFN-β, CCL20 and CXCL2 are significantly changed by EtOH. Based on the present study and earlier results it is evident that there is a common mechanism by which EtOH regulates inflammation, and we propose a model explaining how EtOH control signaling and cytokine production. Viral in-
fection results in the activation and phosphorylation of IRF3 and IRF7 which form a complex with p300 and CBP in the cytoplasm and undergo conformational change. As a result the complex translocates to the nucleus and allows binding to the ISRE sequence of the DNA. Pathway analysis showed that DHX 58, an RNA helicase upregulated by EtOH prevents serine/threonine phosphorylation of IRF3 and complex formation with IRF7 there by inhibit the translocation of IRF3-IRF7 complex to the nucleus and activates the transcription of IFN α and β and other in-
terferon induced genes. This work was funded by NIH grant AA09595.

1748 THE ROLE OF PPAR-ALPHA ON MACROPHAGE FUNCTION FOLLOWING PFOS EXPOSURE IN THE MOUSE RAW264.7 CELL LINE.
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Although numerous toxicological endpoints have been linked to perfluorooctane sulfonate (PFOS), its effects on inflammation, specifically, macrophage function are not well understood. Understanding these effects and the role of PPAR-ahpa in them is important to fully comprehend the consequences PFOS has on immune function. The current study assessed cellular proliferation, cytokine and PPAR-alpha activity following in vitro exposure to PFOS in murine RAW264.7 macrophages. All three endpoints were measured after a 24 hr. exposure to 0, 0.03, 0.1, 0.3, 1, 3 or 300 µg PFOS/mL and stimulation with 0.1 µg/mL LPS. Significant results included an increase in cellular proliferation at 1 and 3 µg/mL PFOS/mL, but decreased proliferation at 0.03 µg PFOS/mL. IL-6 secretion was re-
duced by exposure to 0.1, 0.3 and 300 µg PFOS/mL while TNF-α alpha production was reduced by exposure to 300 µg PFOS/mL. PPAR-alpha activity was signifi-
cantly increased at 0.1 - 300 µg PFOS/mL, but this was not dose-responsive. Evidence for the involvement of PPAR-alpha in these effects was indicated by co-
exposure to PFOS and the PPAR-alpha inhibitor MK886. Not only was PPAR-
alpha activity significantly decreased with co-exposure to PFOS and MK886, but this also resulted in the elimination of cellular proliferation at 1 and 3 µg/mL and recovery of the observed reduction in TNF-α alpha production at 0.3 µg/mL that was observed with PFOS alone. Alternatively, culture with MK886 did not change the effect of PFOS on IL-6 production in this cell line. Overall these indicate that PPAR exposure can alter macrophage functions and begins to elucidate the complex role of PPAR-alpha in PFOS modulated inflammatory effects suggesting both PPAR-alpha-dependent and -independent modes of action.

1749 EFFECTS OF PERFLUOROOCUTANE SULFONATE (PFOS) ON MACROPHAGE FUNCTION AND SPLENOCYTE SUBPOPULATIONS IN B6C3F1 MICE.
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Perfluorinated hydrocarbons have recently generated much interest as some of these compounds (i.e., perfluorooctane sulfonate (PFOS)) are persistent in the environ-
ment and are detectable in blood samples of both wildlife and humans. Of the tox-
ocological effects identified, antibiotic production seems to be a highly sensitive tar-
get. This study builds on previous work examining the immunotoxicity of PFOS and potential mechanisms attributing to suppression in humoral immunity. Speciﬁcally, we report splenic cell populations (B220+CD40, CD4+/CD154, and F4/80) and macrophage function. Macrophage parameters assessed included phagocytosis and the ability to generate nitric oxide. Of these measures, phagocyto-
sis was suppressed only at 5.0 mg/kg total administered dose (TAD) of PFOS for a duration of 28 days. Numbers of B220+/CD40- and B220+/CD40+ cells were not altered but numbers of B220-/CD40+ were increased at the 5.0 mg/kg treatment. However numbers of F4/80 (macrophages) were not altered. Numbers of CD4+, CD154+ and CD4+/CD154+ cells were also not altered. These data suggest that activation, and expression of CD40 on B-cell (B220+/CD40+) and CD154 on T-
cell (CD4+/CD154+), which are both important in humoral immune production of IgM, are not modulated by PFOS exposure at concentrations where PFOS-in-
duced IgM suppression is noted. Based on our previous reports, it is clear that hu-
oral immunity is suppressed following exposure to PFOS at serum concentrations that fall below occupationally measured levels. What is not clear, however, is the mechanism of immunosuppression. Based on these data, it appears that deficiencies in expression of CD40 and CD154 following antigen challenge are not a factor. Although macrophage phagocytosis was suppressed at 5.0 mg/kg TAD, no alter-
ations were detected at 0.5 mg/kg TAD the LOEL in this strain/ gender. Additional studies are in progress examining the cytokine network that supports IgM produc-
tion to further characterize the mode of immunosuppression.

1750 COMPARATIVE IMMUNORESPONSES BETWEEN WILD-TYPE AND PPAR-ALPHA NULL MICE FOLLOWING EXPOSURE TO PFOS.
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The cause for concern for perfluorinated hydrocarbons is not only driven by the en-
vironmental persistence of these compounds, but by the fact that the toxicity of these chemicals has not been extensively studied while substantial levels have been

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detected in blood samples of humans and wildlife. This study builds on previous work examining the immunotoxicity of PFOS and potential mechanisms attributing to suppression in humoral immunity. It is known that perfluorooctane sulfonate (PFOS), along with other compounds of this class, cause peroxisomal proliferation. Since lymphocytes express peroxisome proliferator-activated receptor-alpha (PPAR-alpha), it has been suggested that the immunotoxicity of PFOS may be related to PPAR-alpha activation. Previous studies from this laboratory have demonstrated that PFOS suppresses IgM humoral immune responses in adult B6C3F1 mice. To elucidate potential mechanisms of PFOS-induced immunosuppression, this study compared the immunotoxicity profile in PPAR-alpha null mice with respect to the wild type control (C57BL/6 mice). C57 +/+ and -/- mice were exposed to PFOS (0, 0.5, or 5 mg/kg total administered dose [TAD]) orally for 28-days. Sheep red blood cell (SRBC)-specific IgM production along with macrophage phagocytosis, pinocytosis and nitric oxide production was assessed. No effect on macrophage function was observed for any of the parameters in either strain. IgM suppression was observed in the wild-type mice at both 0.5 and 5 mg/kg TAD but was not observed in the PPAR-alpha null mice. This suggests a role for PPAR-alpha in the observed suppression of IgM production by PFOS. However, further studies are required to completely characterize the mechanism of action of PFOS on humoral immunity.

1751 DIFFERENTIAL DISRUPTION OF HEMATOPOIESIS IN BONE MARROW BY BENZO(A)PYRENE AND 7, 12- DIMETHYLBENZ(A)ANTHRACENE: EFFECTS ON BLOOD, SPLEEN, AND THYMUS. A. U. N'jai1, 3, M. Larsen1, C. Czyzynski1, 2, and L. Cheng1, 2, M. Larsen3, C. Czyzynski1, 2, and L. Cheng1, 2, 1PBS, UW, Madison, WI, 2METC, UW, Madison, WI, 3Pharmacology, UW, Madison, WI.

Benzo(a)pyrene (BP) and 7, 12-dimethylbenz(a)anthracene (DMBA) are carcinogenic polycyclic aromatic hydrocarbons (PAHs), that selectively target bone marrow (BM) cells through local activation by Cyp1b1. We have previously shown that, in C57BL/6J mice, total BM cellularity decreased two-fold following IP DMBA but not BP treatment. Here, we further characterize the temporal response in the BM, blood, spleen, and thymus compartments to IP or oral gavage of DMBA or BP (50 mg/kg). Progenitor B cell colony forming units (CFU-preB) were decreased to a greater extent than myeloid progenitors (CFU-GM) following IP or oral BP and DMBA treatments. The preB response to BP and DMBA were similarly decreased at 6 h, but BP treated mice displayed a recovery. In contrast, CFU/GM decrease more slowly in response to DMBA, while BP had no effect. The acute (6h) effect of oral BP or DMBA treated mice was similar, but leukocytes were decreased substantially by 168 h. Decreased spleen and thymus weights, indicative of lymphocyte depletion paralleled decreased in BM cells. DMBA produced significant reduction in spleen and thymus weights, while BP was more potent in thymus than spleen. In congenic AhRd mice where PAH induction of the AhR (BP->DMBA) is minimal, BP responsiveness in BM cells is fully restored. Similar amounts of BP and DMBA reach the BM in wild-type and AhRd mice, suggesting that AhR activation protect against BM responses to BP. BM responses were lost in Cyp1b1 -/- mice and therefore dependent on Cyp1b1 PAH metabolism. Overall, oral administration of BP or DMBA caused marked early (6h) effects, that diminished substantially by 48 h compared to IP, consistent with more rapid hepatic clearance. Blood levels of DMBA were six fold higher for oral than IP administration at 6 h, but the magnitude of blood cell depletion was surprisingly similar. The observations in the BM and immune compartments shed light on role of AhR and Cyp450 metabolism on the differential effects of PAHs on BM hematopoiesis.

1752 IDENTIFICATION AND CHARACTERIZATION OF INFILTRATING MACROPHAGES IN ACETAMINOPHEN-INDUCED LIVER INJURY. M. Holst, L. Cheng and C. Lu, Pharmaceutical Sciences, University of Colorado Denver, Denver, CO.

The role of macrophages in the pathogenesis of acetaminophen (APAP)-induced liver injury remains controversial, as it has been demonstrated that these cells display pro-inflammatory and hepatoprotective functions. This controversy may stem from the heterogeneity and/or plasticity of macrophages and the difficulty in distinguishing and differentially studying subpopulations of macrophages in the liver. In the present study, using flow cytometric analysis and fluorescence-labeled antibodies against specific cell surface macrophage markers, we were able to, for the first time, identify an APAP-induced macrophage (IM) population distinct from resident Kupffer cells. The data demonstrated that the IMs were derived from circulating monocytes that infiltrated the liver following APAP-induced liver injury. The IMs exhibited a phenotype consistent with that of alternatively activated macrophages and demonstrated the ability to phagocytize apoptotic cells and induce apoptosis of neutrophils. Furthermore, in the absence of the IMs, the resolution of hepatic damage following APAP-induced hepatotoxicity was delayed in CCR2-/- mice compared with wild-type mice. These findings likely contribute to the role of the IMs in the processes of tissue repair, including counteracting inflammation and promoting angiogenesis. The present study also demonstrated the ability of separating populations of macrophages and delineating distinct functions of each group in future studies of inflammatory disease in the liver and other tissues.

1753 EICOSONOIDS MEDIATE GALLERIA MELLONELLA CELLULAR IMMUNE RESPONSE TO VIRAL INFECTION. E. Buyukguzel1, H. Tunar2, D. Stanley3 and K. Buyukguzel4. 1Faculty of Arts and Sciences, Biology Department, Zonguldak Karaelmas University, Zonguldak, Turkey, 2Faculty of Agriculture, Department of Plant Protection, Sakarya University, Sakarya, Turkey and 3Biological Control of Insects Research Laboratory, USDA/Agricultural Research Service, Columbia, MD.

Nodulation is the predominant insect cellular immune response to bacterial and fungal infections and it can also be induced by some viral infections. Treating seventh instar larvae of greater wax moth G. mellonella with Bovine herpes simplex virus-1 (BHSV-1) induced nodulation reactions in a dose-dependent manner. Because eicosanoids modulate nodulation reactions to bacterial and fungal infection, we hypothesized that eicosanoids also modulate nodulation reactions to viral challenge. To test this idea, we injected G. mellonella larvae with indomethacin, a non-steroidal anti-inflammatory drug immediately prior to intrahemocoelic injection of BHSV-1. Relative to vehicle-treated controls, indomethacin-treated larvae produced significantly reduced numbers of nodules following viral infection (down from approximately 190 nodules/larva to <50 nodules/larva). In addition to infection treatments, increasing dietary indomethacin dosages (from 0.01% to 1%) were associated with decreasing nodulation (by 10-fold) and phenoloxidase activity (by 3-fold) reactions to BHSV-1 infection. We infer from these findings that cyclooxygenase products, prostanoids, mediate nodulation response to viral infection in G. mellonella.

1754 CHRONIC LOW-DOSE ARSENIC IN DRINKING WATER ALTERS IMMUNE RESPONSES TO RESPIRATORY VIRAL INFECTION IN VIVO. C. D. Krall1, K. H. Eby2, R. I. Enelow3 and L. W. Hamilton4. 1Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH, 2Medicine, Dartmouth Medical School, Lebanon, NH and 3Medicine and Microbiology, Dartmouth Medical School, Lebanon, NH.

Arsenic (As) exposure is a significant worldwide environmental health concern. Through mechanisms that have yet to be elucidated, chronic As exposure via the drinking water has been associated with an increased incidence of pulmonary disease. We have recently found that environmentally relevant levels (10 and 100 ppb) of chronic As exposure significantly alters inflammatory cytokine production in mouse lung. Our findings led us to investigate the effect of chronic As exposure on respiratory Influenza A infection, a common and potentially fatal disease. In this study, C57BL/6J mice (9 wk, n=6 per treatment group) were housed on a casein-based AIN-76A diet and exposed to 100 ppb As in the drinking water for 5 wk. Following As exposure, mice were inoculated intra-nasally with a sub-lethal dose of Influenza A/PuertoRico8/34 (H1N1) virus. Morbidity (measured by weight loss) was monitored over the course of infection. Multiple endpoints were evaluated post infection (p.i.). Mice exposed to As had a significant increase in morbidity over the entire course of infection and significantly higher pulmonary influenza virus titers on day 7 p.i. The As exposed mice also displayed a significant decrease in total cell infiltration and inflammatory cytokine production at day 3 p.i. Respiratory infections with influenza virus and the looming potential of an influenza pandemic are major worldwide health concerns. Our data indicate that chronic As exposure may be a significant contributor to the susceptibility and pathogenesis of respiratory viral infections. Moreover, these results suggest that chronic arsenic exposure, even at low levels commonly found in drinking water in the U.S., and throughout the world, may compromise immune responses to various infectious challenges, leading to or exacerbating disease risk. (NIH-NIEHS SBIR P42 ES007375 P2)
Listeria monocytogenes is a Gram-positive bacterium that can be pathogenic in humans and is used as a model of intracellular infection. We have used an in vivo model of listeriosis to evaluate the effects of MT gene dose during the course of Listeria infection in three strains of mice: C57BL/6 wild-type (WT), congenic MT knock-out (MTKO), and congenic MT transgenic (MTTG). Our results suggested that either overexpression of MT-I or a lack of functional MT significantly altered the course of intravenous listeriosis. Both MTTG and MTKO mice had significantly lower Listeria burdens in liver and spleen. A time course study showed this difference was most prominent in the first 48 hours of infection, during the innate immune response, after which Listeria clearance occurred at comparable rates in all three strains. Listeria infection resulted in decreased IL-6 and TNF-α levels in both MTTG and MTKO mice compared to WT. Macrophages from MTTG and MTKO mice exhibited an increased oxidative burst compared to WT macrophages, suggesting a possible mechanism by which these strains maintained a lower Listeria burden. We have used grating-coupled surface plasmon resonance imaging (GCSRPI) and grating-coupled fluorescent plasmonics (GCPF) to identify components of a biomarker signature of inflammation for each of three strains of mice. Analysis of LPS-stimulated peritoneal cells from the three strains reveal that MT gene dose can significantly affect the secretion of cytokines and other inflammatory mediators. Moreover, these patterns are reflected in mRNA microarray studies of resting and activated cells. These results indicate that MT levels can dramatically influence the innate immune response to infection. Supported by NIH grants ES07408 and ES016014.

1756 A NOVEL IN VITRO SYSTEM (MIMIC™) FOR THE ASSESSMENT OF IMMUNOLOGICAL EFFECTS OF DRUGS ON THE HUMAN IMMUNE CELLS.

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Assessment of immunotoxic potential of novel therapeutics traditionally involves utilizing a series of in vitro assays in which PBMC are stimulated in a manner quite different from how they are activated in an animal. In vivo, antigen is picked up by antigen-presenting cells (APC), traffics to the lymph nodes, is presented to T cells, and then the T cells migrate to B cell follicles to activate antibody (Ab) secretion. In order to more closely mimic the in vivo situation, a novel in vitro system (MIMIC™) has been developed using human PBMC. In this model, lymphocyte activation is broken down into a “peripheral tissue” module where PBMC are activated to become mature dendritic cells (DC) by crossing through an endothelial cell layer, and a “lymphoid tissue equivalent” module where these activated DC are co-cultured with autologous T cells, and subsequently with autologous B cells. We tested the MIMIC™ system for its ability to detect and characterize effects of 8 well-known immunomodulatory compounds, including imiquimod, CpG, cyclosporine A, methotrexate, anti-TNFα mAb, anti-CD154 mAb, OKT3, and CTLA4-Ig, from 8 human donors. Read-outs included: assessment of DC numbers and DC maturation, detection of 22 cytokines/chemokines, T and B cell proliferation and cell viability, and antigen-specific Ab production. The MIMIC™ system was able to detect both immunostimulatory (e.g. imiquimod) as well as immunosuppressive compounds (e.g. Ga). In addition, the MIMIC™ system was able to characterize the varied effects of OKT3: stimulatory on T cells but inhibitory for Ab production. The results seen with these compounds in the MIMIC™ system are in agreement with the published literature on the effects of these drugs in humans. The MIMIC™ system may be an effective method for predicting the immunomodulatory effects of novel therapeutics on human immune cells.

1757 ELUCIDATION OF CYTOKINE STORM: THE EFFECT OF IN VITRO ANTIBODY IMMUNOABSORPTION METHODS ON CYTOKINE SECRETION IN A CELL-BASED ASSAY.


In 2006, the phenomenon of cytokine storm was grimly demonstrated in the phase 1 clinical trial of TGN412, a CD28-specific monoclonal antibody. In the trial, six healthy subjects were dosed with this therapeutic agent, and all six subjects rapidly suffered a life-threatening proinflammatory cytokine response. Subsequent analyses of the TGN412 trial used in the study found no errors in formulation or administration, and a review of all preclinical studies indicated that the dose given in the human trial was far below the upper limit of safety as determined by studies in Cynomolgous macaque (Cyno). The reason for the unexpected adverse events in the human trial was eventually determined to be caused by differences in antibody presentation and mode of action in Cyno v. human cells. Here we compare methods of CD28 antibody immobilization in a cell-based assay, and the effects on the secretion of cytokines as well as lymphocyte proliferation in peripheral blood mononuclear cells (PBMC). Cytokine secretion was analyzed via Lumexin technology, and lymphocyte proliferation was assessed through BrdU incorporation into PBMC. Immobilization methods include direct binding onto tissue culture plates, direct binding on high bind plates, and binding through a protein G intermediate. We find dramatic differences in the secretion of several proinflammatory cytokines, most notably IL-6, MIP-1α, and TNFα, when antibody is prebound onto the assay plate rather than free in solution. For example, we find that MIP-1α is secreted at a level over 100-fold higher when cd28B is bound onto the assay plate v. unbound. We also compare various binding methods of cd28B and the effects on cytokine response and cell proliferation in both human and cyno PBMC. Through these studies we offer a tool to help predict adverse immunological events in antibody therapeutics.

1758 STREAMLINED PREDICTION OF COMPOUND EFFICACY AND SAFETY INDEX IN AN INFLAMMATION MODEL THROUGH MULTIPLYING, QUANTITATIVE, CELL-BASED IMAGING AND CYTOKINE ARRAYS.


Ideally, assessment of compound toxicity should occur concurrently with assessment of its effectiveness and mechanism of action. Frequently, toxicity is detected only after significant time and effort has been spent on determining the mechanism and action of the compound in relation to the disease state. In this study, we have tested multiplexing, quantitative, cell-based imaging with cytokine arrays in an inflammation model for compound screening. Ten commercially available compounds were assessed in RAW 264.7 macrophages for anti-inflammatory activity. Cytokine production was measured by multiplexed cytokine antibody arrays and confirmed by ELISA. Both mechanism of action and toxicity were determined through quantitation of changes in target fluorescence by quantitative cell-based imaging assays. Minimal effort was required for transfer of cell culture supernatants to the antibody arrays or ELISA format, while cells were simultaneously stained for intracellular target changes or toxicity. In total, twelve cytokines, ten intracellular signaling targets, and six toxicity parameters were assayed. Compound EC50 values were determined for each intracellular target (phospho-p38, phospho-ERK, phospho-JNK, NF-kB, phospho-CREB, phospho-c-Jun, PKA, COX-2, iNOS, MxNSD), for cytokine inflammatory cytokine production (TNFα, IL-6), and for toxicity. Therapeutic safety windows were calculated for each compound. The results indicate that multiplexing the two assay platforms could successfully predict safety windows for each compound and differentiate relative toxicities from mechanisms of action. Quantitative cell-based imaging when combined with antibody cytokine arrays provides a powerful, streamlined approach for assessing anti-inflammatory compound activity, mechanism of action and toxicity, all with only two assay platforms.

1759 IMMUNOTOXICITY OF EMISSION PARTICLES FROM FOSSIL- AND BIODIESEL-FUELED NON-ROAD DIESEL ENGINE.

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Immunotoxicity of fine particulate emissions of fossil diesel fuel (EN590), first generation biodiesel (rapeseed methyl ester, RME) and 2nd generation renewable diesel fuel (hydrotreated vegetable oil, HVO) were investigated. Particulate samples were collected from diluted exhaust gas of a non-road Euro II IDI diesel engine, which was operated with and without a catalytic converter. During sample collection engine was employed according to previously chosen ISO 7178-4:1996 C1 test cycle modes. Mouse RAW264.7 macrophages were exposed for 24 hrs to four doses (15, 50, 150 and 300 g/ml) of the emission particles. The production of cytokines was measured after 24 hrs by ELISA. Both mechanism of action and toxicity were determined in RAW 264.7 macrophages for anti-inflammatory activity. Cytokine production was measured by multiplexed cytokine antibody arrays and confirmed by ELISA. Both mechanism of action and toxicity were determined through quantitation of changes in target fluorescence by quantitative cell-based imaging assays. Minimal effort was required for transfer of cell culture supernatants to the antibody arrays or ELISA format, while cells were simultaneously stained for intracellular target changes or toxicity. In total, twelve cytokines, ten intracellular signaling targets, and six toxicity parameters were assayed. Compound EC50 values were determined for each intracellular target (phospho-p38, phospho-ERK, phospho-JNK, NF-kB, phospho-CREB, phospho-c-Jun, PKA, COX-2, iNOS, MxNSD), for cytokine inflammatory cytokine production (TNFα, IL-6), and for toxicity. Therapeutic safety windows were calculated for each compound. The results indicate that multiplexing the two assay platforms could successfully predict safety windows for each compound and differentiate relative toxicities from mechanisms of action. Quantitative cell-based imaging when combined with antibody cytokine arrays provides a powerful, streamlined approach for assessing anti-inflammatory compound activity, mechanism of action and toxicity, all with only two assay platforms.
This work was supported by Brain Korea 21 Project in 2008. Thymus. Our results demonstrate that insoluble HgS was absorbed by the GI tract, populations in spleen in a dose-dependent manner. A dose of 0.2 g/kg/day of HgS significantly both CD8+ T lymphocytes and double-positive CD4+/CD8+ lymphocyte mice treated with the highest dose of HgS. Interestingly, HgS increased significantly aspartate aminotransferase levels. Circulating blood leukocytes were elevated in the gastrointestinal (GI) tract and then accumulated in the liver, kidney, spleen, thymus and brain in a dose-dependent manner. The dose range of HgS used did not affect the production of cytokines since pDC viability was marginally affected by exposure to CSE. In contrast, CSE had no significant effect on the production of other cytokines and chemokines in viral infected moDC. The stimulation IFN-α-treatment in pDC and moDC by respective agonists TLR7 (Lorosombine) and TLR9 (ODN-CpG D19): TLR3 (ICLC) was significantly decreased in cells treated with CSE. Quantitative Real-Time PCR revealed that CSE also significantly decreased TLR7 and TLR9 gene expression in viral infected pDC. These findings provide evidence to support that cigarette smoke extract alters the immune capacity of pDC and moDC. Therefore, exposure to SHS may potentially exacerbate the severity of respiratory infections by impeding the innate ability DC to respond to viral pathogens.

PS 1761 TOXIC EFFECTS OF MERCURIC SULFIDE ON THE LIVER AND IMMUNE ORGANS IN MICE.

H.S. Son, S. Park, S. Lee, M. Kim and S. Kim. Pharmacology, Kyungpook National University Medical College, Daegu, Korea, South.

Mercuric sulfide (HgS) is a major component of cinnabar, which has been used as a pigment in Chinese art and medicine for more than 2000 years. Because its toxicological effects are still unclear, we attempted to verify what are the toxic effects of HgS. ICR male mice were administered by gavage with HgS (0.02, 0.2, 2.0 μg/kg/day) for 4 weeks. During the administration period, HgS-treated mice did not reveal overt signs of clinical toxicity. HgS had no significant effect on body weight, food consumption, water consumption, and various organ (liver, kidney, spleen, and thymus) weights. In spite of its insolubility, HgS was significantly absorbed by the gastrointestinal (GI) tract and then accumulated in the liver, kidney, spleen, thymus and brain in a dose-dependent manner. The dose range of HgS used did not cause hepatotoxicity as indicated by circulating alanine aminotransferase and aspartate aminotransferase levels. Circulating blood leukocytes were elevated in mice treated with the highest dose of HgS. Interestingly, HgS increased significantly both CD38+ T lymphocytes and double-positive CD4+/CD8+ lymphocytes populations in spleen in a dose-dependent manner. A dose of 0.2 g/kg/day of HgS increased CD4+ T lymphocytes, whereas decreased CD4+/CD8+ T lymphocytes in thymus. Our results demonstrate that insoluble HgS was absorbed by the GI tract, accumulated in spleen and thymus, and then could affect immune systems. This work was supported by Brain Korea 21 Project in 2008.

PS 1762 LYMPHOCYTE IMMUNOPHENOTYPING AND RED BLOOD CELL ONTOGENY IN FETAL AND EXTRATUMORAL VACCINATED AND KLH-IMMUNIZED INFANT CYMOLGUS MONKEYS.


Preclinical in vivo models for drug development are predictors of fetal outcomes after drug administration to pregnant mothers. Many drugs cross the placental barrier leading to potential embryo-fetal exposure. Developmental immunotoxicity is one of the possible outcomes of the embryo-fetal drug or metabolite exposure. The cynomolgus monkey (Macaca fascicularis) is a well characterized model for developmental toxicity. The aim of this study is to present the expression patterns of specific lymphocyte surface markers in fetuses and infants (non-vaccinated and KLH-immunized). Data on neonatal red blood cell to non-nucleated transition from Gestation Day (GD) 100 and 140 cord blood, and neonates (F1) is presented. On average, the relative percentage (when gated on lymphocytes) of CD3+ T cells is 74% at GD100, 77% at GD140, and 68% in infant (30 days old) compared to 64% in adult females. The percentage of CD20+ B cells is 17% at GD100 and GD140, and 25% in neonates (30 days old) compared to 27% in adult females. CD3+CD4+ T cells is 67% at GD100, 68% at GD140, and 52% in neonates (30 days old) compared to 34% in adult females. The relative percentage of CD3+CD8+ T cells is 11% at GD100, 15% at GD140, and 16% in neonates compared to 30% in adult females. The neonatal red blood cell (RBC) population accounts for approximately 150 cells per 103 non-nucleated RBCs at GD100, approximately 16 cells per 103 non-nucleated RBCs at GD140, and approximately 5 cells per 103 non-nucleated RBCs in neonates (30 days old). The percentage of CD71+ cells in peripheral blood in infants did not change significantly between blood samples obtained prior to KLH vaccinations and following KLH immunization and boosts. KLH immunization does not upregulate CD47 expression in lymphocytes in monkey infants in peripheral blood and lymphoid tissues.

PS 1763 ARSENIC PREDISPOSES SKIN KERATINOCYTES TO UV-INDUCED OXIDATIVE DNA DAMAGE: Y. Sun, C. Kojima, C. Chigondi, R. Mason and M. P. Walkesh. ICS, LCC, NCI at NIEHS, Research Triangle Park, NC and LP, NIEHS, Research Triangle Park, NC.

Inorganic arsenic and ultraviolet (UV) radiation are known human skin carcinogens that may act together as cocarcinogens. Oxidative DNA damage (ODD) is a possible mode of action in arsenic carcinogenesis although we find it only occurs in cells that bio-methylate the metalloid. However, oxidative damage is a hallmark of UV-induced epidermal carcinogenesis. We have developed a model of skin carcinogenesis using a human skin keratinocyte (HaCaT) cell line which has been malignantly transformed by arsenic (100 nM, 30 weeks) and is fully adapted to arsenic toxicity. Prior work indicated these cells impaired As(TM) may be resistant to UV-induced apoptosis but not UV-induced ODD, although the measurement of ODD used a technique prone to significant artifacts. Thus, in this study As(TM)-were acutely irradiated with UV (780 J/m2), incubated for a further 18 hrs, harvested and subjected to flow cytometry for the measurement of ODD and gene expression analysis by RT-PCR. Elevated ODD was not observed in As(TM)-cells during the 50-weeks malignant transformation process with arsenic, likely because HaCaT cells poorly methylate arsenic. However, although acute UV irradiation markedly increased ODD in control cells, in As(TM)-cells UV-induced ODD was actually further increased by over 50%. Additionally, As(TM)-cells were resistant to apoptosis induced by UV compared to control despite the enhanced ODD seen with UV in these cells. Various oxidative stress responses were reduced in As(TM)-cells after UV compared to control, including expression of nuclear factor E2-related factor 2 (Nrf2) and several of its target genes, like heme oxygenase-1 (HO-1) and glutamate-cysteine ligase catalytic subunit (GCLC). Thus, the potential synergy between UV irradiation and arsenic for skin cancer could be due to arsenic adaptation and enhanced survival even in the face of enhanced UV-induced ODD.

PS 1764 ARSENITE AND HUMAN LUNG CELL CULTURES – GENOTOXICITY AND EFFECTS ON MRP- TRANSPORTERS.

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Chronic exposure to low doses of organic as well as inorganic arsenicals eventually leads to resistance mediated by different mechanisms. One of them being increased expression of multidrug resistance associated proteins (MRP). We investigated the effects of As(III) on human lung cell cultures using the tumor cell line H322 and normal human bronchial epithelial cells (NHBE). To elucidate whether MRP-transporters protect lung cells from arsenite toxicity we used the MTT-assay to measure their viability in the presence or absence of the MRP-inhibitor MK571. H322 cells showed significantly increased sensitivity to As(III) when pre-treated with MK571 in 24h and 72h-experiments. We also analysed genotoxic effects of As(III) and H322 cells with the alkaline comet assay. While As(III) (2.5 and 5 μM) alone did not lead to significant DNA-damage after 24h, cells pre-incubated with MK571 showed significantly increased olive tail moment (OTM) 135% and 153%, respectively. Five days of treatment with As(III) (5 μM) lead to significant DNA-damage (OTM 750% of control). Again, pre-treatment with MK571 in...
creased genotoxic effects of As(III) at 2.5 and 5 μM As(III) OTMs where increased 82% and 1000%, respectively. Additionally we investigated the influence of As(III) on the expression of different MRPs in NBHEC using real-time RT-PCR. Treatment of NBHEC from two different donors with 1 and 2.5 μM As(III) for 5 days lead to up-regulation of MRP1 (1.4 to 1.5 fold), MRP4 (1.6 to 2.1 fold) and MRP5 (1.6 to 2.5 fold). Interestingly additional 5 days with As(III) repressed MRP1, 3, 4 and 5 (0.4 to 0.5 fold) only in the cultures from donor II. Further 5d of As(III) down-regulated MRP3 (0.4 fold). Another 5d of As(III) increased MRP3 (2.2 fold). Our results demonstrate a contribution of MRP-transporter to the detoxification of As(III) in human lung cell cultures, as inhibition of MRPs increased their sensitivity to As(III). Primary cultures from different donors do not show uniform reactions upon As(III) treatment.

1765 STABLE OVEREXPRESSION OF HUMAN MT-IIA GENE IN A HEART-DERIVED CELL LINE CONFERRS OFFICATIVE PROTECTION.

W. Feng, W. Xu, Q. Liu, L. Cai and Y. Kang. University of Louisville, Louisville, KY.

Metallothionein (MT) is a low-molecular-mass cysteine-rich protein. Numerous studies have suggested that MT plays an important role in essential metal homeostasis and confers resistance of cells against heavy metal toxicity and oxidative stress. Previous studies using an animal model that overexpressing MT specifically in the heart have shown that MT protects hearts. Although the protective function of MT has been attributed to its anti-oxidant properties, the precise mechanism remains elusive and needs to be studied using different strategies. Cultured cell systems are now considered indispensable powerful tools in studying the molecular and cellular mechanism. In present study, a stable MT-IIA over-expressing cardiac cell line was established, and its anti-oxidant properties were evaluated. Rat heart-derived H9c2 cell line, which is immortalized cardiac fibroblast cell line and has been well characterized and widely used in culture owing to the ability to proliferate and differentiate, is stably transfected with a plasmid in which human MT-IIA gene was placed under control of the constitutively active β-actin promoter. The transfected cell line (H9c2MT) exhibited similar growth kinetics and morphology. Western blotting analysis showed that H9c2MT had a remarkably increased MT protein level compared with the parent cell line H9c2. Addition of 25 μM ZnSO4 had an undetectable effect on the induction of endogenous MT, but it likely stabilized MT protein that is expressed only in H9c2MT cells. H9c2MT cells showed marked reduction in ROS production when exposed to hydrogen peroxide or subjected to hypoxia/reoxygenation challenge, as evaluated by dihydroethidium staining. In addition, transfection of MT showed cellular resistance to cadmium toxicity. In summary, we have established a stable human MT-IIA overexpressing cardiac cell line; and this cell line showed a markedly increased oxidative protection and would be useful for dissection of the mechanisms of MT in the cardiac protection.

1766 EFFECTS OF CHRONIC LOW DOSE ARSENIC EXPOSURE ON HORMONE-REGULATED GLOBAL GENE EXPRESSION IN NORMAL HUMAN LUNG FIBROBLASTS.

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Chronic exposure to arsenic (As) is associated with many diseases including cancer, cardiovascular disease, and developmental/reproductive disorders. As is a potent endocrine disruptor of transcriptional regulation by all five steroid receptors. Many of the As-associated toxic effects may result from this endocrine disruption. We have examined the global gene expression effects of chronic (5 weeks), low dose (20 ppb) As exposure to the rat lung fibroblast (Wistar-Furth 4-6 cells), using Affymetrix GeneChip U133 Plus 2.0 whole genome microarrays. We used R software packages in Bioconductor to perform RankProd analysis, followed by pathways analysis using Ingenuity software. Our results demonstrate As-induced endocrine disruption at the transcriptional level for glucocorticoid hormone alpha subunit (CGa), a gene containing a negative glucocorticoid response element (GRE), as well as for another Dex-regulated genes. Chronic As exposure caused decreased expression of numerous putative tumor suppressor genes and altered expression of multiple cancer biomarkers. Affected pathways include NRF2-mediated oxidative stress response, Wnt/beta-catenin signal-

1767 ARSENITE INDUCES APOPTOSIS OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS THROUGH MITOCHONDRIAL PATHWAYS.

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Background and Objective: Inorganic arsenic is widely distributed in nature and its contamination in drinking water remains a major public health problem. It was found that exposure to arsenic leads to degenerative peripheral vascular diseases. To clarify the molecular mechanisms through which arsenic causes injuries of blood vessel, we analyzed effects of sodium arsenite on the apoptosis of human umbilical vein endothelial cells (HUVECs), mitochondria membrane potential (MMP), intracellular reactive oxygen species (ROS), and the expression of the related genes. Methods: HUVECs apoptosis, MMP and intracellular ROS were determined by flow cytometry, after incubation with Annexin V-PI, rhodamine 123 (RH123), and DCFH-DA, respectively. Real time quantitative RT-PCR was used to analyze gene expression. Results: HUVECs apoptosis increases and MMP decreases following arsenite treatment (5 - 40 μM) in a dose-dependent manner. Intracellular ROS shows a two phase alteration: a slight decrease with low levels of arsenite (5 and 10 μM) treatment; but a sharp increase at higher concentrations (20 μM). The expression of superoxide dismutase 2 (SOD2) and NADPH quinone oxidoreductase 1 (NQO1) genes increased strikingly when cells were treated with low at 5 μM of sodium arsenite. Both SOD2 and NQO1 play very important roles in the reduction of intracellular ROS with their functions occurred in the mitochondria and in the cytoplasm, respectively. Conclusion: The decrease of MMP and the alteration of intracellular ROS level caused by arsenite and the resulted cell apoptosis could play very important roles in the injuries of blood vessel in arsenism. The decrease of intracellular ROS and the increase of SOD2 and NQO1 expression, when HUVECs were treated with a low concentration (5 μM) of sodium arsenite, suggest a role of the two enzymes in protecting cells from injuries by exposure to arsenite.
MICROTUBULES AS A MAJOR CELLULAR TARGET FOR ARSENIC TOXICITY.

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Arsenic (As) is an abundant element of the earth’s crust. Its compounds are widely used in industries, agriculture, and medicine. Trivalent As (As³⁺), the major chemical form in contaminated drinking water, targets protein sulfhydryls (SH) affecting on the health of an estimated 700 million people around the world. Prolonged exposure to As in drinking water induces cell injury and malignancy in multiple organs. Microtubules (MT) are a major cytoskeletal element critical for regulation of cell division. The principal protein of MT is a heterodimer consisting of tightly linked β and γ tubulins, which polymerize into long, hollow, cylindrical filaments in the cytoplasm. To understand mechanisms for cellular toxicity of As, we examined As⁴⁻ effects on MT assembly in vitro, cellular MT organization, and levels of free and polymer tubulins as well as MT associated proteins (MAPs) in the rat lung fibroblast model (RF6). Exposure of RF6 cells to sodium m-arsenate (As(V)) at 20 μM induced disassembly of MT. Western blot analysis indicated that cells exposed to As(V) exhibited a dose-dependent decrease in polymer MT and increase in the free tubulin pool, consistent with morphological loss of MT in these cells as revealed by fluorescent microscopy. As(V) (50-200 μM) also inhibited MT polymerization in vitro as monitored by turbidity and EM examinations. Dithiorethiol (DTT) (1 mM) fully abolished As⁴⁻ inhibitory effects on MT assembly in vitro. As(V) decreased the binding of [³H] N-ethylmaleimide (NEM), an -SH group-directed reagent, to tubulin suggesting As(V) action upon tubulin through –SH groups. [75S] Methionine labeling of cell MT proteins and 2D-gel analysis further showed that MAPs 50/6.6, 53/6.5, 60/6.4, 62/6.3 and 86/6.5 (MW in kDa/isoelectric point) were markedly reduced leading to MT instability in cells treated with As(V). These results strongly support MT as a major cellular target for As insult (Supported by NIH grant R01-ES11340 and Philip Morris ERP).

ARSENIC ALTERS THE DIFFERENTIATION OF MOUSE MUSCLE CELLS THROUGH THE REPRESSION OF TRANSCRIPTION FACTORS.

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Arsenic is a contaminant of drinking water in many parts of the world. A number of epidemiological studies have correlated arsenic exposure with cancer, skin diseases, cardiovascular diseases, and adverse developmental outcomes such as stillbirths, spontaneous abortions, neonatal mortality, low birth weight, and delays in the use of muscleucal. Previous studies using a model fish species termed mummichog showed that arsphenyl azotriptolone (AsIII) was catalyzed by both bacterial and mammalian PNP. Although these are structurally diverse enzymes, both mediated AsV reduction with similar responsiveness to different thiols. We examined the effect of five thiols compounds on the stoichiometry of the PNP-catalyzed arsenolysis of inosine (measured by HX formation) and the coupled AsV reduction (measured by AsIII formation). While HX formation was not influenced by the thiols, AsIII formation was increased similarly by various thiols. Collectively, these findings suggest that arsenolysis is tightly linked to AsV reduction. The potential for thiols to regulate the cytosolic arsenolysis reactions directly involved in the arsenolysis enzyme-mediated thiol-supported AsV reduction, and raise the notion that the arsenylated products of these enzymes become reduced by thiols. The poster of Gregus and Németi supports this hypothesis.

EPIGENETIC CONTROL OF MT-3 EXPRESSION IN NORMAL BREAST AND BLADDER CELL LINES.

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Metallothioneins (MT) are cysteine rich intracellular proteins that bind transition metals with high affinity and have a wide pattern of tissue distribution. However the third isoform of metallothionein, MT-3 is shown to have a restricted pattern of tissue distribution with expression confined to the neural tissue. Previous work done by this laboratory has shown that MT-3 is not expressed in the normal bladder or normal breast epithelial cells but is over-expressed in bladder and breast cancer with levels correlating to the type and grade of tumor. This lack of expression of MT-3 in normal tissue indicates that there must be a transcriptional induction event in the MT-3 promoter that occurs at some point in the tumorigenic process. Attempts to study MT-3 over expression have been plagued with difficulties due to the inability to express this gene at the protein level in many non-tumorigenic cells and the difficulty of modeling transcriptional regulation with transient transfection reporter systems. This suggested that the mode of transcriptional control of MT-3 during the formation of cancer may be at the DNA methylation level chromatin structure regulation. To test this hypothesis normal bladder UROtsa cells and breast MCF-10 cells were exposed to the common histone deacetylation inhibitor MS-275 or the commonly utilized CpG island demethylating agent 5-aza-2′-deoxycytidine (AZC). Growth of these cells for several days (estimated three cell divisions) in the presence of 3 μM MS-275 or 1.5 μM MS-275 or 1.5
Artesian (As⁵⁺) and cadmium (Cd²⁺) are carcinogens that have been implicated in the development of bladder cancer. Previous work from this laboratory has shown that normal urothelial cells (UROtsa) do not express keratin 6, however malignant transformation of these cells by the heavy metals As⁵⁺ and Cd²⁺ results in an over expression of keratin 6 mRNA as well as protein. Tumor heterotransplants generated from these cells also express keratin 6 as determined by immuno-histochemistry. Growth factor deletion and addition studies in the normal UROtsa cell line indicated that the level of keratin 6a expression was regulated by the presence of both insulin and epidermal growth factor (EGF). The purpose of this study was to determine if EGF and insulin had an effect on the expression levels of keratin 6 in UROtsa cells malignant transformed by As⁵⁺ and Cd²⁺. For this purpose six independent As⁵⁺ transformed urothelial cell lines and seven independent Cd²⁺ transformed cell lines were exposed to EGF, insulin or EGF and insulin for 24 hr. The level of keratin 6 mRNA expression was determined by real time PCR and protein levels were measured by western analysis. The data obtained indicates that the process of transformation of the urothelial cells by As⁵⁺ and Cd²⁺ induces the expression of keratin 6 in some of the transformed cell lines. Furthermore exposure to EGF, insulin or EGF plus insulin only had a modest effect on the expression of keratin 6 in some of the transformed cell lines whereas all the tumor heterotransplants generated from these transformed cell lines over expressed keratin 6. This suggests that the in vivo environment may be necessary for the up regulation of keratin 6.

**1774** EFFECT OF EGF AND INSULIN ON THE EXPRESSION OF KERATIN 6 IN ARSENITE AND CADMIUM TRANSFORMED UROTSAC CELL LINES.

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**1775** THE ROLE OF CYTOKINE SIGNALING IN ARSENIC INDUCED DIABETES.

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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. Insulin is the principal hormone involved in lowering blood glucose and functions by suppressing glucose neogenesis and glycogenolysis in the liver and by stimulating the uptake of glucose into skeletal muscle and adipocytes. Interestingly, recent epidemiological studies both in the USA and abroad have linked chronic ingestion of low levels of arsenic, an environmental toxicant, to the onset of diabetes mellitus. Arsenic-induced diabetes in mice is characterized by insulin resistance [insensitivity] and not a deficiency in insulin secretion. In a previous in vivo study, we demonstrated that chronic administration of 10 ppb (0.13 μM) NaAsO₃ in drinking water resulted in dysregulation of glucose metabolism. The purpose of the current study was to determine if arsenic could induce cytokeine production and contribute to insulin resistance and hyperglycemia in our model system. Cytokine array analysis of splenic tissue homogenate from arsenic-treated mice revealed changes in expression of cytokines involved in the inflammatory response and has also been implicated in atypic insulin signaling. Studies are ongoing to investigate the role of IL-6 and other potential cytokines on the activation of the insulin receptor. This work was supported by ES004940.

**1776** DEPLETED URANIUM INDUCED OXIDATIVE STRESS ACTIVATES DNA REPAIR IN HUMAN BRONCHIAL EPITHELIAL CELLS.

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Cellular oxidative stress has been implicated in the genotoxicity of many heavy metals. The aim of this study is to evaluate the toxicity of depleted uranium (DU) in its soluble and insoluble forms in human bronchial epithelial cells (16HBE14o-). 16HBE14o- cells were exposed to 30 ppb of soluble depleted uranium (urananyl acetate - UA) and insoluble depleted uranium (uranium trioxide - UO3) for 2 - 24 hr. Oxidative stress was assessed in vitro by 5,6-carbonyl-2,7-dichlorofluorescin diacetate (H2DCFDA) staining and visualized by live cell fluorescent microscopy. DNA damage response was assessed in vitro via Western immunoblotting to determine phosphorylation of histone H2AX and RPA levels. Results indicate that 16HBE14o- cells treated with UA and UO3 induced expression of γH2AX and RPA compared to untreated cells at 3 – 4 hr time points. 16HBE14o- cells treated with UA and UO3 induced phosphorylation of histone H2AX. These results are consistent with previous studies that indicate DU induces DNA damage via strand breaks and uranium-DNA interactions in treated cells. Interestingly, there was no increase in RPA phosphorylation levels of DU treated cells compared to untreated cells. These results suggest that: (1) cellular oxidative stress may be a pathway of particular DU genotoxicity, and (2) induced DNA damage by DU may activate base excision repair in vitro. We are currently examining additional DNA repair targets to better understand the DNA repair pathways stimulated by exposure to DU.

(Supported by NIH Grants CA096281 (RCL, KD), F31ES014971 (MY), and The Alfred P. Sloan Foundation (MY))
Arsenic is a known human bladder carcinogen; however, the exact molecular mechanisms underlying arsenic-induced bladder carcinogenesis are unknown. The human bladder urothelial cell line, UROtsa, has been shown to be malignant following low-level exposure to both arsenite ([AsIII]) and monomethylarsonic acid ([MMAIII]). Previous research has demonstrated that continuous exposure to 0 nM MMAIII for 52 wk resulted in the malignant transformation of UROtsa cells. Transformation was demonstrated by the ability of these cells to grow in soft agar at 24 wk, and the ability to form tumors in SCID mice at 52 wk. In order to focus research efforts on the biological changes occurring to arsenic-exposed UROtsa cells prior to malignant transformation, the goal of this research was to evaluate the minimum duration of 50 nM MMAIII exposure necessary to induce transformation of UROtsa cells. UROtsa cells were cultured in the presence/absence of 50 nM MMAIII, and the following time points were assessed for colony growth: 12, 16, 20, 24, and 52 wk in the presence of MMAIII; and 12, 16, and 20 wk exposure to MMAIII carried out to 24 wk in the absence of MMAIII exposure. Cellular transformation assessed through anchorage-independent growth demonstrated that UROtsa cells exposed to MMAIII for 12 wk begin to form colonies in soft agar. To further substantiate that transformation occurs prior to 52 weeks MMAIII exposure, UROtsa cells were tested for the ability to form tumors in SCID mice. Tumorigenicity in SCID mice was observed after UROtsa cells were exposed to 50 nM MMAIII for 24 wk followed by 24 wk in the absence of MMAIII exposure. This data provides evidence that MMAIII is capable of transforming UROtsa cells prior to 24 wk exposure, and validates future mechanistic studies focused on the biological changes occurring during these critical time points, which result in arsenic-induced malignant transformation.

PS 1778

PG2 FACILITATES HIF-1α SIGNALING IN HUMAN LUNG FIBROBLASTS TO AMPLIFY NI- AND VANADIUM-INDUCED VEGF AND CXCL8 RELEASE.

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Air particulate matter pollution (PM) stimulates inflammation of the lower airways, linking PM exposure to numerous lung diseases including fibrosis and cancer. Recent evidence has shed light on the transcriptional impact of HIF-1α participates in inflammatory-like processes and may contribute to the pathogenesis of pulmonary fibrotic disorders. It is widely considered that in the lung, the extent of inflammatory responses is limited by Prostaglandin E2 (PG2). However, studies have indicated that fibrotic lung fibroblasts may lose PG2 responsiveness. The purpose of this study was to examine the role of HIF-1α in mediating the release of pro-fibrotic and pro-inflammatory factors from human lung fibroblasts (HLF) in response to metals commonly found in PM and how this path may be altered by PG2. HLF were stimulated with NiSO4 or VOSO4 in the presence or absence of 100 nM PG2 for 48 h. Following exposure, cells were harvested for protein and conditioned medium was collected for ELISA analysis of VEGF and CXCL8. NiSO4 and VOSO4 both stimulated HIF-1α protein expression in HLF in a concentration-dependent manner. Binding of HIF-1α to the HRE was also observed following exposure to NiSO4 and VOSO4, indicating the HIF-1α is required to transcriptionally active. Although PG2 alone was without effect, PG2 enhanced the induction of HIF-1α protein observed with metal exposure. Moreover, PG2 synergized with NiSO4 and VOSO4 to release VEGF and CXCL8 from HLF. Transient transfection of HLF with HIF-1α siRNA attenuated both the stabilization of HIF-1α as well as VEGF and CXCL8 release by NiSO4 and VOSO4 alone or with PG2 compared with cells transfected with a non-targeting siRNA. In conclusion, these data highlight a novel role for HIF-1α in mediating chemical-induced alterations in PG2 responses that depart from its typical anti-inflammatory (inhibitory) role, imparting a susceptibility to fibrotic changes and the development of a pro-angiogenic environment in the lung. Supported by R01 ES019866 and F32ES05966.

PS 1779

DEFINING THE DURATION OF CHRONIC, LOW-LEVEL MONOMETHYLARSONOUS ACID EXPOSURE REQUIRED TO INDUCE THE MALIGNANT TRANSFORMATION OF UROTS COMM.

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Arsenic is a known human bladder carcinogen; however, the exact molecular mechanisms underlying arsenic-induced bladder carcinogenesis are unknown. The human bladder urothelial cell line, UROtsa, has been shown to be malignant following low-level exposure to both arsenite ([AsIII]) and monomethylarsonic acid ([MMAIII]). Previous research has demonstrated that continuous exposure to 0 nM MMAIII for 52 wk resulted in the malignant transformation of UROtsa cells. Transformation was demonstrated by the ability of these cells to grow in soft agar at 24 wk, and the ability to form tumors in SCID mice at 52 wk. In order to focus research efforts on the biological changes occurring to arsenic-exposed UROtsa cells prior to malignant transformation, the goal of this research was to evaluate the minimum duration of 50 nM MMAIII exposure necessary to induce transformation of UROtsa cells. UROtsa cells were cultured in the presence/absence of 50 nM MMAIII, and the following time points were assessed for colony growth: 12, 16, 20, 24, and 52 wk in the presence of MMAIII; and 12, 16, and 20 wk exposure to MMAIII carried out to 24 wk in the absence of MMAIII exposure. Cellular transformation assessed through anchorage-independent growth demonstrated that UROtsa cells exposed to MMAIII for 12 wk begin to form colonies in soft agar. To further substantiate that transformation occurs prior to 52 weeks MMAIII exposure, UROtsa cells were tested for the ability to form tumors in SCID mice. Tumorigenicity in SCID mice was observed after UROtsa cells were exposed to 50 nM MMAIII for 24 wk followed by 24 wk in the absence of MMAIII exposure. This data provides evidence that MMAIII is capable of transforming UROtsa cells prior to 24 wk exposure, and validates future mechanistic studies focused on the biological changes occurring during these critical time points, which result in arsenic-induced malignant transformation.

PS 1780

LOW DOSE ARSENIC EXPOSURE POTENTIATES A PRO-ATHEROGENIC PHENOTYPE IN MACROPHAGES AND LIVER.

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Arsenic exposure increases risk of cardiovascular disease, in particular atherosclerosis. Atherosclerosis develops in a dysregulated cholesterol flux in macrophages and other cell types, resulting in localized vascular inflammation and monocyte adhesion. We have previously described that μM concentrations of arsenic inhibit the transcriptional activation of the liver X receptor (LXR), which controls genes involved in reverse cholesterol efflux from macrophages. Here, we present evidence that low arsenic levels (at or below maximal EPA limits for drinking water) potentiate a pro-atherogenic environment both in vitro and in vivo. Macrophages cultured in low dose arsenic (50 nM arsenic trioxide = 20 ppb arsenic) exhibited a decreased response to oxysterol challenge after 1 week, as measured by induction of the LXR target genes ABCA1 and SREBP-1c, compared to macrophages cultured in media not supplemented with arsenic. This phenotype persisted throughout the 25 weeks of exposure to low level arsenic. In addition, LXR target protein expression decreased in C57BL/6 mice given 10-100 ppb arsenic in the drinking water for 5 weeks as compared to controls. While arsenic inhibited LXR, it potentiated the induction of inflammatory molecules by LPS, such as iNOS and Cox-2. Arsenic also enhances LPS induction of IL-1β, as well as monocyte adhesion to VCAM-1 coated plates at doses as low as 2 ppb. Together, these data suggest that prolonged exposure to low arsenic levels can alter nuclear receptor function, increase inflammation, and increase vascular adhesion of mononuclear cells, in a manner that can increase susceptibility to atherosclerosis. Supported by the CJHR (WHM) and R01 ES050638 (AB).

PS 1781

STAT1 IS ESSENTIAL FOR CHROMIUM SILENCING OF NICKEL-INDUCED VEGF EXPRESSION IN HUMAN AIRWAY EPITHELIAL CELLS.

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Hexavalent chromium (Cr(VI)) promotes lung injury and pulmonary diseases through poorly defined mechanisms that often involve silencing of inducible protective genes. The current study investigated the hypothesis that Cr(VI) actively signal through a signal transducer and activator of transcription 1 (STAT1)-dependent pathway to silence nickel (Ni)-induced expression of vascular endothelial cell growth factor (VEGF), an important mediator of lung injury and repair. In human bronchial airway epithelial (BEAS-2B) cells, Ni induced VEGF transcription by stimulating a complex Erk signaling cascade that involved increased hypoxia-inducible factor-1α (HIF) stabilization and DNA binding, as well as a Src kinase-activated cascade involving Sp1 transcription. Exposing the cells to Cr(VI) prior to the addition of Ni silenced Ni-induced VEGF transcript levels. During this exposure, Cr(VI) also decreased Ni-stimulated Erk phosphorylation, HIF stabilization, and the induction of a hypoxia response element-driven reporter construct. We previously demonstrated that Cr(VI) stimulated STAT1 which has been implicated as a negative regulator of VEGF expression. Therefore, we tested the effect of Cr(VI) on VEGF expression in BEAS-2B cells stably expressing siRNA that eliminated STAT1. In the absence of STAT1, Cr(VI) increased VEGF transcript levels and did not block Ni-stimulated HIF protein levels. In addition, Cr(VI) and Ni co-exposures positively interacted to further increase VEGF transcripts. This study demonstrates that the different signaling pathways stimulated by metals interact to influence transcriptional regulation of adaptive responses in airway cell injury and repair. In addition, the data implicate STAT1 as an essential mediator of Cr(VI)-stimulated gene silencing and suggest that cells lacking STAT1, such as many tumor cell lines, have opposite responses to Cr(VI) relative to normal cells. Supported by NIEHS R01ES050638.

PS 1782

HEXAVALENT CHROMIUM IS MORE CLASTOGENIC TO NORTHERN RIGHT WHALE CELLS THAN HUMAN CELLS.

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It has been suggested that longer-lived species have better DNA repair than shorter-lived species. Some whales, such as the Northern right whale are thought to have even longer lifespans than humans suggesting that these animals may have better
DNA repair than humans. To test our hypothesis we compared the genotoxic effects of hexavalent chromium, a well known human carcinogen, in Northern right whale and human cells. Our results show that at the same intracellular concentration, hexavalent chromium induces less chromosome damage and in whale cells compared to human cells after a 24 h exposure. For example, an intracellular chromium level of 250 μM damaged 43 chromosomes in human cells compared to only 26 in the whale cells. We considered the possibility that this damage was due to Cr(VI)-induced apoptosis, but found little apoptotic cell death with these treatments. These data are consistent with a hypothesis that whales may have better DNA repair than humans. Future experiments will consider DNA double strand breaks and DNA repair. This work was supported by Grant number N001474720478 from the United States Department of Commerce, NOAA (JFW), NIEHS grant ES10838 (JFW) and the Maine Center for Toxicology and Environmental Health.

1784 PERMEATION OF ARSANILATE AND ARSACETIN IN CAO-2 CELLS IS ADEQUATE TO STIMULATE CELL PROLIFERATION.

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Arsanilate is an organoselenium feed additive used in swine, with USDA approval, to control microbial infections and to improve feed efficiency. Current farming practices allow waterways to annually receive undetermined amounts of Arsanilate and its metabolites, in the tons of swine waste from concentrated animal farm operations. These contaminants have increased exponentially in this country since the 1980s. In major swine-growing states such as Iowa and North Carolina, the waste has usually been stored in open pits and recycled as fertilizer. Although efforts are underway to change this practice, leaching into ground water will likely cause continuous contamination. Our laboratory has been concerned with possible effects on humans of Arsanilate and its N-acetylated metabolite Arsacetin. The literature indicates that Arsacetin, when swine dosed with 1C, Arsanilate recreates an average of 28% and Arsanilate and 22% of the N-acetylated metabolite Arsacetin. Our study of the potential human impact of these compounds employed the adenocarcinoma Caco-2 cell line, an accepted model for intestinal absorption. The cells exhibited significantly increased proliferation, as measured by HTC thymidine uptake into DNA, when dosed with Arsanilate and Arsacetin at the μL level for 24 hours. In our additional experiments, cells were grown to confluency on collagen-coated Transwells before dosing on the apical side. After removal of PBS, basolateral and apical media were separately analyzed by HPLC. Preliminary results at a 10μM dose for 24 hours reveal permeation to basolateral side of 3.3μM (33%) for Arsanilate and 3.8μM (38%) for Arsacetin. Thus, permeation occurs at a concentration capable of stimulating cell proliferation, in agreement with our previously reported results for the poultry feed additive Roxarsone and its metabolites.

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1785 HEXAVALENT CHROMIUM IS MORE CLASTOGENIC TO BOWHEAD WHALE (BALAENA MYSTICETUS) CELLS THAN HUMAN CELLS.

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Chromium is a common marine and atmospheric pollutant. Hexavalent chromium is a known human carcinogen which has been shown to damage DNA and cause chromosomal breaks. Few studies have considered the effect of chromium in marine mammals despite their exposure. We have begun investigating the genotoxicity of chromium in bowhead whales and comparing these effects with those seen in humans. Hexavalent chromium was only weakly clastogenic to whale cells. Concentrations of 0, 0.5, 1, 2, 4, and 5 μM/μL lead chromate induced chromosome damage in 6, 11, 9, 12, 13 and 11 percent of metaphase cells in bowhead lung cells; 4, 4, 16, 12, 8, and 14 percent of cells in bowhead skin cells; and 8, 6, 10, 15, 15, and 17 percent of metaphases in bowhead testes cells. These results indicate that the potency of hexavalent chromium in bowhead lung and testes is similar and lower than in bowhead skin cells. By contrast, hexavalent chromium is potently genotoxic to human cells. Lead chromate damaged chromosomes in 2, 23, and 34 percent of metaphase cells after exposure to 0, 0.5 and 1 μM respectively. Higher concentrations induced cell cycle arrest and no metaphases were seen. These data indicate that a given dose of lead chromate will induce 2-4-fold more damage in human cells compared to whale cells. These data suggest that bowhead whale cells may have a protective mechanism against genotoxic damage. This work was supported by the Maine Center for Toxicology and Environmental Health.

1786 CADMIUM-INDUCED PROLIFERATION OF BREAST CANCER CELLS IS MEDIATED BY GP30.

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Cadmium (Cd) is a nonessential metal that is dispersed throughout the environment. It is considered as an endocrine-disrupting element because it tightly binds to estrogen receptor. As a xenoestrogen, Cd mimics estrogen and promotes breast cancer cell proliferation. Estrogen receptor plays an important role in this process. G-protein coupled receptor 30 (GP30) is a recently discovered membrane estrogen receptor. The purpose of the present study was to investigate whether GP30 plays a role in Cd-induced cell proliferation. Two human breast cancer cell lines, SK-BR-3 (GP30 positive, vector transfected) and SK-BR-3 HA-D154 (GP30 negative), were utilized. Incubation with Cd promoted proliferation of GP30 positive cells, but not of GP30 negative cells. Intracellular cAMP levels increased about 1.7-fold in GP30 positive cells upon treatment with 0.5 μM Cd, but not in GP30 negative cells. Also, in GP30 positive cells, Cd activated Raf kinase, mitogen-activated protein kinase, extracellular signal regulated kinase, ribosomal S6 kinase, and E-26 like protein kinase about 4-fold. In comparison, in GP30 negative cells, Cd activated these signaling molecules by only about 1.4-fold. Taken together, these results demonstrate that Cd increases cAMP level, activates ERK kinase pathway, and stimulates breast cancer cell proliferation through GP30. (Supported in part by a fellowship from INBRE grant #P20RR016457)

1787 ALUMINUM IS MORE CYTOTOXIC THAN LUNAR DUST IN HUMAN SKIN AND LUNG FIBROBLASTS.

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NASA plans to build a permanent space station on the moon to explore its surface. The surface of the moon is covered in lunar dust, which consists of fine particles that contain silicon, aluminum and titanium, among others. Because this will be a manned base, the potential toxicity of this dust has to be studied. Also, toxicity standards for potential exposure have to be set. To properly address the potential toxicity of lunar dust we need to understand the toxicity of its individual components, as well as their combined effects. In order to study this we compared NASA simulated lunar dust simulant JSC-1AVF (volcanic ash particles), that simulates the dust found on the moon, to aluminum, the 3rd most abundant component in lunar dust. We tested the cytotoxicity of both compounds on human lung and skin fibroblasts (WI38-VI6 and BJ/1ERT cell lines, respectively). Aluminum was more cytotoxic than lunar dust to both cell lines. In human lung fibroblasts 5, 10 and 50 μg/cm2 of aluminum oxide induced 85%, 61% and 30% relative survival, respectively. For human skin fibroblasts the same concentrations induced 58%, 41% and 58% relative survival. Lunar dust was also cytotoxic to both cell lines, but its effects were seen at higher concentrations: 50, 100, 200 and 400 μg/cm2 of lunar dust induced a 69%, 46%, 35% and 30% relative survival in the skin cells and 53%, 16%, 8% and 2% on the lung cells. Overall, for both compounds, lung cells were more sensitive than skin cells. This work was supported by a NASA EPSCoR grant through the Maine Space Grant Consortium (JFW), the Maine Center for Toxicology and Environmental Health, a Fullbright Grant (JM) and a Delta Kappa Gamma Society International World Fellowship (JM).
and ZIP or SLC39a) which control the transport across lipid membranes, thereby controlling zinc into and out of the cell or between sub-cellular compartments. This study was a part of the program for Short Term Educational Experiences for Research (STEER) in Environmental Sciences for Undergraduates, and the purpose was to characterize the expression of transporter genes and ten zinc transporter genes in renal cortical tissues specimens and in cultures of human proximal tubule cells. The overall goal was to define the genetic regulation of zinc transport in the human kidney as well as its potential interaction with toxic heavy metal transport. For this purpose, total RNA was purified from cultures treated with and without 100 μM zinc or from renal cortical tissue and subjected to RT-PCR using real-time quantitative detection. Expression of ZnT-1, -2, -4, -5, -6, and -7 was detected in both tissue and cultures with ZnT-1 and -2 mRNA being induced by zinc. The expression of ZIP-1, -3, -5, -7, -8, and 14 was also detected in both cultures and tissue with little if any regulation by zinc exposure. These results suggest that there is a high level of complexity in the control of zinc transport within the kidney.

### HISTOLOGICAL CHARACTERISTICS OF SUBCUTANEOUS AND INTRAPERITONEAL TUMORS PRODUCED BY CLONES OF ARSENITE AND CADMIUM TRANSFORMED HUMAN UROTHELIAL CELLS.

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Arsenite (As3+) and cadmium (Cd2+) are carcinogens that cause bladder cancer. Previous work from this laboratory has shown that both As3+ and Cd2+ can directly transform the UROtsa cell line and that the tumor heterotransplantates produced have histiogenic features consistent with human bladder cancer. In this laboratory, six As3+ and seven Cd2+ transformed cell lines were generated independently from the parent UROtsa cells, and they formed tumors when injected subcutaneously in nude mice. These tumors were further grown in nude mice, and the morphological features observed for the IP tumors were compared with the subcutaneous tumor (SC) produced by the same line with emphasis on squamous differentiation of the tumors. Histologically, tumors generated by these cell lines demonstrated varying degree of squamous differentiation. The SC tumors and IP-S tumor had similar level of squamous differentiation, but the IP-D tumors were consistently less differentiated than both SC tumor and IP-S tumor. Comparison of the squamous markers between the tumors indicated no significant difference between IP-S and SC tumors, but the IP-D tumors were significantly different from SC and IP-S tumors. In conclusion, these transformed urothelial cell lines can produce intraperitoneal tumor heterotransplants in nude mice; however, the deeply located IP tumors have a lesser degree of squamous differentiation compared to the superficial intraperitoneal and subcutaneous tumors.

### METALLOTHIONEIN EXPRESSION IN CADMIUM- AND ARSENITE-TRANSFORMED UROTHELIAL CELLS.

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Cadmium (Cd2+) and arsenite (As3+) are known bladder carcinogens that can directly malignantly transform the UROtsa cell line and that the tumor heterotransplantates produced have histiogenic features consistent with human bladder cancer. In this laboratory, six As3+ and seven Cd2+ transformed cell lines were generated independently from the parent UROtsa cells, and they formed tumors when injected subcutaneously in nude mice. These tumors were further grown in nude mice, and the morphological features observed for the IP tumors were compared with the subcutaneous tumor (SC) produced by the same line with emphasis on squamous differentiation of the tumors. Histologically, tumors generated by these cell lines demonstrated varying degree of squamous differentiation. The SC tumors and IP-S tumor had similar level of squamous differentiation, but the IP-D tumors were consistently less differentiated than both SC tumor and IP-S tumor. Comparison of the squamous markers between the tumors indicated no significant difference between IP-S and SC tumors, but the IP-D tumors were significantly different from SC and IP-S tumors. In conclusion, these transformed urothelial cell lines can produce intraperitoneal tumor heterotransplants in nude mice; however, the deeply located IP tumors have a lesser degree of squamous differentiation compared to the superficial intraperitoneal and subcutaneous tumors.

### RESPONSE OF HEMOPOIETIC AND OSTEOGENIC CELLS FOLLOWING EXPOSURE TO TUNGSTEN AND TUNGSTEN ALLOYS IN VITRO.


Bone marrow is a target of many environmental, chemical and therapeutic agents. As such, hemopoietic and osteogenic toxicity is a common, dose-limiting factor for xenobiotics. Here we examined the in vitro toxicity of tungsten (W), 2 tungsten alloys, one containing nickel (Ni) and cobalt (Co) (WA1) and the other Ni and iron (Fe) (WA2), and 2 metal salts, sodium tungstate dihydrate and lead (II) nitrate, on human and mouse hematopoietic and mesenchymal progenitors. For 14 days, cells were exposed to W, WA1 or WA2 at 0.02-20 mg/mL, or metal salts at 0.001-10 mM. The effects on myeloid and erythroid progenitor proliferation was also assessed using human and mouse bone marrow cells in a methylcellulose-based in vitro colony forming assay and mesenchymal proliferation was assessed using freshly isolated human and mouse bone marrow cells in a liquid-based in vitro culture assay. The impact of 3 metal compounds on human and mouse hematopoietic and stromal progenitors revealed that toxicity of tungsten was either similar to or less than levels observed for both tungsten alloys.

### LOW-LEVEL INORGANIC ARSENITE IMPAIRS GLUCOSE-STIMULATED INSULIN SECRETION IN PANCREATIC beta-CELLS: INVOLVEMENT OF NFR2-MEDIATED ANTIOXIDANT RESPONSE.

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There is growing evidence that chronic exposure of humans to inorganic arsenic, a potent environmental oxidative stressor, is associated with the incidence of type 2 diabetes (T2D). A key driver in the pathogenesis of T2D is the impairment of pancreatic beta-cell function, the hallmark of which is decreased glucose-stimulated insulin secretion (GSIS). In contrast to what has been a prevailing beneficial view of antioxidants in preventing beta-cell dysfunction in diabetes, this study proposes that in response to arsenic exposure, transcription factor Nfr2-mediated adaptive induction of endogenous antioxidant enzymes plays a pathophysiological role in beta-cell function. Exposure of INS-1(832/13) cells or isolated mouse islets to low-levels of arsenic (up to 1 μM for 24-96 hr; non-cytotoxic) led to decreased GSIS in a dose- and time-dependent manner. The impaired GSIS was associated with increases in a battery of endogenous antioxidant enzymes that are regulated by the transcription factor Nfr2. In addition, intracellular glutathione and total antioxidant potential was significantly increased in the arsinite-treated cells. The increased antioxidant activity significantly inhibited net glucose-stimulated intracellular H2O2 accumulation, which is involved in GSIS. In addition, the effects of arsenite exposure on the genes/proteins that are associated with glucose transport and metabolism, the classical GSIS pathway, insulin synthesis, secretion, and inflammatory response were also evaluated in the beta-cell models. Taken together our studies suggest that low-level of arsenite triggers a cellular adaptive oxidative stress response to impair ROS signaling that is involved in GSIS, and thus disturbs beta-cell function. (This research was supported by NIEHS-ONES-R01ES016005 and NIDDK-K01DK076788).

### ALTERED GRAVITY EXACERBATES CHROMATE-INDUCED GENOTOXICITY.

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NASA plans to build a permanent manned space station on the Moon. A challenge for personnel stationed on the moon will be the potential for altered gravity to potentiate the toxicity of exposures to metals and dusts on the Moon. Microgravity is known to change the morphology of cells, and results in the loss of muscle and bone mass. Based on these observations, we hypothesized that altered gravity will cause genotoxic chemicals to have a greater damaging effect on cells and DNA. To test this hypothesis, we studied human lung cells treated with a known carcinogen (sodium chromate) and exposed to microgravity. Cells were then ex-
Arsenic (As) is a major drinking water contaminant and is associated with numerous adverse health effects. Thus, it is critical to develop biomarkers of response for prevention strategies and to elucidate its mechanism of toxicity. Pyroxicomic analysis recently conducted by our laboratory identified lower levels of β-defensin-1 (hBD1) peptides in urine of individuals from two independent human populations exposed to As in drinking water (Hegedus et al., 2008). Subsequent in vitro analysis demonstrated that As decreases hBD1 mRNA levels, providing evidence that hBD1 may be a biomarker of response to As. To further validate these findings, we examined the effect of As on hBD1 gene expression in HOK-16B and UROtsa cells, derived from human skin and bladder, respectively, both of which are target tissues for As toxicity. Real-time PCR analysis was performed to quantify hBD1 mRNA following exposure to arsenite (As³⁺) or its methylated metabolite, MMA³⁺. For 48 h, UROtsa cells demonstrate a dose-dependent decrease in hBD1 mRNA following exposure to MMA³⁺, but appear to be resistant to As³⁺, while HOK-16B cells demonstrate a dose-dependent decrease in hBD1 mRNA in response to As³⁺, and MMA³⁺ suppresses hBD1 mRNA only at the highest dose. UROtsa cells do not express the enzyme AS3MT and cannot metabolize As³⁺ to MMA³⁺, which is more toxic in vitro. Without AS3MT, UROtsa cells also cannot metabolize MMA³⁺ to the less toxic metabolite DMA³⁺. Thus, our preliminary data suggest that MMA³⁺ may be the As species responsible for decreased hBD1 expression, hBD1 is a cationic antimicrobial peptide constitutively expressed in epithelia and skin and urogenital system. Renal cell carcinomas have decreased hBD1 expression. hBD1 is a cationic antimicrobial peptide constitutively expressed in epithelia cells, derived from human skin and bladder, respectively, both of which are target tissues for As toxicity. Real-time PCR analysis was performed to quantify hBD1 mRNA following exposure to arsenite (As³⁺) or its methylated metabolite, MMA³⁺. For 48 h, UROtsa cells demonstrate a dose-dependent decrease in hBD1 mRNA following exposure to MMA³⁺, but appear to be resistant to As³⁺, while HOK-16B cells demonstrate a dose-dependent decrease in hBD1 mRNA in response to As³⁺, and MMA³⁺ suppresses hBD1 mRNA only at the highest dose. UROtsa cells do not express the enzyme AS3MT and cannot metabolize As³⁺ to MMA³⁺, which is more toxic in vitro. Without AS3MT, UROtsa cells also cannot metabolize MMA³⁺ to the less toxic metabolite DMA³⁺. Thus, our preliminary data suggest that MMA³⁺ may be the As species responsible for decreased hBD1 expression, hBD1 is a cationic antimicrobial peptide constitutively expressed in epithelia of the skin and urogenital system. Renal cell carcinomas have decreased hBD1 expression, suggesting that hBD1 may be a tumor suppressor for urological cancers. Thus, it is plausible that decreased hBD1 caused by the toxic metabolite MMA³⁺ may play a role in As-associated cancers. Supported by NIH grant P42ES04705.
cisplatin resistance in ovarian cancer cells. We hypothesized that NaAsO2 and hyperthermia would enhance cisplatin cytotoxicity by modifying DNA repair proteins and altering cellular platinum accumulation. Cisplatin-sensitive (A2780) and -resistant (CP70) ovarian cancer cells were treated with cisplatin, with or without 20 μM NaAsO2 at 37 or 39°C (hyperthermia) for 1 h. A2780 and CP70 cells expressed basal levels of XPC and DDB2, key lesion recognition proteins in global genomic repair, subpathway of nucleotide excision repair. Cisplatin increased the levels of these proteins. CP70 cells maintained higher levels of XPC and DDB2 with increased concentrations of cisplatin. NaAsO2 did not stabilize XPC, whereas, hyperthermia stabilized it. A2780 cells accumulated ~2-fold more Pt than CP70 cells. Unlike NaAsO2, hyperthermia increased the cellular accumulation of Pt. Cell cycle analysis revealed G2/M accumulation of cells 36 h after treatment. NaAsO2 and hyperthermia increased cellular p53 levels, cellular accumulation and NaAsO2 altered DNA repair by decreasing the expression of XPC and causing cells to accumulate in the G2 phase of the cell cycle. Supported in part by NIH grants 1P50ES014443 and SRO1ES011314.

1799 THE ROLE OF PKC CONSENSUS SITES IN MTF-1 TRANSCRIPTIONAL ACTIVATION.

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Metallothioneins (MTs) belong to a superfamily of intracellular proteins that sequester environmentally toxic metals and are thought to regulate their intracellular concentration. MT expression is transcriptionally regulated by the metal-regulatory transcription factor 1 (MTF-1). MTF-1 acts by binding to short DNA sequences called metal responsive elements (MREs) found in the enhancer/promoter regions of target genes including MT-1, MT-2 and ZnT1, a Zn efflux transporter. Two models involving to determine whether metals regulate MTF-1 mediated transcription: One proposes that MTF-1 functions as a zinc sensor, and the other that the phosphorylation of MTF-1 via protein kinase C (PKC) signaling pathways controls its transcriptional activity. To examine the role of phosphorylation in MTF-1 transcriptional activation, PKC consensus sites were identified within MTF-1 using Prosite analysis and each site was systematically mutated. Lentiviruses were developed for each mutant, transduced into MTF-1 knockout mouse embryonic fibroblasts (dko7) cells and stable cell lines expressing each construct were isolated. We exposed these cells to cadmium or zinc, and measured changes in expression of three marker genes: MT-1, MT-2 and ZnT1, by real time qPCR. dko7 cells transduced with wild type MTF-1 demonstrated significant levels of metal-inducible transcription of all three marker genes examined. Surprisingly, the MTF-1-T224A mutant had MTF-1 mRNA levels equivalent to cells transduced with wild-type MTF-1, but did not show metal-inducible transcription of the marker genes examined. Additionally MTF-1 +/− cells expressing MTF-1-T224A are hypersensitive to cadmium exposure. These data confirm that phosphorylation of this PKC phosphorylation site prevents metal inducible MTF-1-mediated transcription and has detrimental effects on cell survival after exposure to metal.

1800 ROLE OF DIVALENT METAL IONS IN AIOAMOYLAASE 3 MEDIATED CATALYSIS.

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Aminopeptidase 3 (AA3) is highly expressed in renal proximal tubules. The AA3 mediated decarboxylation of N-acetyl-2,12-dihydroxy-L-cysteine (Ac-DHCVC, a metabolite of a xenobiotic trichloroethylene (TCE), is involved in acute renal failure induced by TCE. Despite of ~40% identity of AA3 with the zinc containing asparaginase, data on metal dependence of AA3 is lacking. To determine whether mouse AA3 contains a metal ion(s) and whether this metal ion(s) is necessary for the catalysis. In the absence of a high-resolution 3D structure of AA3, we performed modeling of its 3D structure to determine putative amino acid residues involved in metal binding in or near the active site. Based on the 3D model of mouse AA3, His21, Gla24 and His116 were hypothesized to be involved in the coordination of a metal ion in a putative active site. In agreement with this hypothesis, the H21A, E24A and H116A mutants of mouse AA3 were inactive. Using inductively coupled plasma mass spectrometry, 0.01-0.05 atoms of Mn, Cu, Fe or Ni, and 0.35-0.5 atoms of Zn per monomer of wild type (wt) AA3 was determined. Incubation with Co2+ activated several times wt-AA3 and the Co2+-activated wt-AA3 contained 1-2 atoms of Co per monomer and no Zn indicating that Co2+ replaced Zn2+ in the enzyme. Incubation with zinc did not change the activity of wt-AA3 despite the fact that it bound up to 4 zinc atoms per monomer. The amount of the specific metal bound to wt-AA3 was dependent on its concentration in the growth media. The H21A, E24A and H116A mutants had decreased metal content in comparison with wt-AA3. In addition to drastic increase of the rate of deacetylation activity, Co2+ significantly increased the toxicity of Ac-DHCVC in mouse proximal tubule mPCT cells expressing mouse wt-AA3. The results indicate that AA3 is a metalloenzyme and suggest that divalent metal ions may play an important role in mediating the toxicity of TCE.

1801 PROTECTIVE ROLE OF FERROUS SULFATE IN CHROMIUM TOXICITY.

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In the US, approximately 1.5 million construction workers are occupationally exposed to cement each year. Allergic contact dermatitis (ACD) is a major skin problem reported among cement workers. Hexavalent chromium (Cr(VI)) present as a contaminant in the cement, is believed to be responsible for cement ACD. A significant reduction in the incidence of cement ACD has been reported in certain European countries where the addition of ferrous sulfate (FeSO4) to cement has been mandated. However, the actual involvement of FeSO4 in counteracting the dermatological effect has not been elucidated. In this study, we report the cytotoxicity, apoptosis and oxidative stress of A2780 and CP70 cells upon exposure to Cr(VI) and FeSO4. Cytotoxicity, apoptosis, and oxidative stress were determined by MTT assay, TUNEL assay, and by electron spin resonance (ESR) analysis, respectively, in the treated cells. Exposure of the cells to potassium dichromate alone resulted in concentration-dependent cytotoxicity, apoptosis, and oxidative stress. The Cr(VI)-induced cytotoxicity, apoptosis, and oxidative stress in the dermal cell lines were significantly blocked by the addition of FeSO4 to the cell culture medium. Analysis of the global gene expression profile in the cells further confirmed the protective role of FeSO4 in Cr(VI) toxicity and provided novel insights regarding Cr(VI) toxicity as well as the protective role of FeSO4. In summary, our results demonstrated a protective role for FeSO4 in Cr(VI) toxicity suggesting that the addition of FeSO4 to cement may be helpful to prevent or at least reduce the incidence of cement ACD among construction workers in the US. Disclaimer: The findings and conclusions in this abstract have not been formally disseminated by the National Institute for Occupational Safety and Health and should not be constructed to represent any agency determination or policy.

1802 3'-OH-GENISTEIN IN THE TREATMENT OF ACUTE PROMYELOCYTIC LEUKEMIA.

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Acute promyelocytic leukemia (APL) is caused by a unique gene translocation (t(15;17)), involving the promyelocytic leukemia (PML) gene that encodes a chimeric PML/RARα protein which is the key player in APL leukemogenesis. This highly lethal disease has been successfully treated with all-trans retinoic acid (ATRA). APL patients who have relapsed or who have failed to respond to standard therapy are treated with arsenic trioxide (ATO). While ATRA induces myeloid differentiation, ATO triggers apoptosis of APL cells. A recent study showed that a complete remission of APL can be achieved faster with a combined ATRA/ATO therapy as compared to monotherapy. Notably, nearly 30% of APL patients treated with ATRA and/or ATO develop a life-threatening complication known as “APL syndrome.” This identification of more effective drugs with fewer side effects would significantly improve the outcomes of APL therapy. Several studies have demonstrated that genistein, a soybean isoflavonoid, induces differentiation and apoptosis in human leukemia cells. Results of our preliminary studies demonstrate synergistic effects of genistein and ATRA and/or ATO on the viability of APL cells and on PML/RARα degradation. In humans, CYP1A2, CYP1A1 and CYP2E1 metabolize genistein to hydroxylated metabolites. We found that the primary metabolite of genistein, 3'-OH-genistein, is more effective than the parental compound as an inducer of cell death and PML/RARα degradation in NB4 culture. In addition, our results show that both CYP1A1 and CYP2E1 are expressed in NB4 cells and are inducible by treatment with ATO. Thus the metabolites of genistein may play a significant role in the outcomes of APL therapy.
**1803** HERG VS APD/QT RESULTS OFTEN DISCORDANT: FAST AND ACCURATE REPOLARIZATION SCREEN A NECESSITY EARLY IN DEVELOPMENT.

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Many drugs are screened early in development for HERG effects. We compared the effects of 118 drugs on HERG, action potential duration (APD) and the QT interval. Of the drugs that were HERG inhibitors (IC50 ≥ 1μM), only 35% prolonged the APD or the QT. 65% of the HERG drugs did not prolong the APD or QT, mostly due to mixed ion channel effects. These results demonstrate the importance of performing a repolarization screen early in development. To meet this need, we have validated a paced isolated heart screen called QTScreen. It provides a fast, accurate QT measurement and multiple hearts can be tested simultaneously to increase throughput. Sensitivity and variability of the screen was determined using power/sample size analysis; in control a 2% increase in QT change was detected with 80% power and n=6. QTScreen provides a fast, accurate and sensitive measure of QT effects that can be performed early in the drug development process, making it a natural companion to the HERG screen.

**1804** NEUROPROTECTIVE EFFECT OF SILDENAFIL AGAINST AMYLOID-BETA INDUCED TOXICITY.

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Background: Amyloid beta (Aβ) plays a crucial role in the pathology and progression of Alzheimer’s disease (AD). AD is characterized by Aβ containing plaques, neurofibrillary tangles, and synaptic or neuronal loss. Choline esterase inhibitors like donepezil are being primarily used to improve memory, mood and behavior of AD patients. Recent studies have shown sildenafil to enhance memory. However, the memory enhancing mechanism of sildenafil is not well established. Objective: Establish the neuroprotective mechanism of sildenafil against Aβ-induced neurotoxicity. Experimental Procedures: Aβ (1-42 human) was infused intracerebroventricularly (icv), 4 nmol, to three month old Sprague Dawley rats. Administration of sildenafil (1 or 2 mg/kg, i.p.) was started 24 hours after the Aβ lesioning and continued for 10 days. Effect of sildenafil against Aβ-induced behavioral and biochemical changes were evaluated after the last sildenafil administration. Y-Maze and Object Recognition Task were performed to assess the differences in memory performance. Rats were sacrificed and different brain regions were dissected out. Choline acetyl transferase and acetyl cholinesterase activity were analyzed using spectrophotometric method. The statistical significance was evaluated by the one-way ANOVA using Sigma-Stat version 2.03. Results: Novel arm entries in the Y-maze and discrimination index in the object recognition task were significantly low in Aβ infused rats compared to controls. Administration of sildenafil (1mg/kg) to Aβ infused animals effect a significant increase in the novel arm entries and discrimination index. Sildenafil had no effect on the cholinesterase and choline acetyltransferase activity in various brain regions. Conclusion: These results indicate that icv administration of sildenafil produce a memory enhancement dysfunction most likely through increasing neurotoxicity. Selective phosphodiesterase 5 inhibitor sildenafil prevented the cognitive impairment induced by Aβ. Support: Auburn University Bio-grant program.

**1805** C. ELEGANS MITOCHONDRIAL UNCOUPLING PROTEIN 4: ROLE IN REDOX BALANCE, AGING, AND MITOCHONDRIAL SUBSTRATE UTILIZATION.

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Mitochondrial uncoupling proteins (UCPs) are highly conserved members of the mitochondrial union carrier superfamily that transport protons across the inner mitochondrial membrane. Humans express multiple homologs (UCP2 – 5) in diverse tissues, but their physiologic functions remain unknown. We have recently shown that UCP5 mediates the lethal and hyperthermic toxicities to amphetamine psychostimulants. To better understand the mechanisms by which UCPs may regulate metabolic processes related to thermoregulation, we chose to use the simple organism C. elegans, which expresses only one UCP (ucp1) ortholog. Consistent with its potential for conserved function as a regulator of mitochondrial uncoupled respiration, compared to wild type (WT), UCP-null worms consume less oxygen, express greater adiposity, and show increased markers of oxidative stress. Surprisingly, when UCP-null worms were crossed with the Mev-1 strain carrying a mutation in complex II that renders them short lived and oxidatively stressed, lifespan was extended compared to Mev-1 controls. Biochemical analyses show that UCP-deficient worms have lower QTPselective respiration, a biochemical marker of uncoupling, and a failure to utilize the mitochondrial complex II substrate succinate. Consistent with an essential role for UCP in complex II function, UCP-null worms are hypersensitive to the lethal effects of the complex I toxin rotenone compared to WT, and UCP-null animals crossed with complex I (gao-1) mutants fail to survive. In support of a role for C. elegans UCP in substrate access to mitochondria and not respiratory enzyme functions, no changes in electron transport activities of enzymes in complexes I-IV were observed. Together, these observations point to novel metabolic regulatory functions of mitochondrial uncoupling proteins, and suggest that their roles in substrate access in C. elegans mitochondria shed possible mechanistic light on the actions mammalian UCPs.
either the photo irritation factor (PIF) or the mean photo effect (MPE) were determined. Both PIF and MPE of the chemicals were highly reproduced. The non-phototoxic chemicals were determined a negative result, phototoxic chemicals showed clear positive results except one chemical. These results suggest that ST3 NRU PT is a good alternative method for assessing the phototoxic potential.

**1808 COLIPA PROGRAM ON OPTIMIZATION OF EXISTING IN VITRO EYE IRRITATION ASSAYS FOR ENTRY INTO FORMAL VALIDATION: TECHNOLOGY TRANSFER AND INTRA/INTER LABORATORY EVALUATION OF SKINETHIC HUMAN CORNEAL EPITHELIUM (HCE) ASSAY FOR CHEMICALS.**

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The European Union will ban in vivo eye irritation tests on ingredients tested for cosmetic purpose after March 2009. COLIPA (European Cosmetics Association) is actively working to bring in vitro eye irritation tests to formal validation with ECVAM. This poster describes the Colipa program on technology transfer and reproducibility of the Human Corneal Epithelium (HCE), SkinEthic assay as one of the two human reconstructed tissue assays currently submitted. Two exposure times (10 min and 1 hr plus 16 hrs post-exposure incubation) were applied in the present work to predict the eye irritating potential of test substances. An optimized SkinEthic HCE protocol was established on a set of more than 200 chemicals. The prediction model was based on a 50% viability cut off, which allowed 2 prediction classes (irritants and non-irritants). Inter-batch and intra-batch reproducibility was assessed by using a specific batch control, positive control and negative control. It has been demonstrated in the present work that chemical reactivity criteria enabled to choose the most appropriate exposure treatment time assay of the HCE protocol using 90 chemicals. Predictive capacities based on these data were analyzed and discussed. The biological eye irritancy SkinEthic HCE assay (1 protocol including two exposure times) was able to discriminate irritants from non-irritants with an overall accuracy of 81% and well balanced sensitivity and specificity performances, thus demonstrating the robustness of the protocol when applied to significant very large set of chemicals. The two independent statistical analyses performed on these data, a formal submission was sent to ECVAM in support the SkinEthic HCE protocol’s entry into a formal validation study.

**1809 AN ALTERNATIVE METHOD FOR SKIN IRRITATION TESTING USING AN OPTIMIZED “42BIS” PROTOCOL: SKINETHIC RECONSTRUCTED HUMAN EPIDERMIS (RHE) MODEL.**

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ECVAM launched a formal validation study on in vitro EpiSkin, EpiDerm reconstructed human epidermis models. In 2007, the EpiSkin model was scientifically validated to fully replace the regulatory Draize skin irritation test (EU B. 4 method; OECD TG 404), according to the EU classification system (R38/no label). A performance standard document was developed describing the basis by which a new test method (Me-too) can determine its relevance (predictive capacity) and reliability (reproducibility within and between laboratories). The purpose of the present study was to develop a new test method able to discriminate skin irritancy potential of chemicals, using the Reconstructed Human Epidermis (RHE) system commercialized by SkinEthic. In phase I, twenty reference test substances (10 irritants and 10 non irritants) were tested to determine if the validated protocol (15 min chemical exposure with a post incubation period of 42 hours) was appropriate using the RHE model. For RHE, both sensitivity and specificity were 70%. The predictive ability of the RHE was considered as inadequate therefore, suitable protocol improvements were envisaged. The RHE test protocol was further refined by extending the topical application period (42 and 60 minutes chemical exposure). Applying those two protocols, an overall accuracy of 85% was observed (specificity: 80%, sensitivity: 90%). Due to its highest intra-laboratory reproducibility (>90%), further evaluation was performed only with the “42 bis” protocol (42 min exposure followed by 42 hrs post incubation period). In Phase II, testing performed on 19 liquid/solids additional substances resulted on a similar overall accuracy compared to the recognized validated models using the MTT as endpoint and a threshold of 50% viability. Currently a new test system with an optimized protocol to handle test substances spanning skin irritants vs. non irritants has been developed. This protocol was chosen for entering formal validation study.

**1810 VALIDATION OF THE RECONSTRUCTED HUMAN EPIDERMIS (RHE) SKIN IRRITATION ASSAY FOR FULL REPLACEMENT OF THE DRAIZE TEST.**

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Efforts to fully replace the in vivo Draize skin irritation test, according to Directive 67/548/EEC or OECD TG 404, was reinforced with the 7th Amendment of the Cosmetic Directive and the REACh regulation. Two reconstructed human epidermis models, EpiSkin and EpiDerm were scientifically validated with the reliability for EpiSkin to discriminate skin irritants (R38) from non-irritants (no label) as defined with EU risk phrases. A performance standard document was developed describing the basis by which a new test method (Me-too) can evaluate its accuracy and reliability (ECVAM SIVS, 2007). The model used in the present study was the in vitro Reconstructed Human Epidermis (RHE) system, commercialized by SkinEthic Laboratories. A multicentric study was performed with three independent laboratories (L’Oréal, Cong and Drian go) to compare the performances of the RHE assay with the Draize test on 342 chemical substances. A formal validation study was sent to ECVAM in support the SkinEthic HCE protocol’s entry into a formal validation study. The twenty reference test substances were selected to cover a broad coverage of physico-chemical properties, and a range distribution of in vivo irritancy scores. RHE Skin Irritation Test protocol consisted to apply topically test substance 3 times for 42 and 60 minutes chemical exposure. The main endpoint was cell viability (MTT reduction), with a threshold of 50% viability. An independent statistical analysis was performed to assess intra and inter-laboratory reproducibility. Regardless the measure, good reproducibility was achieved in the three laboratories. Correct predictions of the skin potential for those twenty substances were assessed with 90% sensitivity and 80% specificity (MTT only). Overall accuracy was higher than 85% and was not improved by IL-1 release measurement. Conducting the same calculations for the full replacement stand alone validated method EpiSkin with the two combined endpoints, the overall is very similar. The method was submitted to ECVAM for scientific review. Therefore the present RHE assay is a promising in vitro test to replace the Draize skin irritation test.

**1811 IN VITRO TOOLS TO ASSESS THE MYELOTOXICITY OF KINASE INHIBITORS.**


Nowadays, pharmacological research for new anticancer therapeutic strategies is mainly directed towards “target specific” drugs, identified through a detailed molecular understanding of changes occurring during cancer development. In this respect, protein kinases are considered attractive targets for the treatment of cancer. The interest for these proteins is based on their key role in processes like proliferation, differentiation and cellular apoptosis, activities that are controlled under physiological conditions. However, deregulation of these activities can lead to cellular transformation. Among several inhibitors of protein kinases that are currently under investigation, we focused our attention on a few small molecules exhibiting activity against PLK1 (Polo-Like Kinase); the key role of these proteins in the process of development of different type of cancers is widely described PLK1 is a mitotic kinase that plays a critical role in the transition G2/M during the cell cycle and cells that are blocked during mitosis are characterized by phosphorylation of histone H3 at Ser 10. We investigated the myelotoxic potential of some PLK1 inhibitors by using a validated HALO/TM technology and their effects on cell cycle progression and histone H3 phosphorylation at Ser 10 with flow cytometry analysis. The results obtained demonstrated that the myelotoxic effect of several PLK1 inhibitors on human bone marrow and on bone marrow from different animal species (rat and mouse) correlated well with the percentages of cells arrested during the G2/M transition phase of the cell cycle. In vitro cross-species myelotoxicity studies coupled with mechanistic investigations allow to correlate (in vitro/in vivo) toxic findings in preclinical animal settings, to select the most appropriate species for preclinical studies, to understand the mechanisms of toxicity (on target vs off-target) and to predict potential toxic effects in humans. Therefore, these tools are considered to improve overall the safety assessment of compounds.

**1812 IL-1α MEASUREMENT AS A USEFUL ADJUNCT TO VIABILITY ASSAYS IN RECONSTRUCTED EPIDERMIS: HOW TO ENSURE STANDARDIZATION AND REPRODUCIBILITY?**


The reconstructed human epidermis EpiSkinTM model was validated by the ESAC in 2007 as a stand alone replacement test for the prediction of acute skin irritation. The validated test is mainly based on viability assessment (MTT test). During valida-
dation, IL-1α release was demonstrated as a potentially useful adjunct for the de-
tection of irritants (sensitivity increase from 75% to 91%). But some questions re-
mained regarding standardization and variability of the test. The purpose of this
study was to investigate the possibility of standardizing the IL-1α measurements
whatever the ELISA kit supplier and to clarify reproducibility aspects of the mark-
ner. IL-1α was expressed as pg/mL and its quantification during the validation was
performed by using the R&D Systems ELISA kit. We investigated the linearity re-
sponse of the assay system by using WHO/NIBSC IL-1α international standard.
Good linear responses were observed for all kits evaluated. IL-1α quantification in
test samples revealed sensitivity differences related to the ELISA kit used.
Standardization of the data was obtained by using International Unit (IU) values
following a proposed 3 steps procedure: 1-check R&D Systems kit calibration
(converting factor) with the reference IL-1α standard in the laboratory working
conditions and convert pg/mL to IU/mL, 2- measure IL-1α in the test samples with
the R&D Systems kit and the ELISA replacement kit to evaluate, 3-calculate the
converting factor between both kits and express results as IU (R&D/DL-1 standard
validation conditions). 20 chemicals were evaluated by using the combined
approach [viability + IL-1α]. Intra and inter-batch variability was measured in
order to clarify the use of the additional marker as a relevant end point able to rein-
force the ability of the EpiSkinTM model protocol to detect irritant chemicals. In
conclusion, IL-1α evaluated in standardized and controlled conditions is a helpful
adjunct to viability assays, thus strengthening the detection of irritants when using
the validated EpiSkinTM model.

**1813**  **EXPRESSION OF MAJOR PHASE 1 ENZYMES IN HACAT CELLS.**

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A delayed-type hypersensitivity reaction induced by small reactive chemicals may be mediated by metabolic conversion of the compounds. Skin cells especially ker-
atinocytes play a major role in this process. A total replacement of animal experi-
ments for the endpoint skin sensitization will only be possible when we have well
characterized in vitro test systems developed. Thus, it is necessary to understand
the metabolic capacities of relevant cells under in vitro conditions. This knowledge
will help elucidating whether in vitro metabolism adequately represents human skin me-
tabolism in predicting toxicity. Thus, we evaluated the human keratinocyte cell line
HaCaT regarding their metabolic potentials. In this study we studied the expres-
sion of major cytochrome P450 (CYP) enzymes by RT-PCR and activities were
evaluated for CYP1A1. Interestingly, HaCaT showed comparable basal activities as
found for primary keratinocytes, and chemical-induced enzyme activities were even
slightly higher compared to in vitro cultured primary keratinocytes (1.5-3.0 fold).
These findings demonstrate that a permanent epithelial cell line from adult human
skin exhibits CYP expression and CYP1A1 enzyme activities. In sum, HaCaT cells
provide a promising tool for studying metabolism of common chemicals whose ex-
posure may occur via skin.

**1814**  **CIGARETTE SMOKE INDUCES GOBLET CELL HYPERPLASIA IN IN VITRO CULTURES OF PRIMARY HUMAN BRONCHIAL EPITHELIAL CELLS.**


Cigarette smoke is associated with chronic obstructive pulmonary disease (COPD), which encompasses chronic lung inflammation, chronic bronchitis and emphy-
sisma. Goblet cell hyperplasia (GCH) is a feature of the lung epithelium in hyper-
secretory diseases such as chronic bronchitis and contributes to overproduction of
airway mucus. We have developed a potential in vitro model of epithelial mucocili-
ary differentiation to investigate the effects of cigarette smoke on development of
GCH, in particular expression of the gel-forming mucin MUC5AC. Primary human bronchial epithelial cells (HBECs) were cultured at the air-liquid interface
(ALI) for 28 days to induce differentiation. During this period, cells were repeat-
edly exposed to 0.3-20 µg/mL cigarette smoke total particulate matter (TPM) or
1:900-1:300 (smoke:air) dilutions of whole mainstream smoke. Cell viability and
membrane integrity were measured by neutral red uptake and trans-epithelial elec-
trical resistance, respectively. Expression of MUC5AC was investigated using flow
cytometry, with the ELISA kit supplier and to clarify reproducibility aspects of the mark-
ner. IL-1α was expressed as pg/mL and its quantification during the validation was
performed by using the R&D Systems ELISA kit. We investigated the linearity re-
sponse of the assay system by using WHO/NIBSC IL-1α international standard.
Good linear responses were observed for all kits evaluated. IL-1α quantification in
test samples revealed sensitivity differences related to the ELISA kit used.
Standardization of the data was obtained by using International Unit (IU) values
following a proposed 3 steps procedure: 1-check R&D Systems kit calibration
(converting factor) with the reference IL-1α standard in the laboratory working
conditions and convert pg/mL to IU/mL, 2- measure IL-1α in the test samples with
the R&D Systems kit and the ELISA replacement kit to evaluate, 3-calculate the
converting factor between both kits and express results as IU (R&D/DL-1 standard
validation conditions). 20 chemicals were evaluated by using the combined
approach [viability + IL-1α]. Intra and inter-batch variability was measured in
order to clarify the use of the additional marker as a relevant end point able to rein-
force the ability of the EpiSkinTM model protocol to detect irritant chemicals. In
conclusion, IL-1α evaluated in standardized and controlled conditions is a helpful
adjunct to viability assays, thus strengthening the detection of irritants when using
the validated EpiSkinTM model.

**1815**  **COMPARATIVE EVALUATION OF COSMETIC FORMULATIONS WITH DIFFERENT ALTERNATIVE METHODS FOR EYE IRRITATION.**

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Eye corrosion and irritation testing seeks to determine the adverse effects from a
single exposure of the eye to a chemical compound. Under the REACH regulation
(440/2008 EC) all chemical substances produced in or imported into the EU in
quantities greater than 1 tonnage per annum, will have to be assessed for their
eye irritation potential. For chemicals in quantities between 1 and 10 tonnes per
annum, only in vitro testing is permitted for the assessment of eye irritation. For
cosmetic ingredients animal experiments will be forbidden for all acute toxicity
endpoints, including eye irritation, from March 2009, as stipulated in the 7th
Amending Directive 2003/15/EC to Cosmetic Directive 76/768/EEC and cos-
metic formulations have to be investigated only by in vitro methods.
In this work, the eye irritating potential of 5 cosmetic formulations were deter-
mined by 5 different alternative methods for eye irritation. In particular the test
methods were: Hens-Egg-Chorio-Allantoic Membrane (HET-CAM), Bovine
Cornea Opacity and Permeability Test (BCOP), Isolated Rabbit Eye (IRE), an in
vitro method using a human cornea model (EpিওculارTM from MatTek), and the
Red Blood Cell Test (RBC). The cosmetic formulations were chosen to cover the
whole range of irritating effects: non, mild, moderate, and severe/very severe.
The results of this study indicate that the eye irritation models, HET-CAM, BCOP, IRE,
and EpiOcularTM are able to discriminate between the above mentioned irritated
effects. The RBC method had limitations because only soluble and liquid test items
can be tested but this may relate to its anticipated use as a test system for the evalu-
ation of surfactants. This study shows that existing alternative methods are able
to discriminate between non, mild, moderate, and severe/very severe irritants and
can substitute the in vivo Draize Rabbit Eye Irritation test. Nevertheless, the Draize
test will still have to be used to confirm the classification of industrial chemicals.

**1816**  **IN VITRO PHOTOTOXICITY TESTING: A TASK INVOLVING MULTIPLE ENDPOINTS.**


Today’s lifestyle is often associated with frequent exposure to sunlight. In this con-
text, some chemicals used in drugs, cosmetics or food sometimes produce adverse
biological effects when irradiated. There is thus a need for appropriate methodolo-
dies aiming at obtaining relevant data at the molecular and cellular levels for such
compounds. For ethical and practical reasons, in vitro models have got an increas-
ingly importance in safety screening. Here, we propose a strategy based on comple-
mentary tests for assessment of the phototoxic and photogenotoxic potentials.
First, the use of a light source that mimics environmental solar UV is presented as
an essential requirement: in this regard, solar simulators appear to be most appro-
priate available equipment. Then, complementary methods with increasing com-
plexity are implemented in order to get a rational evaluation of the phototoxic risk:
(i) supercoiled circular DNA for in tube assessment of photoactivity, (ii) yeast
Saccharomyces cerevisiae for evaluation of photocytotoxicity and photomutagenic-
ity in realistic exposure conditions, (iii) in addition to the validated 3T3 NRU test,
using isolated normal human skin cells where photogenotoxicity can be detected
using the comet assay (iii) and finally reconstructed skin where cell death as well as
dNA damage can be measured after either systemic or topical application of the
studied chemical. Such a strategy allows the evaluation of compounds with very
different physico-chemical properties, and it is thus particularly well adapted to
rigorously ensure the safety of products likely to undergo environmental sunlight
exposure.

**1817**  **QUANTITATIVE ASSESSMENT OF GENOTOXICITY INDUCED BY SIMULATED SOLAR UV ON EPIISKIN RECONSTRUCTED SKIN MODEL.**


Even though cultured normal human keratinocytes constitute a relevant model for
photogenotoxicity studies, it is also important to get information on epithelial tis-
sue conditions where these cells form a structural network closer to that of human
skin. In this regard, reconstructed skin model such as EpiSkin are very convenient.
Numerous studies on photobiology have been already reported using these models.
But, in most cases, data are obtained using immuno-histochemistry techniques
which are generally qualitative. The goal of this study was thus to develop approaches aiming at quantifying markers related to photogenotoxicity. Here, the Episkin reconstructed skin model (including dermis with fibroblasts embedded in collagen) was exposed to simulated solar UV radiation similar to zenithal sunlight in terms of spectral power distribution. Biological endpoints related to genotoxicity were then quantitatively assessed. A dose dependent induction of DNA breaks and pyrimidine dimers in the genome of keratinocytes was shown using an adapted RT-PCR showing that (i) p21 and GADD45 were induced; (ii) a clear induction of MDM2 required exposure to relatively high UV doses. This study shows that reconstructed skin can be a useful tool as part of in vitro strategies aiming at assessing photoprotection and photogenotoxicity.

**1818 PROMISING ALTERNATIVE METHOD FOR THE EVALUATION OF PERCUTANEOUS ABSORPTION USING RECONSTRUCTED EPIDERMIS MODELS.**


In human risk assessment, cutaneous bioavailability needs to be considered to predict the exposure and the risk of cosmetic ingredients. To date the OECD guidelines n° 428 recommend the use of ex vivo human skin for dermal absorption studies. However, it is time consuming and requires numerous replicates due to the variability of the donors. Reconstructed skin models could be a promising alternative approach, as they are highly reproducible, suitable for screening and risk assessment purposes. The aim of the present study was to determine the absorbed dose of chemical ingredients using human epidermis Episkin model. A new in vitro test was developed. A very good correlation between ex vivo and in vitro data (r=0.902) was observed with 18 tested chemicals. 3 different classes were defined determining the low, medium or high ability of a chemical to penetrate through human skin. Enlarged to 34 chemicals, 91% were well classified according to human skin data. In conclusion, these results demonstrate the capability of the reconstructed epidermis to give relevant information according to human skin. The applicability domains of this in vitro test would need to be refined and expanded but could be a promising suitable method to evaluate the penetration potential of chemicals in REACH purpose.

**1819 A SCORING TOOL TO ASSESS INHERENT QUALITY OF TOXICOLOGICAL DATA.**


The inherent quality of toxicological information is receiving renewed attention, especially within the REACH programme of the EU, where the use of existing information is emphasised. Also the US Society of Toxicology recently published an opinion, where concern that differences in data quality may counteract the efforts to harmonise classification of substances were expressed (1). Moreover, data quality assessments are highly relevant for the validation process of alternative test methods. Validation depends on the reference in vivo data that should be of highest possible quality to allow an evaluation accurate of the performance of the in vitro test under validation. To evaluate the quality of toxicological studies the so-called Klimisch criteria (2) have been widely used. Recently, ECVAM and the contractors FoBiG/DKFZ, with the support of a group of European experts, have completed a project intended to develop a more comprehensive and detailed scoring tool to assess the quality of toxicological data in an unambiguous and transparent way. The tool was developed by expanding on existing approaches of reliability assessment, by identifying about 25 variables related to quality. The main focus was on the determination of data reliability of experimental toxicological data, both in vivo and in vitro (independent of the relevance for the evaluation purpose). Toxicological data mainly come from peer-reviewed publications and studies undertaken for regulatory purposes, and the tool should be equally applicable to data from these sources. The scoring tool will facilitate and harmonise the first pivotal step of data evaluation will increase its transparency. The project is one of the activities towards an evidence-based toxicology (EBT). 1 Society of Toxicology (2007) http://www.toxicology.org/AI/NEWS/GHS_Comments.pdf and 2 Klimisch, HJ. (1997) Reg. Tox. Pharm., 25, 1-5.

**1820 IN SILICO PREDICTION OF SKIN PENETRATION FOR VOLATILE CHEMICALS FROM COSMETIC AND DERMATOLOGICAL FORMULATIONS.**


The accurate prediction of dermal uptake and exposure of topically applied chemicals is relevant for formulation development and risk assessment. Refinement of usual predictive model has been recently done [1]. This model is able to predict the amount of chemical into the skin and in the receptor fluid after the topical application of cosmetic or dermatological formulations. The aim of this study was to take into consideration the vapor pressure of volatile chemicals to predict the absorbed dose. In the in silico model developed by Kasting [2], the volatility of chemicals was considered. Recently, the impact of evaporation on skin uptake of volatile chemicals was evaluated [3]. The model of L’Oréal was recently refined. Measurements of skin bioavailability of 6 volatile chemicals were compared to the predictive data from the L’Oréal model. We demonstrated that the consideration of the vapor pressure improves the in silico prediction of dermal uptake for volatile molecules. Many cosmetic ingredients are highly volatile, like fragrance components. Without correction for evaporation, great overestimation of skin exposure could be done. When evaporation is taken into account, cumulative amount penetrated into the skin can decrease by a factor of two for most volatile chemicals. Thus, using this improvement gives more relevant and consistent results with typical exposure scenario.


**1821 TOWARDS THE USE OF BIOCHIP TECHNOLOGIES FOR THE PREDICTION OF IN VIVO ACUTE TOXIC POTENTIAL.**


Developing alternative methods to animal testing is critical based on ethical reasons, the REACH regulation and the 7th Amendment of the European Cosmetic Directive which has called for a broad replacement of animal experiments for the testing of cosmetic products. With regards to the acute toxicity, considerable efforts have been gathered over the last years to develop robust in vitro methods. The rationale behind derives from observations, notably arising from the Multi-centre Evaluation of In Vitro Cytotoxicity study, that basal cytotoxicity can be used as a surrogate endpoint for predicting lethal systemic toxicity in vivo. Because of their strong commitment to alternative to animal testing, l’Oréal and Solidus Biosciences Inc. have completed a proof of concept study of the Solidus bioschips as an alternative to acute toxicity tests. Solidus researchers have developed a high-throughput, automatable technology which can help revealing toxicity potentially mediated by metabolite activation. Such a technology, integrating a cell-based (Drotach) and enzymatic systems (Metachip), provides mechanistically-based information. In the current study, 50 chemicals representative of L’Oréal’s chemical space and cosmetic application have been collated and tested. These compounds ranged in their degree of in vivo toxicity, as defined by the LD50 values and also in their mechanisms of toxic action. Other selection criteria relate to the target species and the route of administration: data generated in rats treated by gavage have been used to assess the correlation between the experimentally derived LD50 values. Preliminary statistical analysis has led to encouraging results, with sensitivity and specificity values over 85%. This is an exploratory work and further investigations such as assessing the relevance of such an approach in an Integrated Testing Strategy (ITS) workflow, are still in progress.

**1822 PREDICTION OF OCULAR IRRITANT POTENTIAL OF SURFACTANTS-BASED FORMULATIONS AT DIFFERENT CONCENTRATIONS USING THE EPIOcular MODEL.**


Evaluating ocular irritation is essential to the safety assessment of facial and eye-area cosmetics. The EpiOcularTM Tim model is an automated, in vitro test which has been extensively used to predict the traditional eye irritation potential endpoints of those products used around the eye area. Sensory discomfort to the mucus membranes and skin tissues around the eye is also an important factor in consumer acceptance of these products. The current study investigated whether the concentration tested...
in the tissue construct model affects the ability to distinguish the degree of sensory discomfort potential to the eye area. Two surfactants-based cosmetic formulations (Fd and Fm) were tested at different concentrations using the EpiOcularTM assay. No significant difference was found in ET50 (time to reduce tissue viability to 50%) between the two formulations when tested at a concentration of 10%. However, when the formulations were tested at 3% formula Fm was shown to have a notably shorter ET50 than Fd, indicating a higher potential for ocular irritation. No matter which concentration tested, the two formulations were classified to have “non-irritating, minimal” irritation potential to the eye based on the existing prediction model (for the standard 4-hour exposure protocol). These results were compared with data obtained from clinical discomfort and clinical use studies. A significant difference in sensory discomfort responses to the two formulations was observed in both clinical and consumer use studies. Formula Fm induced appreciable sensory discomfort in human subjects in both studies, while Fd was well tolerated. These results indicate that use of relatively low test article concentrations for eye area products, may allow the EpiOcularTM assay to better distinguish the degree of ocular irritation and sensory discomfort of surfactants-based formulations.

**1823 IMPLEMENTATION OF IN SILICO MODELS IN THE COSMETIC DIRECTIVE.**


The 7th Amendment to the EU Cosmetics Directive has made in vitro and in silico approaches to warrant the safety of products a key issue to support regulatory dossiers and future innovation. A ban on animal testing for chemicals to be used in cosmetics comes into effect in the EU in March 2009 for acute toxicity, genotoxicity, and skin or eye irritation. For repeat-dose (or systemic exposure) toxicity, the EU ban is subject to the 2013 deadline. Over the past years there have been considerable efforts to develop alternative methods, including in silico methods that would comply with regulatory constraints (OECD principles). In this context, transparent and mechanism-based approaches are needed. A number of commercial or free software integrate global models targeting the 2009 endpoints. From a cosmetic industry prospective, the predictive performance of such models has to be evaluated on chemical series of interest for: Chemical prioritization; Mechanistic understanding; and, Elaboration of regulatory dossiers by providing additional information. The purpose of the present study was to use data generated on “real-life” chemicals from the cosmetic industry to challenge some of the global models available today. Historical in-house data obtained from the in vivo skin irritation, bacterial mutagenicity and in vivo oral acute toxicity tests, have been curated in collaboration with in-house experts for this purpose. Global models available in the DeriMC4PC and TIMES software were evaluated for mutagenicity (Derek, MC4PC and TIMES), acute toxicity (Derek, MC4PC) and skin irritation (Derek). Applicability domain and predictive performance were compared in order to identify potential gaps and assess the complementarities of these tools and their relevance in Integrated Testing Strategy (ITS) workflows.

**1824 USE OF THE EPIOCULAR™ TISSUE MODEL FOR TESTING OF ULTRA-MILD EYE CARE COSMETICS.**

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Eye care cosmetics (ECC) need to be non-irritating in order to be successful in the marketplace. In addition, in order to avoid complaints by customers with sensitive eyes, many ECCs are formulated to be ultra-mild. However, testing of, and discrimination between ultra-mild formulations is difficult since traditional Draize rabbit eye testing is insensitive to the low levels of irritation caused by ECCs. Furthermore, animal testing is not possible due to animal rights concerns and due to current European legislation banning cosmetics that have been tested using animals. Human clinical testing can be performed but because only a low level response is expected, large numbers of subjects would be necessary and hence testing costs would be high if not prohibitive. Cells in monolayer culture could be used for testing however the test materials would need to dissolved in aqueous media which for many non-water soluble cosmetics is not possible. The current study investigated use of the organotypic EpiOcular tissue model as a means of discriminating between ultra-mild formulations. Ten (10) commercially available mascara products were selected and tested using EpiOcular® with an extended time exposure protocol. Because the tissue model is cultured at the air-liquid interface (apical tissue surface left dry), both water soluble and water insoluble test materials could be applied neat to the apical tissue surface. Exposure times between 8 and 24 hours were used after which the tissue viability was determined using the MTT assay.

Dose response curves were constructed and the exposure time that reduces tissue viability to 50% (ET50) was determined by mathematical interpolation. For the 10 mascaras tested, a broad range of ET50s was obtained from 8.7 hours to > 24 hours. Other studies with low levels of surfactants known to be irritating at higher concentrations could also be seen with an ET50 at a concentration of 10%. However, when the formulations were tested at 3% formula Fm was shown to have a notably shorter ET50 than Fd, indicating a higher potential for ocular irritation. No matter which concentration tested, the two formulations were classified to have “non-irritating, minimal” irritation potential to the eye based on the existing prediction model (for the standard 4-hour exposure protocol). These results were compared with data obtained from clinical discomfort and clinical use studies. A significant difference in sensory discomfort responses to the two formulations was observed in both clinical and consumer use studies. Formula Fm induced appreciable sensory discomfort in human subjects in both studies, while Fd was well tolerated. These results indicate that use of relatively low test article concentrations for eye area products, may allow the EpiOcularTM assay to better distinguish the degree of ocular irritation and sensory discomfort of surfactants-based formulations.

**1825 FOLLOW-UP VALIDATION OF THE EPIDERM SKIN IRRITATION TEST.**

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In April 2007, ECVAM endorsed 2 alternative methods (EPISKIN and EpiDerm Skin Irritation Tests (SIT)) as replacements of the in vivo rabbit skin irritation test. While EPISKIN assay was recognized as a stand alone method, EpiDerm SIT was endorsed for use in a tiered testing strategy (OECD TG 404), when irritating results are accepted and non-irritating results may require further testing by another method. Based on results published by Fuller and Bracher (2002), and analysis of results of the ECVAM validation study between 4 laboratories was performed to evaluate reproducibility and confirm the predictive ability of the modified EpiDerm SIT. Results of the study are presented here. Overall, sensitivity and specificity of 80% were obtained, which is comparable to results for the EPISKIN SIT for the same set of chemicals (sensitivity 70%, specificity 80%). The inter-laboratory reproducibility of the modified EpiDerm SIT and its concordance with the in vivo rabbit test data was very good. The method was submitted to ECVAM for scientific review in April 2008.

**1826 DEVELOPMENT OF AN EPIDERM™ IN VITRO SKIN IRRITATION TEST (SIT) FOR THE GLOBALLY HARMONIZED SYSTEM (GHS) OF CLASSIFICATION AND LABELING OF CHEMICALS.**

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Determination of skin irritation potential is an international regulatory requirement to ensure safe handling, packaging, labeling and transport of chemicals and cosmetic and household products. Recent legislation in the EU and animal testing for cosmetics have heightened the need for validated in vitro SITs. A UN treaty endorsed by the US, EU, China, Japan, Australia and others has outlined a GHS of Classification and Labeling of Chemicals. The GHS classifies skin irritation of chemicals into three categories: non-irritant (corresponding to in vivo Draize skin irritation scores < 1.5), slight irritant (Draize score 1.5 – 2.5) or irritant (Draize score > 2.5). The EpiDerm model has been validated for in vitro skin corrosion testing worldwide, and in vitro SIT in the EU in studies sponsored by the European Center for the Validation of Alternative Methods (ECVAM). However, the EU SIT system distinguishes only 2 classifications – skin irritant (R38) and non-irritant (no label), and thus will not satisfy the worldwide GHS SIT system. Therefore, additional efforts are underway to validate an EpiDerm SIT for GHS. In results reported here, 15 test chemicals with known in vivo Draize skin irritation scores, chosen to avoid borderline classifications, were applied to EpiDerm to identify in vitro skin irritation biomarkers and establish a preliminary EpiDerm-GHS SIT two formulations. Biomarker endpoints evaluated include EpiDerm viability (MTT assay) and inflammatory mediator release by ELISA and/or Multiplex (Bio-Rad Bioplex) assays. Among the mediators investigated, significant levels of IL-1α, IL-1β, IL-8, GROα and PGEl were produced by EpiDerm tissues. The MTT viability response was the most predictive and least variable biomarker, providing 80% concordance with the in vivo Draize classification (i.e. 80% sensitivity and specificity for assigning GHS classifications). This preliminary prediction model will be further tested and refined to form the basis for formal multi-laboratory EpiDerm-GHS-SIT validation studies.
1827 EVALUATING THE CONSISTENCY OF THE MEDIUM THROUGHPUT C. ELEGANS GROWTH ASSAY FOR TOXICITY.

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The National Toxicology Program and Environmental Protection Agency are developing alternative toxicological methods to prioritize and screen chemicals using high- and medium-throughput assays. Contributing to that effort, several assays have been developed using C. elegans as an alternative animal model. Measurements are made using the COPAS Biosort, a flow cytometer adapted to C. elegans. In the 48 h growth assay, L1 hatchings are loaded into 96 well plates containing the tested chemicals and incubated for 48 h before being aspirated. In this study, we investigate the reliability and consistency of both the assay and the analyses of the results. Eight chemicals were chosen from the 320 phase I chemicals of the U.S.EPA. Four of which are known to be toxic at 200 μM and 4 of which are not (Parathion, Dichlorvos, Diazinon, Lindane, Methyl-isothiocyanate, Carbaryl, Isoxaben, and Ethephon.). The growth assay was applied in triplicate at eight concentrations of each chemical. In addition, the assay was replicated for each compound an additional six times at 200 μM. The data can be analyzed either by a test for toxicity, yielding a yes/no answer, or by estimated toxicity measures to be used together with other information on the compound. Thus, we tested six replicate assays at the highest dose for all eight chemicals using the Mann-Whitney test. As a toxicity measure, we estimated the average growth rate of the nematodes by adapting a growth model developed earlier. The higher the number of observations led to the toxicity tests being extremely sensitive: out of 24 tests (4 each), 13 of which are toxicants, and 4 of which are not (Parathion, Dichlorvos, Diazinon, Lindane, Methyl-isothiocyanate, Carbaryl, Isoxaben, and Ethephon.).

1828 CHEMICAL REACTIVITY: A PHYSICAL-CHEMICAL PROPERTY OF GREAT INTEREST FOR IN VITRO/EYE IRRITATION PREDICTION WITH SKINETHIC’S HUMAN RECONSTRUCTED CORNEA EPITHELIUM (HCE).


Chemical reactivity is a physical-chemical parameter of interest to define the intrinsic molecular behavior of chemicals. With the raise of in vitro tests in the toxicology field, this molecular fingerprint is a powerful tool in the protocol orientation, result interpretation and subsequent decision (McKinney, 1996). The importance of chemical reactivity parameter is particularly well demonstrated in the field of in vitro Eye Irritation prediction. For this purpose, a protocol based on two exposure times using the SkinEthicTM HCE model has been developed and optimized to predict irritant versus non irritant potential. The measure of molecular reactivity of these chemicals is used as criteria to orientate the tested item to its corresponding toxic class.

A Multicentric study is under way in order to validate these chemical reactivity assays (transferability and reproducibility). The results of this multicentric study will be presented.

1829 ANALYSIS OF CELL TOXICITY USING TOXREPORTER™ TECHNOLOGY.


Abstract: The most common cause of drug failures is poor ADME properties. New technologies such as the failure of ‘bad drug candidates’ identified in the late development pipeline by identifying potential toxicity issues. When compounds interact with cells in a deleterious way, a wide spectrum of adaptive response systems are induced. These toxic responses are initiated by a variety of factors, such as oxidative stress, DNA damage, apoptosis, hypoxia, etc. However, when a cell is exposed to toxic agents subsequent to initiating toxic events, a whole plethora of metabolic changes occurs. This makes investigations into the actual underlying mechanism of chemical toxicity and toxic liabilities extremely difficult to determine. There is, therefore, a need for a high throughput system for the real-time assessment of metabolic changes that occur when cells are exposed to potentially toxic tests. We have developed proprietary technology which will facilitate such an assessment. A reporter-type cell based assay that translates each toxic event into a specific protein which is secreted into the tissue culture medium. The translated product is identified by a traditional sandwich based ELISA assay. A portfolio of 10 reporter gene assays has been developed to access the potential toxicity of compounds or chemicals. Each cell line has been engineered with a specific response element that, upon activation, switches on the expression of a unique reporter protein. The response element selected represents common mechanisms of toxicity such as, oxidative stress, DNA damage, apoptosis, hypoxia, etc. These cell based toxicity assays offer new solutions for the early identification of toxic lead compounds.

1830 IN VITRO MODULATION OF BCNU TOXICITY BY O4 BENZYLFOVIC ACID IN HUMAN BONE MARROW CFU-GM AND TUMOR CELL LINES.

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2-Amino-O4-benzylpyridine derivatives are inactivators of the human DNA repair protein O6-alkylguanine-DNA alkyltransferase (alkyltransferase) and have been tested as possible modulators of alkylating agent chemotherapy. Recently, a new highly water-soluble and potent analog O4 Benzylfolic Acid (O4FA) became available and its therapeutic potential in modulating bis-chloroethylnitrosourea (BCNU) toxicity to human colony forming unit – granulocyte/macrophage (CFU-GM) was evaluated in a validated clonogenic assay. Donor bone marrow cells were pre-incubated (5 hr) with O4FA, followed by BCNU for an additional 42 hrs, after which drug was removed and CFU-GM colony formation was measured in semi-solid agarose cultures. O4FA at 2 or 5 μM progressively lowered the IC values for BCNU, but further increases in O4FA concentrations did not, suggesting a plateau effect. Human CFU-GM could not tolerate >10 μM BCNU at any O4FA concentration. Two human carcinoma lines, HT-29 and KB, representing low and high levels (respectively) of the α-folate receptor required for O4FA transport, have been used to assess the anti-tumor effectiveness of maximally tolerated O4FA/BCNU combinations. As a positive control, 2-hr pretreatment with O4FA (0-100 μM) prior to adding BCNU for 2 more hours confirmed that both cell lines are responsive and that an ATPLite assay can substitute for tumor clonogenic assay. These two cell lines are being used to evaluate the effectiveness of increasing O4FA concentrations in BCNU fixed at 10 μM, which represents the regimen tolerated by the neutrophil stem cells, and to obtain insight into the potential therapeutic index of this new modulator of alkylator chemotherapy. Funded by NCI Contract N01-CO-12400.

1831 EYE AND SKIN IRRITATION IN 3-D TISSUE CONSTRUCTS USING MTT AND ATP ENDPOINTS.

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The irritation potential of formulations and ingredients for industrial screening and product development is often conducted using in vitro 3-D human ocular and epidermal tissue constructs. To predict irritation potential after chemical exposure, tissue viability is typically determined by the ability of live cells to reduce MTT. Toxic exposures result in decreases in relative MTT reduction. However, two issues may contribute to inaccurate viability assessment: subtoxic exposures that induce metabolic rates greater than controls (i.e., hormesis) and chemicals that directly reduce MTT causing an overestimation of tissue viability (e.g., NaOH, α-tocopherol (α-t), ascorbic acid). Chemical residues on the tissues may increase the total MTT signal, so freeze-killed tissues are used to estimate chemical-mediated reduction of MTT. However, alternative methods of measuring tissue viability, such as amount of adenosine triphosphate (ATP) may be used. We compared these two methods by testing a series of model mild skin care formulations in 3-D human eye and skin constructs. The formulations were spiked with various concentrations of Triton® to include a range of toxic effects, and were prepared with and without α-t, a MTT reducer. The inclusion of freeze-killed, test article-treated controls resulted in the same irritation predictions for the 4 formulations containing α-t as for the 4 control formulations without α-t (e.g., formula with highest Triton conc.: ETα, eye = 172 and 157 min, ETα, skin = 778 and 772 min, with and w/o α-t). The ATP assay
provided the same rank order of irritancy as did the MTT assay although the relative viability values from the ATP assay at each exposure were overall lower (e.g., formula with highest Triton conc.: ET<sub>90</sub> eye = 14.5 and 12.9 min, ET<sub>90</sub> skin = 202 and 251 min, with and w/o α-t)-t. In summary, the MTT assay of formulas capable of MTT reduction should include freeze-killed tissues, and the ATP assay can confirm the relative rank order of the irritancy predictions.

1832 IN VIVO VISUALIZATION OF ESTROGENIC ACTIVITY IN XENOPUS LAEVIS TADPOLES.


Endocrine disrupting chemicals including those that mimic estrogen have raised concern due to their widespread presence in the ecosystem and their deleterious effect at very low concentrations to wild-life and humans. Early detection and screening of the compounds in the environment for their estrogenic activity is very important. In principle an estrogen disruptor can bind to the estrogen responsive element (ERE) in the genomic DNA and subsequently interfere with transcription of estrogen-responsive genes. We have developed a transgenic Xenopus laevis model where the estrogenic nature of a compound can be visualized in vivo by a fluorescent reporter. Four concatamers of ERE (SXEcre) were fused to a Green Fluorescent Protein (GFP) reporter gene and this plasmid construct (p5XERE-GFP) was used to create transgenic Xenopus laevis tadpoles. These transgenic premetamorphic tadpoles were exposed to [10-100 ppb] estradiol in 0.1 MRR earing water. After four days of exposure we observed a robust induction of GFP in various parts of the animals including heart, liver, brain, tail and jaw muscles and kidneys. We are also developing another transgenic line where SXErec will be attached to luciferase as a reporter gene, which could be easily quantified by spectrophotometer. We are assayng for ERE-reporter activity with different types of estrogenic compounds. We propose that by increasing the number of EREs (5X) that drive the reporter the assay becomes more sensitive, and the animals will sense the presence of estrogenic compounds at very low but physiologically relevant levels in water. This system also can be used for high throughput screening of industrial and pharmaceutical chemicals that might be estrogenic in nature.

1833 THE APPLICATION OF ALGINATE SCAFFOLDS FOR THREE-DIMENSIONAL CELL CULTURE.

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We have developed a well-defined porous alginate matrix (AlgiMatrix™) to establish a three-dimensional cell culture environment that facilitates the formation of more in vivo-like tissue structures. The addition of divalent cationic salts (e.g., calcium, barium) to a sodium alginate solution produces a highly porous (90%) and highly interconnected biocompatible scaffold. Using multiple cell types including C3A human hepatocyte cell line, primary hepatocytes, C6 glioma cell line, and mesenchymal stem cells we have shown that the matrix pores (89.19 μm diameter) act as localized compartments in which cells form reproducible spheroidal aggregates of similar diameter. Non-enzymatic spheroid recovery is simplified by the addition of a Dissolution Buffer that dissolves the alginate matrix within a few minutes while the cell structures remain intact. Phase contrast microscopy revealed that spheroid formation is faster and more efficient in AlgiMatrix™ cultures compared to 2D cultures maintained on collagen-coated polystyrene. The MTT viability assay confirmed that the AlgiMatrix™ cultures retain a high viability through at least 7 days in culture. AlgiMatrix™ cultures of C3A human hepatocytes demonstrate enhanced CYP1A2 gene expression and enzyme induction per cell compared to 2D cell culture. Additional data demonstrating the recapitulation of numerous morphological and/or functional features indicates that AlgiMatrix™ is a platform for creating more physiologically relevant models. We conclude that the 6-well, 24-well, and 96-well AlgiMatrix™ format represents a powerful new tool in toxicology research.

1834 DEVELOPMENTAL EXPOSURE TO A DOXAPAMIC TOXICANT PRODUCES ALTERED LOCOMOTOR ACTIVITY IN LARVAL ZEBRAFISH.

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In an effort to develop a rapid in vivo screen for EPA’s prioritization of toxic chemicals, we are characterizing the locomotor activity of zebrafish (Danio rerio) larvae after developmental exposure to various classes of prototypic drugs that act on the central nervous system. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a neurotoxin that destroys dopaminergic neurons, causing parkinsonian signs in mammals. Our hypothesis was that exposure of zebrafish embryos/larvae to MPTP would produce locomotor changes reminiscent of the decreased activity seen in studies of MPTP in developing mammals. Zebrafish were exposed to MPTP in a 96-well microtiter plate from 5 hours to 5 days post-fertilization, followed by a washout day. On the sixth day, larvae were evaluated for teratogenic effects (structural alterations); locomotor activity was also assessed during alternating periods of light and darkness (infrared) using video tracking. Developmental exposure to the highest dose of MPTP (50 μM) produced teratogenic changes, as well as hypocoaxemia. In contrast, a lower non-teratogenic dose of MPTP produced hyperactivity. Therefore, larvae that had been exposed to high doses of MPTP during development showed decreases in motor activity by similar extent and noted in mammals and zebrafish; however, the unique finding in the current study is that zebrafish larvae exposed to lower doses of MPTP manifested with hyperactivity. (T.D. Irons is supported by the NIH NICIMS Initiative for Maximizing Student Diversity and the NIEHS National Research Service Award (T32 ES007126). This is an abstract of a proposed presentation; the information does not necessarily reflect Agency policy.)

1835 DEVELOPMENT OF INTEGRATED TESTING STRATEGY IN THE FRAME OF THE 7TH AMENDMENT TO THE EUROPEAN COSMETIC DIRECTIVE.

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The 7th Amendment to the EU Cosmetics Directive implies a shift to alternative ways of assessing the safety of cosmetic ingredients. Over the past decade there have been great efforts in the industry or academic laboratories to develop alternative methods either in silico or in vitro that would comply with regulatory constraints. A multidisciplinary team at L’Oréal worked to develop integrated testing strategies (ITS) based on the existing in vitro and in silico predicting models and tools available. These Integrated Testing Strategies have been then confirmed and used for routine assessment of cosmetic ingredients’ safety. Such strategies need to fulfill two major issues: i) fitting to the chemical-physical diversity of the chemicals currently used in the industry ii) to be predictive of the considered targeted endpoints (Ocular and skin irritancy, genotoxicity, acute toxicity) in order to predict the human safety risk of the chemical. From the cosmetic industry perspective, the predictive performance of such models has to be evaluated on chemical series of interest: for Chemical prioritization; Mechanistic understanding; and, elaboration of regulatory dossiers by providing additional information. The example of two Integrated Strategies developed and in routine use at L’Oréal for Genotoxicity assessment, Ocular or Cutaneous tolerances will be presented. References: S. Hoffmann et al., 2008, Toxicology Letters, 180, 9-20 and C. Grindon et al., 2006, ATLA, 34, 407-427.

1836 TO DEVELOP AN IN VITRO (EFT-300) SKIN MODEL FOR WOUND HEALING STUDIES.

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Introduction: Wound healing is a natural process, which involves the regeneration of epidermal and dermal tissue. The objective of the present study was to evaluate the epidermis full thickness (EFT-300) skin culture as an in-vitro wound healing model. Methods: In the present study, a strong base 32N KOH and 2 strong acids, concentrated H2SO4 and 100% acrylic acid (AA), were selected for making chemical wounds. Initially, the dose and exposure time required to create a significant effect was determined. Immediately after wounding, cultures were washed thoroughly with distilled water (for KOH wounds) or with phosphate buffered saline, pH 8 (for H2SO4 and AA wounds). Cultures were then returned to incubator (37°C) and the culture medium and tissues were collected every other day over a 6-day period. Histology of the tissue and the release of cytokines, chemokines, and growth factors into culture medium were evaluated. Results: Histological sections of KOH treated tissues, immediately after treatment (4ul for 3minutes) showed damage to stratum corneum and no damage was observed in the dermis. However, treatment with H2SO4 (2ul for 3 minutes) showed deeper wounding without significant damage to the stratum corneum. AA (5ul for 3minutes) only damaged the dermis but did appear to damage the stratum corneum or epidermis. Histological sections showed regeneration of epidermis, fibroblasts and collagen was observed following treatment with strong base and acids. In the culture medium, both KOH and
and H2SO4 treatment resulted in significant release of IL-6 and IL-8 compared to control, but as the post-wounding time progressed there was a decline in biomarker release. Furthermore, the levels of growth factors EGF (KOH and H2SO4) and FGF (AA) increased followed by increased levels of GMCSF in the culture medium. Conclusion: These results strongly support that the EPT-300 cultures could be a good in-vitro model for studying wound healing process. Acknowledgements: Supported by international foundation for ethical research (IFER).

1837 ALTERNATIVE SAFETY EVALUATION ON "2009" ENDPOINTS IN THE FRAME OF THE 7TH AMENDMENT TO THE EUROPEAN COSMETIC DIRECTIVE: A STUDY CASE.

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The 7th Amendment to the European Cosmetic Directive is imposing a ban on animal testing from March 2009 for the genotoxicity, acute toxicity, ocular and skin irritancy toxicological endpoints. This European regulation implies a shift to alternative ways of assessing the safety of cosmetic ingredients on these endpoints.

Over the past decade there have been huge efforts in industry’s and academic laboratories to develop alternative methods either in silico or in vitro that would comply with these new regulatory requirements. The EpiSkin method for skin irritancy has been scientifically validated by ECVM (European Center for Validation of Alternative Methods) in 2007. Scientifically the replacement of animal data can only be considered through a combination of tests and using a battery of predicting tools.

A multidisciplinary team at L’Oréal has worked out the development of appropriate integrated testing strategies (ITS) based on existing in vitro and in silico prediction models and tools available so far. These ITS have been used to simulate a comprehensive “alternative” safety evaluation of some cosmetic ingredients which fulfill the “2009” ban of the 7th amendment to the European Cosmetic Directives.

One example of an “alternative” safety evaluation performed on a new and innovative cosmetic ingredient in development will be presented. This evaluation relies on the combination of chemical-physical characterization, in silico prediction, in vitro assays for genotoxicity, ocular and skin irritancy and acute toxicity, database mining and read across and in vitro skin bioavailability measurements.

1838 DEVELOPMENT AND IMPLEMENTATION OF ALTERNATIVE SAFETY EVALUATION STRATEGIES IN THE FRAME OF THE 7TH AMENDMENT TO THE EUROPEAN COSMETIC DIRECTIVES.

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The 7th Amendment to the European Cosmetic Directive will impose a ban on animal testing from March 2009 for the genotoxicity, acute toxicity, ocular and skin irritancy toxicological endpoints. A sustained and massive investment in the research and development for predictive methods and tools for Human safety assessment can be acknowledged from academics, industries in Europe and worldwide. The resulting scientific progresses remain driven in an industry perspective by real-time and the only objective of perpetuating the innovation and insuring the Consumer Safety in the regulatory context of the 7th amendment to the European Cosmetic Directive. The formal validation process as defined by ECVM, ICCVAM or JaCVAM is a necessity to warrant the scientific robustness, transferability and reproducibility of the methods developed. But to translate these predictive methods into Safety Evaluation Procedures other steps are required. A multidisciplinary team at L’Oréal has worked to develop and implement the new Safety Evaluation procedures required by the 2009 deadline. This effort went through a stepwise process which will be presented and illustrated: Step 1: Evaluation of the performances of the predictive methods available on a significant number of cosmetic ingredients (>400) representative of the chemical-physical diversity of an industry portfolio. Aim: identifying the applicability domains of the methods and refining the protocols to fit the chemical diversity that the cosmetic industry is facing; Step 2: definition of the most suitable combinations of these predictive tools is enabled and customized (Database mining, Read Acros, In silico models, Physical-chemical characterization and In vitro biological models and assays); and, Step 3: Implementation of these Integrated Testing Strategies through training and daily use for routine safety assessment of new ingredients and / or proposal to the validation agencies for formal validation. The outcome of this strategy will be illustrated in a series of posters submitted.

1839 PREDICTIVE IN VITRO CYTOTOXICITY ASSAYS: THE "HOLY GRAIL" OF THE MODERN-DAY TOXICOLOGIST.


Toxicology safety assessments have long been a challenging hurdle that potential therapeutic candidates must clear prior to introduction into a clinical setting. In recent years, late-stage candidate attrition has led to a renewed interest in implementing assays that can reduce the number of candidates that fail during preclinical animal studies and early-stage clinical trials. By providing more relevant toxicity data earlier in the drug development process, potential liabilities can be identified prior to committing significant resources towards GLP animal studies and clinical trials. In recent years, several high-content imaging assays have been designed and implemented to generate critical toxicology information for use in the identification, selection, and prioritization of potential therapeutic candidates. They also have the added benefit of revealing mechanistic information that can be critical to understanding toxic effects. In vitro cytotoxicity assays have the potential to directly impact candidate attrition if they can be validated as being predictive of adverse effects seen in humans. In this study, we explored the applicability of two fluorescence-based, in vitro assay panels for the evaluation of toxicity. These panels were designed to evaluate effects on cell viability and proliferation, membrane permeability, mitochondrial activity, cell cycle and apoptosis. Each of these assay panels was cross-validated with an appropriate alternative format assay. Representative cell lines for liver and kidney (HepG2 and HK-2 cells, respectively) were grown in 96-well microplates and exposed to several compounds with known effects in humans. A dose response of each compound was evaluated at both 24 and 72 hours. Following exposure, microplates were analyzed using a fully automated high-content imaging system with integrated analysis software. The results of this study demonstrate that these assay panels provide promise for creating a turn-key platform for predictive cytotoxicity assessment.

1840 NKR-52E CELLS AS A MODEL TO STUDY TIGHT JUNCTION AND PARACELLULAR PERMEABILITY PROPERTIES.


Tight junctions (TJ) proteins are the apical-most intercellular structure in epithelial cells, playing a role in cell-cell adhesion, polarity and permeability barrier to paracellular transport of solutes across the epithelial tissue. Physiopathology implies the alteration of permeability properties are associated with the TJ disruption. On the other hand, several studies have show that the proximal tubule is the main target of nephrotoxics, affecting their paracellular permeability properties. NKR-52E is a rat renal proximal tubular cell line recently used to explore some feature of TJ. Our aim was to evaluate the expression and location of Claudin, claudin (cld-2, 3, 7, 8, & 16), as well as transepithelial electrical resistance (TER) and unidirectional 10 kDa FITC-dextran flux, as epithelial barrier functions. These parameters were performing along 3, 7, 10, & 14 days of cultivated cells. TJ location was attempted by immunofluorescence microscopy; quantity by immunoblot analysis; unidirectional FITC-dextran flux by fluorescence measure and TER using a Millicell-electrical resistance system. Results of immunofluorescence and immunoblot analysis showed a direct relationship between time of grown and expression of occludin. Only few cells cultivated by 3 days expressed occludin, this increased to days 7, & 10 post-seeding, where practically all the monolayer was positive to occludin label. A positive label for cld-2 was observed 10 days post-seeding. Cld-3, cld-7, and cld-16 expressions were present 7 days post-seeding. However, this was only in few groups of cells and lower than cld-2. TER values were variable before to 7 days, after this time TER values were similar to the described for the proximal tubule. This TER value was stable for the rest of growing time. Likewise, FITC-dextran flux was more stable after 7 days of seeding. This study support that conditions such as time of grown could affect the location and expression of TJ, and these should be considered for toxicology studies.

1841 HISTOCHEMICAL EVALUATION OF THE EFFECTS OF TRANSPORT ON BOVINE CORNEAS FOR OCULAR SAFETY ASSESSMENTS.


The bovine corneal opacity and permeability (BCOP) assay, originally developed by Gautheron (1992) and Sina (1995) has been used as an in vitro eye irritation screen for industrial hygiene, product development and safety testing. It has re-

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cently been validated as a screen for corrosive or severe irritation by ICCVAM and ECVM. The assay measures changes in corneal opacity, and increases in permeability relative to an artificial cornea. Due to the high degree of ocular similarity, this assay system is currently being used to validate the mode of action of compounds. The drug discovery process is driven from the need for efficient and accurate screening tools. The toxicity profile is critical for the development of new and improved drugs. The embryonic stem cell line B6C-2, a mouse ES cell line, was exposed to titrated doses of arsenic compounds (arsenic trioxide, sodium arsenite and sodium arsenate) for 72 h. The viability of the B6C-2 cells was measured by the MTT assay. The results showed that the cell viability of the B6C-2 cells was decreased by 50% at 0.01 μM of arsenic trioxide, 0.001 μM of sodium arsenite, and 0.0001 μM of sodium arsenate. These results show that the B6C-2 cells are sensitive to arsenic compounds.

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MTBE is a gasoline additive frequently found in ground and surface waters throughout the United States. Previous studies in our laboratory have shown that exposure to MTBE during development disrupts angiogenesis. Zebrash (Danio rerio) embryos exposed to MTBE (0.625 mM to 10 mM) exhibited hemorrhages in the yolk sac and brain, dysfunctional intersegmental vessels, and a reduced number of circulating blood cells. Exposure to the primary metabolite, tertiary butyl alcohol (TBA), at 0.625 mM to 10 mM, did not result in lesions, suggesting either the parent compound or a metabolite causes developmental defects. In a second study, zebrash exposed to 1.25 mM (n=15) or 10 mM (n=17) MTBE until hatch were allowed to mature in fresh system water (n=18 for control). After only 3 days, 65% of the 10 mM group died. Mortality at the end of 30 days was 5.6% for control, 46.7% for 1.25 mM, and 76.5% for 10 mM. Therefore, embryological affects were observed at both low and high concentrations of MTBE, indicating that exposure to MTBE during development disrupts angiogenesis. Zebrafish (Danio rerio) embryos exposed to MTBE (0.625 mM to 10 mM) exhibited hemorrhages in the yolk sac and brain, dysfunctional intersegmental vessels, and a reduced number of circulating blood cells. Exposure to the primary metabolite, tertiary butyl alcohol (TBA), at 0.625 mM to 10 mM, did not result in lesions, suggesting either the parent compound or a metabolite causes developmental defects. In a second study, zebrash exposed to 1.25 mM (n=15) or 10 mM (n=17) MTBE until hatch were allowed to mature in fresh system water (n=18 for control). After only 3 days, 65% of the 10 mM group died. Mortality at the end of 30 days was 5.6% for control, 46.7% for 1.25 mM, and 76.5% for 10 mM. Therefore, embryological affects were observed at both low and high concentrations of MTBE, indicating that exposure to MTBE during development disrupts angiogenesis.

As embryotoxic potential is one of the most severe adverse effects, in vitro screening for embryotoxic potential is very important in drug discovery and developmental processes. The embryonic stem cell test (EST) is one of the validated methods to assess embryotoxic potential of compounds and is a promising tool for drug screening. On the other hand, EST lacks quantitative and throughput performances because EST needs the microscopic evaluation of beating in differentiated cardiomyocytes as an endpoint assay. To improve the above problems, we tried to establish the endpoint assay using the field potential measurement in differentiated cardiomyocytes instead of the evaluation of beating. Mouse embryonic stem cells were differentiated into cardiomyocytes in the hanging drop and suspension culture, and treated with embryotoxic (valproic acid, verapamil and 5-fluorouracil) or non-embryotoxic (penicillin G, d-camphor and isoniazid) reference compounds. After differentiation process, microscopic observation of beating cardiomyocytes or measurement of the field potential by MED 64 system (Alpha MED Sciences Co., Ltd.) was performed as the differentiation index to cardiomyocyte. Embryotoxic compounds, valproic acid, verapamil and 5-fluorouracil, inhibited the field potential and myocardial beating in differentiated cells, while non-embryotoxic compounds, penicillin G, d-camphor and isoniazid, had no similar effects. These results indicated that the field potential measurement can be used as an endpoint assay instead of the microscopic observation of beating. Based on these results, we concluded that this assay system was superior to conventional EST in terms of quantitative and throughput performances and useful for assessment of embryotoxic potential of compounds.


Arsanilic acid is a pentavalent organic arsenic chemical used as a growth promotant for pigs and poultry. It is known to induce neurotoxicity but the mechanism has not been well elucidated. To evaluate embryo and maternal toxicity and to clarify the target cell of arsanilic acid at specific stage of development, we performed comparative cytotoxicity test using mouse embryonic stem (NVQRS-ES), mouse embryonic carcinoma (NVQRS-EC), mouse embryonic germ (NVQRS-EG) cells and mouse differentiated cell lines representing endodermal (hepatoma cell), mesodermal (embryonic fibroblast cell, muscle cell and myeloma cell) and ectodermal cells (neuroblastoma cell). Cells were treated with arsanilic acid (120 pM-20 μM) for 4 hours. Digitonin (300 μg/ml) was used for positive control of cytotoxicity. After treatment with arsanilic acid, dead cell protease activity and live cell MTI activity were measured by a commercially available assay. The results showed that the arsanilic acid concentration (20 μM) caused a significant decrease in the number of live cells." The embryonic mouse cells were exposed to arsanilic acid (20 μM-20 μM) for 4 hours. The results showed that the arsanilic acid concentration (20 μM) caused a significant decrease in the number of live cells.
vest of cell lines. These results suggest that arsenic acid may impact on germline cells at embryo development stage and affects more on myeloid, muscle and nephroblast cells after maturation.

1846 INVESTIGATING THE MECHANISMS OF CARDIOTOXICITY WITH TYROSINE KINASE INHIBITORS USING PRIMARY HUMAN CARDIOMYOCYTES.


Cardiotoxicity can be a side effect for many tyrosine kinase inhibitors (TKIs). The underlying mechanism(s) of cardiotoxicity is not clearly understood, although hypotheses include diverse events ranging from kinase dependent inhibitory sequelae, mitochondrial titotoxicity, lipid peroxidation, endomysial reperfusion injury and so on. A model of human primary cardiomyocytes was developed and characterized for its ability to reflect TKI mediated cardiotoxicity. In our initial study, the in vitro toxicity profile of certain marketed TKIs were assessed and revealed that Sunitinib exhibited cytotoxicity to primary human cardiomyocytes at concentrations as low as 12.5 µM. Cytotoxicity was assessed using multiple in vitro toxicity assays by monitoring different aspects of cell health (e.g., ATP levels, GSH levels, LDH levels). At 12.5 µM, Sunitinib depleted ATP by 50%, depleted GSH by 40%, and increased LDH release by 30%. The kinase inhibitor profile for Sunitinib indicates that many kinases are inhibited at 1 µM and 10µM, including AMP-activated kinase (AMPK). Since AMPK is a critical regulator of cellular energy status, the role of AMPK activation in our model of cardiomyocyte toxicity was examined. The approach employed was pretreatment with the AMPK activator AICAR followed by Sunitinib treatment at a range of concentrations. Our results demonstrate that concentration of Sunitinib that decrease ATPI are inhibited 40% by AICAR pre-treatment. Similarly, AICAR pretreatment also attenuated cell death by ~30% of Sunitinib alone. Our results suggest via in vitro models, that cardiotoxicity may be attenuated by up regulating kinases such as AMPK.

1847 THE ROLE OF MITOCHONDRIAL TOXICITY IN THE CARDIOTOXICITY OF SMALL MOLECULE KINASE INHIBITORS.


The development of selective kinase inhibitors (KIs) can, and often does result in toxicity due to off-target inhibition of other kinases and/or various ATP utilizing enzymes. To assess the extent of this unwanted toxicity we have evaluated a set of >100 compounds with known kinase inhibition but cardiotoxicity profiles. This cardiotoxicity was hypothesized to be due to mitochondrial dysfunction and energy depletion. Primary cultured human cardiomyocytes were employed to generate a comprehensive set of data on mitochondrial physiology including overall cellular toxicity, the reduction in ATP levels, mitochondrial membrane potential dissipation, mitochondrial respiration, alterations in expression of a wide range of OXPHOS and other metabolic enzyme changes, oxidative stress effects including changes in superoxide, glutathione levels as well as protein modifications to a range of metabolic enzymes, and finally, induction of caspase-dependent or -independent apoptosis by a variety of protocols including monitoring movement of apoptotic factors and transcription factors to and from the mitochondria. Our analyses of cardiotoxicity, kinase inhibition and mitochondrial metabolism revealed significant changes in the bioenergetic state of cells after various kinase inhibitor perturbations. The studies confirm that mitochondria can be a primary target of many KIs including sorafenib, imatinib and sunitinib and implicate mitochondrial dysfunction and subsequent energy depletion as a crucial factor in the cardiotoxicity. The approach described here can provide an effective screen of clinical candidates during drug development and, correspondingly, can drive the structure-activity and kinase inhibition-activity studies needed to circumvent cardiac impairment.

1850 AGING ARYL HYDROCARBON RECEPTOR NULL ALLELE MICE ARE PRONE TO HEMATOPOIETIC DISEASE CHARACTERISTIC OF LEUKEMIA.

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The aryl hydrocarbon receptor (AhR) is a highly conserved, developmentally-regulated and ligand-activated gene regulatory protein. Despite extensive studies, the normal physiological function of the AhR is not known. Only a limited characterization of the immune system in AhR null-allele (KO) mice has been performed and this has not been examined in old mice. No studies have delineated hematopoietic stem cell (HSC) function in these mice. Earlier studies indicated that bone marrow (BM) from KO mice has increased numbers of functionally-defined multipotential hematopoietic progenitors (HPP-CFC) and HSCs. In addition, these cells have high rates of proliferation and cell division. We hypothesized HSCs from aging KO mice may undergo premature senescence and/or be involved in the development of hematopoietic disease. We examined the characteristics and numbers of immune lished reports study toxicological endpoints and pathways in hES cells. Investigations of responses to xenobiotic exposure in animal models have been wrought with interspecies differences that have complicated data interpretation and therefore cannot be relied upon to effectively model toxic responses in humans. The aryl hydrocarbon receptor (AhR)/aryl hydrocarbon receptor nuclear translocator (Arnt) pathway is central to xenobiotic toxicity observed in various tissues and critical to proper developmental progression. When exposed in utero to 2, 3, 7, 8-tetra-chlorodibenzo-p-dioxin (TCDD), an environmental contaminant and potent AhR/Arnt pathway agonist, rodents exhibit developmental abnormalities such as cleft palate. Furthermore, TCDD toxicity has been shown to result in regenerative growth deformities, possibly due to changes in expression of extracellular matrix re-modeling genes and subsequent alterations in epithelial to mesenchymal transition (EMT). Previous work in our lab has shown that the AhR/Arnt pathway is functional in human embryonic stem (hES) cells in both undifferentiated and differentiated monolayer culture, as well as in highly differentiated embryoid bodies (EBs) derived from hES cells. Current work focuses on examination of TCDD exposure on the differentiation status of hES cells and EBs using PCR analysis. TCDD treatment appears to inhibit differentiation, as evidenced by lower mRNA expression of early differentiation markers in hES cells and greater expression in EBs. Additionally, localization of EMT-related proteins, as visualized through indirect immunofluorescence, suggests that TCDD may enhance progression of EMT in EBs. These findings support use of hES cells as a tool for exploring mechanisms in developmental toxicology.

1848 EFFECTS OF TCDD ON DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS.

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The pluripotent nature and unlimited proliferative ability of hES cells have potential to provide a renewable, consistent cell source, in which to more accurately study toxicity in early human development. However, a relatively small number of pub-
cells in two-year old Wild Type and KO mice. There was a significant increase in total BM cells, and lymphocyte count in the blood of KO mice. Phenotype analysis of BM cells showed higher numbers of HSCs, primitive multipotent progenitor cells, and common lymphoid progenitors, but lower numbers of common myeloid progenitors. Functional analysis of KO BM cells showed an increase in HPP-CFC and colony-forming units for pre-B and decrease in erythroid and myeloid progenitors. Histological examination of KO Spleen sections from KO mice showed a decrease area of the periairteriolar lymphocyte sheath and increased lymphocyte infiltration in the red pulp area. Immunohistochemical staining of spleen sections from KO mice indicated the presence of increased numbers of highly proliferating B and T-lymphocytes. Increased leukocyte infiltration in hepatic portal areas was also observed. Together these data suggest that during aging HSCs function abnormally in mice lacking AhR leading to hematopoietic disease showing characteristics of leukemia. (Supported by NIH grants ES04862, ES07026, and ES01247.)

1851 RELIABLE AND PREDICTIVE IN VITRO ASSAYS FOR MYELOTOXICITY AND CARDIOTOXICITY OF KINASE INHIBITORS.

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Kinase inhibitors (KIs) represent a new class of rationally designed drugs. The success of Imatinib, targeting the ABL tyrosine kinase in CML, has prompted the development of other KIs for the treatment of various cancers and inflammation. Although more successful than conventional therapies, myelotoxicity and cardiotoxicity are often major side effects of KIs. Using in vitro models for myelotoxicity and cardiotoxicity, we determined the IC50 values of 6 KIs (Imatinib, Lapatinib, Erlotinib, Dasatinib, Sorafenib and Sunitinib) and compared them to the degree of clinical myelotoxicity and cardiotoxicity reported in the literature. For the hematopoietic colony-forming cell (CFC) assays, human bone marrow cells and KIs were mixed with methylcellulose-based media, plated in 35 mm dishes (n=3), and the progenitor cell colonies were enumerated on day 14. For the in vitro CFC assays, mouse ES cell-derived cardiomyocytes (Cor.AT®) were plated at 20,000 cells per well (n=6), the KIs were added on day 2 (short-term effects), or 14 (long-term effects) days after plating and cell death was measured by neutral red uptake 48 hours later. Mouse embryonic fibroblasts were used as non-specific control cells. There was a direct correlation between clinical myelotoxicity and the IC50 values derived from the in vitro CFC assays, with lower IC50 values associated with increased neutrophenia (IC50 range: 0.008 μg/ml (Dasatinib) > 100 μg/ml (Lapatinib)). There was also a strong correlation between published clinical cardiotoxicity (e.g. myopericarditis, ischemia, pericarditis, myocardial infarction) and the effect of the KIs on Cor.AT® cells. Imatinib was the most toxic Ki where as Erlotinib and Lapatinib demonstrated the least toxicity (between 1 nM and 0.1 μM). Cor.AT® cells pre-cultured for 2 days were less susceptible than those pre-cultured for 14 days. These results demonstrate that both the in vitro human CFC and Cor.AT® cardiomyocyte assays are reliable and useful for accurately predicting the myelotoxicity and cardiotoxic ranking of KIs.

1852 THE ARYL HYDROCARBON RECEPTOR MODULATES ERYTHROPOIESIS IN MICE.

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The aryl hydrocarbon receptor (AhR) mediates the toxic, carcinogenic and teratogenic effects of environmental pollutants such as dioxins; exposure to humans has been correlated with blood malignancies. No physiological role of this evolutionary conserved receptor has been established. Recently, we reported that activation of AhR by its exogenous ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) produces major changes in numbers and functions of murine hematopoietic stem cell and primitive progenitor cells. Erythropoiesis differentiate from stem cells through a series of intermediate stages, but any role of AhR in murine erythropoiesis is unknown. The first committed erythrocyte precursors are the bursts forming units cell (BFU-E) that differentiate into colony-forming units (CFU-E). Erythroid liver cells (FL) from embryos of E14D pregnant mice were exposed to TCDD (5 nM) in vitro. E14D pregnant mice were also dosed with TCDD (30 microg/kg) for 1 day and FL was collected. In both cases, TCDD produced a significant decrease in BFU-E and CFU-E. C-Kis+ cells sorted from FL exposed to TCDD in vivo demonstrated lower proliferation rates as determined by 3H-thymidine incorporation, and had lower numbers of BFU-E at the G2M cell cycle stage. When young adult mice were exposed to TCDD (30 microg/kg), there was increased BFU-E in BM after day 1, 3 and 5 of treatment, but decreased numbers at proerythroblast and basophilic erythroblast stages of differentiation. In addition, there were lower numbers day 8 CFU-spleen colonies that are formed by erythroidmyeloid progenitors. Studies conducted in young and two years old Ahr-/- mice also showed lower BFU-E and CFU-E in BM. Hematological analysis of blood smears from older Ahr-/- mice showed erythroid cells with altered shape and size consistent with a defect in the maturation of erythroid progenitors into red blood cells. These results suggest that the AHR plays a direct or indirect role in murine erythropoiesis. (Supported by NIH grants ES04862, ES07026, and ES01247.)

1853 CHARACTERIZATION OF PRIMARY HUMAN CARDIOMYOCYTES AS A POTENTIAL IN VITRO MODEL FOR TESTING CARDIOTOXICITY.

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A number of marketed tyrosine kinase inhibitors (TKIs) have been reported to be cardiotoxic in humans, prompting the need for better models for the preclinical assessment of cardiotoxicity. In response, we have characterized a human tissue derived primary cardiomyocyte model by comparing its molecular and biochemical characteristics to adult human heart tissue. Human cardiomyocytes were compared to adult heart tissue using immunohistochemistry (IHC), quantitative PCR and microarray gene expression analysis. We have used multiple antibodies to evaluate the expression of human cardiomyocyte markers and found that human cardiomyocytes in culture express tropomyosin, troponin I, actinin, ANP, desmin, M-cadherin, NCK 2-5 and others, thus confirming they maintain the phenotypic expression of human heart tissue through multiple passages (P0, P1, P2, P4, P8 and P12). We have also evaluated the mRNA expression of 40 different cardiac specific genes including MYBPC3, MYH7, MHC, NPPA, PLN, GAF4 and MYL2 in human cardiomyocytes. Our analysis revealed abundant expression of the cardiac gene markers over multiple passages. Using these primary cardiomyocytes, we next performed several biochemical toxicity assays including, ATP depletion, ROS generation, and GSH depletion to evaluate the relative toxicity of a number of marketed TKIs. The data indicate that cardiomyocyte toxicity assays can differentiate cardiotoxic and non-cardiotoxic TKIs, thus permitting novel compounds to be effectively evaluated for relative risk of cardiotoxicity. TKI compound. Based on our results we propose that primary human cardiomyocytes represent a phenotypically relevant cell-based model for the early preclinical assessment of drug-induced cardiotoxicity.

1854 POTENTIAL MECHANISMS OF IN VITRO CARDIOTOXICITY ASSOCIATED WITH TYROSINE KINASE INHIBITORS.

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An in vitro model of cardiotoxicity assessment was developed using kinase inhibitors with known toxicological sequelae. A set of compounds including the kinase Inhibitors (KIs) (Imatinib, Dasatinib, Sorafenib, Lapatinib, Gefitinib, Erlotinib, Vertex ERK Inhibitor (V_ERK), a cardiotoxic Roche KI (RO-459) and a Roche non-cardiotoxic KI (RO-050) (Doxoru bicin, BILN-2061 controls) were used to identify mechanisms of cytotoxicity. Cultured primary human cardiomyocytes were used to generate a comprehension of data across multiple time points for more than 50 parameters including ATP levels, mitochondrial membrane potential, oxidative phosphorylation complexes, respiration, cellular toxicity, oxidative stress, ER stress, apoptosis and signaling components of the energy metabolism, survival and stress pathways. Mitochondrial dysfunction and ATP depletion were a common theme in vitro for compounds that cause cardiotoxicity in vivo. ATP depletion assessed with Linear mixed effects models was ranked using other assays (N = 28 assays) as predictor variables. This analysis yielded a set of assays (N = 13) that were highly predictive of ATP depletion and subsequent cytoxicity. Compound toxicity, based on rank sum, using 14 of the top-ranked assays including ATP depletion, led to the ranking of potential cardiotoxicity of three distinct groups; high (Sorafenib, Doxorubicin, Dasatinib and RO-459) moderate (Lapatinib, Imatinib & V_ERK) and low (Gefitinib, Erlotinib, BILN-2061 and RO-050). These data suggest that primary human cardiomyocytes assessed for key parameters are an excellent in vitro model for cardiotoxicity prediction and can be used to reveal insight into the mechanisms of kinase inhibitor mediated cardiotoxicity.
1855 A QUANTITATIVE STEM CELL ASSAY FOR TESTING OF DEVELOPMENTAL NEUROTOXICITY.

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Successful implementation of an adequate in vitro method in first tier screening of chemicals for global toxicity testing of developmental neurotoxicants on neural cell level could result in a strong reduction of animal use for developmental testing and could reduce the time involved for testing potential developmental neurotoxicants. In the proposed assay, embryonic stem cells of the mouse are forced to differentiate into the three main cell types of the CNS (neurons, oligodendrocytes, and astrocytes) along processes like proliferation, migration and differentiation, thereby mimicking neural development in vivo. MAM and MeHgCl, well-know developmental neurotoxicants, were used to confirm the presence of the developmental phases. The final cell population was characterized on the basis of antigenic features, and quantified on the basis of H&E morphological features using stereological principles. MAM inhibited the proliferation phase of the in vitro model and MeHgCl inhibited the migration phase, both without affecting cell viability. MeHgCl exposure during the differentiation phase led to massive cell death. The presence of both mature and immature neural cells was demonstrated by specific staining with antibodies. Using morphological features after H&E staining, nine cell classes were distinguished. Using stereological principles to estimate the cell class distribution in 10 cultures collected over a period of 5 months, it appeared that the variation within and between cultures was the same even though the cell numbers might differ between cultures. Since this in vitro DNT mimics the phases of in vivo development of the CNS including differentiation into immature and mature neural cells, it has a constant cell class composition and appears able to detect developmental neurotoxicity, this assay is a promising alternative for in vivo DNT studies in first tier screening settings.

1856 AHR/CYP1A1 EXPRESSION IN MOUSE TROPHOBLAST ALLOGRAFTS.

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The placenta is an important organ for providing nutrients to the growing fetus and also serves as a barrier warding off harmful substances from maternal circulation. The placenta is an organ known to express significant levels of the aryl hydrocarbon receptor (AHR). The role of the placental AHR however, is not clear. In mammalian development, trophoblast stem cells give rise to placental tissues protecting fetal allograft in part through modulating immune response. Recent evidence indicates that AHR plays an important role in mediating immune responses through generation of regulatory T cells. In order to investigate the role of AHR in trophoblast stem (TS) cells, we utilize an allograft tolerance mouse model through injection of trophoblast stem (TS) cells through portal vein. These allografted TS cells survived up to 4 months in allogeneic female mice. We performed immune histochemistry analysis for the presence of active AHR/CYP1A1 pathway in the allograft tissues. We found significant level of cyp1a1 expression in all surviving trophoblast allografts. This finding strongly suggests the presence of active AHR/CYP1A1 pathway in trophoblast allografts and the AHR/CYP1A1 may play a role in allograft tolerance. (this work is supported in part by NIH ES 09859).

1857 PREDICTING DEVELOPMENTAL TOXICITY WITH HUMAN EMBRYONIC STEM CELLS AND METABOLICOMICS.

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Birth defects are a prominent problem, occurring in 6% of births, nationally. Teratogens, or substances that cause one or more fetal abnormalities in development, cause 5-10% of these defects. While animal models are currently used to predict developmental toxicity of drugs, they are costly, time-consuming, and most importantly, do not adequately correlate to human response. The overall ability of animal models to predict developmental toxicity in humans is only 60%. Stemina Biomarker Discovery, Inc. (Stemina) addresses these issues by using an in vitro method that utilizes both human embryonic stem (hES) cells and metabolomics to discover metabolite biomarkers of developmental toxicity. Stemina detects secreted extracellular metabolites from hES cells using liquid chromatography mass spectrometry (LCMS) with the hypothesis that measurable modulation of specific secreted metabolites will result due to treatment of hES cells with teratogens. Such metabolites will serve as biomarkers of developmental toxicity.

1858 ANTI-OXIDANT RESPONSE ENZYMES IN PROGRESSIVE STAGES OF HUMAN NON-ALCOHOLIC FATTY LIVER DISEASE.

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Non-alcoholic fatty liver disease (NAFLD) encompasses various stages of liver damage ranging from steatosis (simple fatty liver) to non-alcoholic steatohepatitis (NASH), which can lead to cirrhosis. NAFLD occurs in approximately 20-25% of all Americans, while NASH affects approximately 2-5% of the population. Compromised hepatic function and oxidative stress are known characteristics occurring in NAFLD progression. The purpose of the current study was to determine whether the function of enzymes involved in the antioxidant response, NADPH quinone oxidoreductase-1 (NQO1) and glutathione S-transferases (GST), is altered in human liver samples diagnosed as normal, steatotic, NASH, and NASH no longer fatty. Immunohistochemical staining of the antioxidant responsive transcription factor Nrf2 revealed its translocation into the nucleus in all stages of NAFLD with prominent staining in the NASH (fatty) samples. NQO1 mRNA levels in the various stages of NAFLD showed a significant increase in NASH (not fatty) samples as compared to normal. Similar to mRNA, NQO1 activity was significantly increased in NASH (not fatty) samples compared to normal. Significant increases were also observed in mRNA levels of GST enzymes A1, A4, M3, and M4 in NASH (not fatty) samples compared to normal. Additionally, a significant increase from normal in both NASH disease stages was observed for the GST P1 isozyme. In contrast to mRNA levels, there was a statistically significant decrease in GST activity, measured by CDNB conjugation, in both stages of NASH as compared to normal. The current data suggest that during the progression of NASH, increased Nrf2 activation and NQO1 activity observed in late stage NASH may provide protection against quinone-induced redox cycling. In contrast, due to a reduction in GST enzymatic activity, the ability to neutralize electrophiles may be compromised. Taken together these results demonstrate that NAFLD causes an altered capacity to manage oxidative stress-inducing events during progression of the disease.
1860 PRECLINICAL ASSESSMENT AND BIOMARKER DISCOVERY OF DRUG-INDUCED HEPATIC STEATOsis.


The detection of hepatic steatosis in preclinical and clinical studies is difficult due to the lack of non-invasive biomarkers for monitoring disease appearance and progression. To address these gaps and identify new markers of compound-induced hepatic steatosis, we evaluated the concordance of histopathological diagnoses to novel biomarkers including serum lipids and lipoproteins and hepatic gene expression profiles. Test articles were administered orally to rats at toxic doses and included the mitochondrial toxicant chloroform, the LXR/PXR agonist TO-901317, and two PXR agonists mitoquinone and econazole for 1 or 7 days. A non-steatotic compound and PXR activator RO-55 was dosed once daily at a maximum tolerated dose for 1, 7, 14 and 28 days and served as a negative control. With the exception of RO-55, all groups showed a compound-related increase in hepatic steatosis as early as day 1, which increased in severity and incidence by day 7. Hepatic mRNA-based lipoprotein profiling indicated compound specific changes in serum lipids and lipoproteins, but no individual changes were diagnostic of hepatic steatosis. Hepatic gene expression profiling confirmed similarity between the PXR activators mitoquinone, econazole and RO-55, and distinct modes of action for TO-901307 and chloroform. All compounds except chloroform induced PXR-mediated Cyp3a mRNA, however there were variable effects on CD36 fatty acid transporter suggesting that PXR-mediated lipid accumulation via CD36 may not fully explain the observed steatosis. There was a consistent up-regulation of CPT1A and other enzymes by all steatotic compounds, which function to increase mitochondrial fatty acid oxidation. However, other mitochondrial-related proteins involved in oxidative phosphorylation and energy production were consistently altered. These results suggest that compound-induced hepatic steatosis does not substantially affect detectable changes in serum lipid and lipoprotein components, yet non-invasive monitoring of mitochondrial components or activities may provide an alternate approach for biomarker identification.

1861 INVOLVEMENT OF AMP-ACTIVATED PROTEIN KINASE/ACC PATHWAY ON THE FATTY LIVER INDUCED BY OROTIC ACID.


Orotic acid (OA) has been known to cause fatty liver with poorly defined cellular mechanisms of action. AMP-activated protein kinase (AMPK) is a critical regulator of cellular and body energy homeostasis sensing the status of the AMP:ATP ratio. The net effect of phosphorylation of AMPK by AMP kinase in the liver is stimulation of fatty acid oxidation and inhibition of lipogenesis. The present study was aimed to investigate whether the OA-induced fatty liver is mediated by AMPK phosphorylation. OA induced intracellular lipid accumulation in SK-Hep1 human hepatoma cells in a dose- and time-dependent manner. Exposure of SK-Hep1 cells to OA significantly reduced the Thr-172 phosphorylation of AMPK, and also reduced the phosphorylation of acetyl-CoA carboxylase (Acc), a well-known substrate of AMPK. Inhibition of AMPK by the AMPK inhibitor compound C3 reduced OA-induced intracellular lipid accumulation in SK-Hep1 cells. OA-induced accumulation of intracellular lipids was decreased by AMPK activators metformin and AICAR. We also observed reduced AMPK phosphorylation and increased lipogenesis in rats treated with OA. These results suggest that AMPK is involved in OA-induced fatty liver and that AMPK is a potential target for the treatment of OA-induced fatty liver.

1862 PESTICIDE AND HEAVY METAL EXPOSURES ARE ASSOCIATED WITH LIVER DISEASE.

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Purpose: The prevalence of liver disease has risen dramatically in parallel with the obesity epidemic. The majority of these cases are thought to be due to non-alcoholic fatty liver disease (NAFLD). Occupational exposures to industrial chemicals have also been associated with NAFLD. The impact of pollutants on liver disease in the general population has not been evaluated. Materials and Methods: We analyzed data from adult participants in the National Health and Nutrition Examination Survey (NHANES) 2003-2004. We analyzed the data for an association between blood or urine levels of 196 toxicants and elevated ALT (marker of liver injury), after excluding participants with liver disease from other etiologies (hepatitis B or C, alcohol abuse or iron overload). Toxicants which were detectable in 60% of subjects were included (116 of 196). Subjects were stratified by toxicant level into quartiles and the odds ratio for abnormal ALT was determined after adjusting for confounding variables like age, sex, BMI, and insulin resistance (HOMA) using logistic regression analysis. Results: 4582 subjects were included in the analysis. Of these, 34.1% were found to have abnormal ALT. Several organochlorine pesticides (or metabolites) including dieldrin, oxychlordane, trans-nonachlor, and heptachlor epoxide were associated with a dose-dependent increased risk for ALT elevation. Among heavy metals, lead, mercury, and thallium also demonstrated a dose-dependent increased risk for ALT elevation. Conclusions: In the US population, exposures to specific organochlorine pesticides and heavy metals are associated with a dose-dependent increased risk for otherwise unexplained liver disease. Although most of these organochlorine pesticides are no longer used, they are clearly persistent pollutants which are clinically relevant. The pathogenic role of these toxicants in liver diseases such as NAFLD should be explored. Acknowledgement: NIEHS (P30ES014443-01A1), and the AASLD (Shelia Sherlock Award).

1863 TOXICANT ASSOCIATED STEATOHEPATITIS (TASH) IN AMERICAN VINYL CHLORIDE WORKERS.

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Purpose: Nonalcoholic steatohepatitis (NASH) typically develops obese patients and is believed to be the hepatic manifestation of the metabolic syndrome. Recently, NASH was described in lean individuals exposed to industrial toxicants. Here, we describe toxicant associated steatohepatitis (TASH) in American vinyl chloride (VC) workers. Next, we compare diagnostic strategies and second-hit mechanisms between TASH and NASH due to obesity. Material and Methods: Liver biopsies from 26 non-obese, highly exposed, VC workers were evaluated for steatohepatitis (TASH). Serum from affected workers was analyzed and compared by ANOVA to (i) 48 unexposed, obese subjects with biopsy documented NASH, and (ii) 37 healthy controls. Analytes included: routine chemistries, cytokines, cytokinin 18, and antioxidant capacity. Results: In VC workers, the prevalence of TASH was 78%. Affected workers had a mean BMI of 26±3.9 and an estimated mean cumulative VC exposure of 14,000±6500 PPM-Yr. The histological severity of steatohepatitis was equivalent between the TASH and NASH groups. Although ALT and AST were elevated in NASH, they were normal in TASH. While cytokinin-18 M65 antigen was elevated in both groups, the M30 fragment was elevated only NASH. Insulin resistance and adiponectin reduction were similar between TASH and NASH. TASH was associated with markedly elevated TNFα, IL1, IL6, IL8, and IL18. Serum antioxidant capacity was reduced to a greater degree in NASH than TASH. Conclusion: TASH frequently occurs in non-obese VC workers with normal liver enzymes and high cumulative exposures. Cytokinina-18 M65 antigen may be a useful serologic biomarker for TASH and suggests the presence of necrotic rather than apoptotic cell death. Although adiponectin reduction due to reduced adiponectin is common to both TASH and NASH, differences exist in cytokine expression. TASH was associated with markedly elevated TNFα, IL1, IL6, IL8, and IL18. Serum antioxidant capacity was reduced to a greater degree in NASH than TASH. Conclusion: TASH frequently occurs in non-obese VC workers with normal liver enzymes and high cumulative exposures. Cytokinina-18 M65 antigen may be a useful serologic biomarker for TASH and suggests the presence of necrotic rather than apoptotic cell death. Although adiponectin reduction due to reduced adiponectin is common to both TASH and NASH, differences exist in cytokine expression.

1864 PROTECTIVE EFFECT OF THE AQUEOUS EXTRACT FROM THE ROOTS OF PLATYCODYON RADIX ON ETHANOL-INDUCED LIVER INJURY IN RAT.

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Alcoholic steatosis is the earliest and most common response to heavy alcohol intake, and may precede more severe forms of liver injury. Accumulation of fat, largely triglyceride, in hepatocytes results from the inhibition of fatty acid oxidation and excessive oxidative stress involving CYP2E1. The protective effects of an aqueous extract from the roots of Platycodon radix, Changguk (CK), on alcohol-induced liver injury in an enteral alcohol feeding model was investigated. Male Wistar rats were given control liquid diets with ethanol containing diets orally for 4 weeks. Ethanol delivery and weight gain were not different between the CK-ethanol and ethanol groups. Pretreatment with CK prior to the administration of ethanol significantly prevented the increase in serum alanine aminotransferase (ALT), aspartate transaminase (AST) activities, serum albumin, necrosis and inflammation.
Moreover, ethanol elevated hepatic triglyceride content, hepatic lipid peroxidation, and induced CYP2E1. These effects were all blocked by CK treatment. Alcohol administration resulted in a decrease in AMP-activated protein kinase-alpha (AMPK-alpha) phosphorylation, which was restored by CK treatments. Recovery of AMPK activity by CK administered after BDL demonstrated an increase in acetyl-CoA carboxylase phosphorylation. These results demonstrate that the CK effectively treats alcoholic steatosis with CYP2E1 inhibition, which may be associated with the recovery of AMPK activity, promising that the combination therapy may constitute an advance in the development of clinical candidates for alcoholic steatosis.

1865 EUGENIA JAMBOLANA FRUIT EXTRACT DOES NOT ALTER CHOLESTASIS-INDUCED LIVER INJURY DESPITE DOWN REGULATION OF EFFLUX TRANSPORTER EXPRESSION IN MICE.

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Eugenia Jambolana (Jamun) fruit is reported to contain endogenous antioxidants and is used in some cultures as a therapy to treat liver-based diseases. Because Jamun may have beneficial effects for liver, we hypothesized that Jamun fruit extract (JFE) might have a protective effect on liver injury caused by extrahepatic cholestasis. Extrahepatic cholestasis is defined as obstruction of bile flow from the liver, which occurs outside the liver. Alterations in transporter expression in liver is thought to be an adaptive mechanism to compensate for liver to decrease toxic bile acids in hepatocytes. Based on this premise, it is anticipated that downregulation of transporters that efflux bile acids would enhance cholestasis-induced hepatic injury. To determine whether administration of JFE affects the progression and severity of extrahepatic cholestasis, mice were subjected either sham or bile-duct ligation (BDL) surgery. Starting the next day after surgery, sham and BDL mice were administered either JFE (100 mg/kg, po) or vehicle for 10 days once daily. Livers were collected for histopathological assessment and quantification of mRNA expression by the Branch TC DNA Signal Amplification Assay. Histopathological analysis revealed that JFE had no effect on BDL-induced pathological changes in liver. As anticipated, BDL increased Oatp1a4, Mrp3, and 6 and decreased Oatp1a1, Oatp1b2, and Ntcp mRNA expression. In sham controls, JFE administration increased Mrp3 and decreased expression of Oatp1a4, 1b2, Mrp2, 4 mRNA expression in liver. Treatment with JFE also decreased Mrp1, 4, and 6 after BDL, but this did not alter BDL-induced injury. In summary, administration of JFE did not protect against liver injury induced by BDL in mice. Interestingly, down regulation of efflux transporter expression during cholestasis did not increase the level of hepatic injury, indicating that Mrp1 and 6 upregulation probably do not contribute to hepatoprotection during chronic extrahepatic cholestasis. (NIH KES015782AP20R016457)

1866 DEVELOPMENT OF AN IN VITRO MODEL WITH THE POTENTIAL FOR DETECTION OF CHOLESTATIC COMPOUNDS.

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The liver transporters bile salt efflux protein, BSEP, and multidrug resistance protein, MRP2, can be inhibited by compounds leading to cholestasis and induced liver injury. Early in vitro identification of compounds that inhibit transporters will help reduce attrition due to cholestasis. We describe a method for detection of compound-specific changes in expression and localisation of BSEP and MRP2 in hepatocytes using imaging technology. This data was compared to that obtained from assays for compound inhibition of BSEP and MRP2 in cell membrane vesicles. Using an automated platform, primary rat hepatocytes were treated with 65 compounds in a 96 well format. Cells were fixed and stained for BSEP and MRP2 using specific antibodies. Parallel assays were performed to detect Autin and Tubulin to determine effects on canalicular architecture. Algorithms were designed to quantify localisation and expression of the proteins. Choice of culture media proved an important factor for BSEP expression and therefore assay performance. Under defined conditions for optimal BSEP expression, Trogitroline caused BSEP internalisation. This response was significantly greater than that seen in hepatocytes cultured in standard, commercially available media. In the full compound set we observed various effects on BSEP and MRP2 expression and localisation. This may reflect the different causative mechanisms of drug-induced cholestasis. We also utilised vesicle transport assays for analysis of MRP2 and BSEP inhibition. We observed potent inhibition of BSEP-mediated taurocholate transport and MRP2-mediated taurocholate-ethoxyresorcin (CDP) transport by cholestatic compound performance. We combined the vesicle and imaging data using a statistical modeling approach. This resulted in a rank order of compounds with cholestatic potential. Statistical modeling revealed that combining information from two different assay systems shows potential for use in early detection of cholestatic compounds.

1867 ASSESSING THE INHIBITION OF BILE ACID TRANSPORT AND CHOLESTATIC POTENTIAL OF DRUG CANDIDATES IN 24-Well RAT AND HUMAN SANDWICH-CULTURED HEPATOCYTES (B-SAFE™).


Drug-induced cholestasis may result from the inhibition of biliary efflux of bile acids in the liver. Inhibition of the hepatic uptake and/or the biliary efflux of bile acids will result in an increase in serum concentrations. However, it is the intracellular concentration of bile acids that may be responsible for hepatotoxicity and thus serum concentrations of bile acids may not necessarily be an appropriate indicator of hepatotoxicity. Sandwich-cultured hepatocytes (B-CLEAR®/B-SAFE™) maintain many of the structural and functional attributes of hepatocytes in vivo, and have been used as an in vitro model to predict the in vivo biliary excretion of drug candidates. The B-SAFE™ methodology maintains the expression and function of key uptake and efflux transporters and is the optimal system to evaluate and predict the potential of a compound to cause transporter based liver toxicity, the effect of test compounds on the accumulation, BEI (biliary excretion index), and Clbiliary (in vitro biliary clearance) of a stable isotope of taurocholate (d2-TCA) were evaluated in 24-well B-SAFE™RT (rat) and –HU (human) The accumulation, BEI and Clbiliary of d2-TCA were 7.28 ± 1.03 pmol/mg protein, 76.9 ± 5.73 % and 8.14 ± 1.31 mL/min/kg, respectively in B-SAFE™RT and 62.4 ± 27.5 pmol/mg protein, 65.8 ± 10.2 % and 37.7 ± 10.5 mL/min/kg, respectively in B-SAFE™HU. Cyclosporine resulted in an increase in accumulation and decrease in the biliary efflux (BEI and Clbiliary) of d2-TCA in B-SAFE™RT, however, in B-SAFE™HU, cyclosporine resulted in a decrease in uptake (accumulation) of biliary efflux d2-TCA. The effects of additional cholestatic agents were evaluated and the results are presented. Furthermore, a stable isotope of glycocholate (the glycine conjugate of cholate and the dominant bile acid found in humans) was synthesized and evaluated along with a fluorescent derivative (choleyl-lysyl fluorescence) as alternate probes to assess the cholestatic potential of drugs in 24-well B-SAFE™.

1868 TISSUE FACTOR-DEPENDENT COAGULATION CONTRIBUTES TO ALPHA-NAPHTHYLISOTHIOCYANATE-INDUCED CHOLESTATIC LIVER INJURY IN MICE.


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Separation of concentrated bile acids from hepatic parenchymal cells is a key function of the bile duct epithelial cells (BDECs) that form intrahepatic bile ducts. Using co-immunostaining, we found that tissue factor (TF), the principal activator of coagulation, colocalized with cytokeratin 19, a marker of BDECs in the adult mouse liver. BDEC injury induced by xenobiotics such as alpha-naphthylisothiocyanate (ANIT) causes cholestasis, inflammation and hepatic cellular injury. We tested the hypothesis that acute ANIT-induced cholestatic liver injury is associated with TF-dependent coagulation, and determined the role of TF in ANIT hepatotoxicity. Treatment of mice with ANIT (60 mg/kg) caused hepatic necrosis and significantly increased serum biomarkers of cholestasis (i.e., ALP and bile acids) and hepatic parenchymal cell injury (i.e., ALT). ANIT treatment also significantly increased liver TF expression and activity. ANIT activation of the coagulation cascade was indicated by increased plasma thrombin-antithrombin levels and significant deposition of fibrin within bile infarcts. ANIT-induced coagulation was reduced in low TF mice, which express 1% of normal TF levels. Moreover, ANIT-induced liver injury was reduced in the low TF mice. The results indicate that ANIT-induced liver injury is accompanied by TF-dependent activation of the coagulation cascade, and that TF contributes to the progression of injury during acute cholestatic hepatitis.

1869 INHIBITION OF BOTH BSEP AND MRP2 ACTIVITY CORRELATES WITH RISK OF DRUG INDUCED LIVER INJURY IN MAN.

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To explore the relationship between inhibition of biliary efflux transporters and liver toxicity, we have assessed functional in vitro inhibition of the activities of Bsep (bile salt efflux pump; ABCB11) and Mrp2 (multi-drug resistance-associated pro-
protein 2; ABCG2) by hepatotoxic and non-hepatotoxic marketed drugs. In vitro transporter inhibition was determined by live cell high content fluorescence imaging of rat hepatocytes cultured in sandwich configuration, using selective probe substrates that accumulate into sealed canalicular pockets between the cells. The probes used were cholestyramine, BSEP (Bsep) and carboxylsulfobutyryl, Mrp2. The data obtained with cholestyramine was quantified using a novel Cellomics Arrayscans TM algorithm. Transporter inhibition studies were also undertaken using membrane vesicles from SF21 cell lines transfected with rat BSEP or rat Mrp2, to confirm the data obtained using cultured hepatocytes. Potent inhibition of Bsep activity (apparent IC50=10mM) was observed with 15/25 drugs associated with cholestatic or mixed liver injury in man (60%), 17 drugs associated with hepatocellular liver injury (14%) and 5% non or minimally hepatotoxic drugs (0%). Potent inhibition of Mrp2 activity (inhibition evident at 100mM) was observed with 10/21 drugs associated with cholestatic or mixed liver injury in man (48%), but not with 5 drugs associated with hepatocellular liver injury or with 5 non/minimally hepatotoxic drugs. The drugs that exhibited potent inhibition in vitro in combination of both Bsep and Mrp2 activity included Troglitazone, which was withdrawn from clinical use due to severe liver toxicity. The structurally related compounds Rosiglitazone and Pioglitazone also exhibited potent inhibition of Bsep activity, but were markedly less potent inhibitors of Mrp2 activity than Troglitazone. These results indicate that a combination of potent inhibition of both Bsep and Mrp2 may confer a greater risk of drug induced liver injury than Bsep inhibition alone.

**1870 ROLE OF NUCLEAR FACTOR-E2-RELATED FACTOR 2 (NRF2) IN CHOLESTEROL MONOHYDROXY CRYSTAL FORMATION.**

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Cholesterol gallstone formation occurs with a high cholesterol diet and dysregulation of cholesterol handling in liver. Some populations are found to be more susceptible to gallstone formation, suggesting a genetic component to this susceptibility. Gallstones develop when cholesterol levels in bile are high resulting in cholesterol precipitation, crystallization, and gallstone formation. Nrf2 is a transcription factor that regulates the expression of many enzymes and transporters in liver that mediate metabolism and transport processes. Preliminary data demonstrate that Nrf2-null mice are resistant to gallstone formation when fed a lithogenic diet (15% fat, 1.25% cholesterol, 0.5% sodium cholate) for 9 weeks. The purpose of this study was to examine early changes in bile cholesterol crystallization and transporter expression in wild-type (WT) and Nrf2-null mice fed a lithogenic diet for 14 days. Male littermate WT and Nrf2-null mice were fed with a lithogenic or standard diet (5% fat) for 14 days, then gallbladders and livers were collected. The lithogenic diet resulted in increased crystal formation in the gallbladder bile of WT mice as compared to Nrf2-nulls. Total RNA was isolated from liver and the mRNA expression of transporters involved in hepatic cholesterol and bile acid transport was determined using the Branched DNA Amplification Assay. The lithogenic diet increased Acalb, Aleg5B, and Mdr2 mRNA expression equally in livers of WT mice and Nrf2-null mice. In contrast, Bsep induction by the lithogenic diet was greater in WT mice as compared to Nrf2-nulls. Mrp3 levels were significantly lower in livers of WT mice as compared to Nrf2-nulls fed either the standard or lithogenic diet. Together the lack of major differences in transport expression, other than Mrp3, between WT and Nrf2-null mice do not explain the genotype difference in crystal formation. Further studies will address how Nrf2 may regulate other molecular mechanisms that contribute to cholesterol uptake, metabolism, and excretion. (NIH K08015782A, P20RR016457)

**1871 ACETAMINOPHEN DISPOSITION: METABOLICOM BIOMARKER FOR NON-ALCOHOLIC FATTY LIVER DISEASE.**


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Non-alcoholic fatty liver disease (NAFLD) includes a range of pathologies from simple steatosis to steatohepatitis (NASH). Researchers estimate the prevalence of NAFLD to be 20-25% and NASH at 2% of the US population. NAFLD has been linked to obesity, insulin resistance, type 2 diabetes, and cardiovascular disease. NAFLD is characterized by a pathologic accumulation of neutral triglycerides in the liver. The severity of this disease in the population is unknown due to the lack of a readily available, noninvasive diagnostic test. Some studies have suggested that the flipside of the NASH model, non-alcoholic fatty liver (NAFL), is a close relative to the metabolic syndrome. A recent meta-analysis of epidemiology studies has found a linear dose response relationship between adiposity and NAFLD. Additionally, prospective studies have found that a high intake of refined carbohydrates and a high ratio of saturated fatty acids to polyunsaturated fatty acids is a predictor of NAFLD. The intake of alcohol, which is known to cause liver injury, is a risk factor for NAFLD. However, alcohol consumption is not a major risk factor for the development of NAFLD and NASH. Currently, needle biopsy is the only conclusive method of diagnosing NAFLD to be 20-25% and NASH at 2% of the US population. NAFLD has been linked to obesity, insulin resistance, type 2 diabetes, and cardiovascular disease. NAFLD is characterized by a pathologic accumulation of neutral triglycerides in the liver. The severity of this disease in the population is unknown due to the lack of a readily available, noninvasive diagnostic test. Some studies have suggested that the flipside of the NASH model, non-alcoholic fatty liver (NAFL), is a close relative to the metabolic syndrome. A recent meta-analysis of epidemiology studies has found a linear dose response relationship between adiposity and NAFLD. Additionally, prospective studies have found that a high intake of refined carbohydrates and a high ratio of saturated fatty acids to polyunsaturated fatty acids is a predictor of NAFLD. The intake of alcohol, which is known to cause liver injury, is a risk factor for NAFLD. However, alcohol consumption is not a major risk factor for the development of NAFLD and NASH.
Inhaled silica exposure is known to cause various health effects in animals, including alterations in lung elasticity and cellular infiltrates. These effects are likely due to the release of inflammatory mediators. To further investigate these effects, we exposed mice to silica and assessed changes in lung mechanics and cellular infiltrates. We observed increased lung compliance and neutrophil infiltration, suggesting a role for neutrophils in the inflammatory response. These findings highlight the importance of understanding the mechanisms underlying silica-induced lung injury and the potential for developing therapeutic strategies to mitigate these effects.

**Methods:** We used C57BL/6J mice and exposed them to silica via intratracheal instillation. Lung function was assessed using a small animal plethysmograph, and lung tissues were collected for histological and immunohistochemical analysis.

**Results:** We observed significant changes in lung mechanics, with increased lung compliance and decreased airway resistance. Additionally, we found increased infiltration of neutrophils and mononuclear cells in the lung tissues.

**Conclusion:** These findings suggest a role for neutrophils in the inflammatory response to silica exposure. Further studies are needed to investigate the mechanisms underlying these effects and to develop potential therapeutic strategies.

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**REFERENCES:**


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**Additional Information:** For more information on silica-induced lung injury and potential therapeutic strategies, please refer to the following resources:

MRP function may vary from protein content. IL-1beta significantly inhibited MRP-mediated CDF efflux in both NHBEC cells and A549 cells. IL-6 showed tendency to suppress MRP activity in both cell types. TNF-α inhibited MRP transport activity only in NHBEC and not in A549 cells. In summary, major proinflammatory mediators IL-1beta, IL-6 and TNF-α can affect MRP expression and function in lung cells. The response of primary human lung cells was different between MRP protein content and functional activity, so these cytoxins may act as inhibitors of MRP function.

1879 EVIDENCE THAT PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR β/δ (PPAR β/δ) IS NOT UPREGULATED BY THE APC/β-CATENIN PATHWAY OR DOWNREGULATED BY NONSTERoidal ANTI-INFLAMMATORY DRUGs (NSAIDS).

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The role of PPARβ/δ in colon carcinogenesis remains controversial, as some studies indicate that PPARβ/δ can either attenuate or potentiate disease. One hypothesis suggests that PPARβ/δ is upregulated by the APC/β-catenin pathway and a related interest in these models suggests that PPARβ/δ is downregulated by NSAIDs. The present study critically examined these ideas using in vivo and in vitro models. Expression of PPARβ/δ was not different in colon or intestinal polyphs from wild-type or APCmin heterozygous mice. Expression of PPARβ/δ was also not significantly different in human colon cancer cell lines with different mutations in APC and/or β-catenin. Differences in the expression of either PPARα or PPARγ were also not observed in any of these colon cancer models. Expression of PPARβ/δ was also examined in vivo and in vitro following exposure to different NSAIDs. No difference in the level of PPARβ/δ was found in colon nuclear extracts from wild-type or APCmin heterozygous mice following treatment with NO-donating aspirin. While NSAIDs inhibited cell growth of the human colon cancer cell lines, expression of PPARβ/δ was similar in RKO and DLD1 human colon cancer cell lines in response to a broad concentration range of either celecoxib, indomethacin, NS398 or APCmin heterozygous mice following treatment with NO-donating aspirin. While NSAIDs inhibited cell growth of the human colon cancer cell lines, expression of PPARβ/δ was similar in RKO and DLD1 human colon cancer cell lines in response to a broad concentration range of either celecoxib, indomethacin, NS398 or nimesulide. Interestingly, expression of PPARα was increased in the human colon cancer cell lines by several NSAIDs at the highest concentration examined. Results from these studies show that PPARβ/δ is not upregulated by the APC/β-catenin pathway. Further, these studies show that PPARβ/δ is not downregulated by NSAIDs suggesting that the mechanisms underlying the chemopreventative effects of NSAIDs do not include downregulation of PPARβ/δ.

1880 DIET-INDUCED OBESITY EXACERBATES INFLAMMATORY/OXIDATIVE STRESS RESPONSES IN MICE EXPOSED TO CIGARETTE SMOKE.

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Smoking and obesity are two of the most important, preventable risk factors for human morbidity and mortality. Our hypothesis is that oxidative stress is the unifying mechanism that leads to worse outcomes of these lifestyle-induced diseases with other environmental toxicants and the genome, resulting in a variety of chronic human diseases. To identify the key biological pathways that define environmental and lifestyle susceptibility factors, we performed parallel exposures of normal C57BL/6 and diet-induced obese (DIO) C57BL/6 mice to cigarette smoke, either main-stream (MS) or sidestream (SS), for 5h/d for 8 days. Blood carboxyhemoglobin levels of ~90% were used to determine a maximum tolerated exposure for MS smoke (250μg/L WTPM) and SS smoke (8μg/L WTPM). Bronchoalveolar (BAL) cytokine indicated a strong neutrophil response with MS smoke, which was 3.5-fold higher in DIO mice but absent with SS smoke exposure. Levels of inflammatory cytokines in BAL fluid also increased after MS smoke exposure in regular and DIO mice (IL-12, MCP-1, MIP-1β) and some displayed significant increases in only DIO mice (IL-1β, TARC, VEGF). Microarray analysis revealed that DIO reprograms the lung's transcriptional response, altering both the number of genes and the specific molecular pathways induced or suppressed by smoke exposure. Despite the lack of a measurable inflammatory response with SS smoke in the BAL fluid, the microarray data demonstrate significant increases in several genes associated with SS smoke exposure, such as Cxcl5 and Cyp1b1, as well as unique gene changes that aren't seen with MS smoke. In addition, we identified more than 500 genes whose expression levels are significantly different between regular and DIO sham control animals, indicating potential susceptibility factors. These results show enhanced and unique stress responses in obese mice, which make them more sensitive to environmental exposure of lung toxicants. Supported

1881 IRON-MEDIATED PROINFLAMMATORY RESPONSE IN LUNG ENDOTHELIAL CELLS.


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Activation of iron turnover in lung has been shown recently to produce oxidative stress followed by increase in leukocyte-endothelial interaction in alveolar microvessels. To clarify the iron-sensitive mechanisms mediating the proinflammatory effects we exposed rat pulmonary endothelial cells (RPAECs) to Fe2+ pulse in the presence of the ionophore perithione and assessed (i) Fe2+ influx, (ii) cell shape profile, (iii) relative electrical resistance of cell monolayer (REEREM), (iv) depletion of cell thiols, (v) redox-dependent nuclear translocation of p53, (vi) expression of ICAM-1 adhesion molecules, and (vii) spatial re-arrangement of the structural proteins, e.g., caveolin-1, F-actin, gelsolin, and pho-caldesmon. The produced effects were monitored with live cell imaging, EVOM endothelial voltommetry, immunofluorescence confocal imaging, immunoblotting techniques, and Thioro-Glo-probing of thiol. Incubation of RPAECs with 30 μM Fe2+ ni-triloacetate over 10 min resulted in depletion of cell thiols followed by nuclear translocation of p53 and p50/p65 kB. Iron-induced alterations were characterized by cell polarization, formation of membrane rafis and microvilli-like membrane projections abundant with ICAM-1 adhesion molecules, pho-caveolin-1, pho-caldesmon, and F-actin proteins. The associated re-arrangements in cytoskeleton were revealed by alternation of membrane fluorescence measured by decrease in the REEREM values. These effects were mitigated in the presence of N,N,N-bis (2-hydroxybenzyl) ethylenediamine-N,N'-dicarboxylic acid, a polynuclear iron chelator, and 2’7367, an inhibitor of Rho kinase. We have suggested that proinflammatory response of RPAECs to Fe2+ pulse proceeds via redox-sensitive mechanisms which mediate re-arrangement of cell structural proteins, i.e., caveolin-1 and F-actin, and ICAM-1 adhesion molecules, and increase in trans-endothelial permeability.

1882 EVALUATION OF SAFETY AND EFFICACY OF TNFR1-SELECTIVE ANTAGONISTIC MUTANT TNF AS A NOVEL ANTI-INFLAMMATORY DRUG.

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Tumor necrosis factor (TNF) is a major inflammatory cytokine that plays a central role in host defense and inflammation via two receptor subtypes, TNFR1 and TNFR2. Elevated serum levels of TNF correlates with the severity and progression of the inflammatory diseases such as rheumatoid arthritis. So far, anti-TNF antibody and soluble TNFRs have been used to treat various inflammatory diseases. However, these therapies can cause serious side effects, such as bacterial and virus infection and lymphoma development because they also inhibited the host defense function mediated by TNFR1. Therefore, we hypothesized that selective inhibition of TNFR1/TNFRI could avoid undesirable side-effect of inhibiting antiviral immunity. With this in mind, we succeeded in generating PEG (polyethylene glycol)-modified TNFR1-selective antagonistic mutant TNF, designated PEG-R1antTNF as a novel anti-TNF drug candidate. In this study, we have evaluated the safety and efficacy of PEG-R1antTNF. In a murine collagen-induced arthritis model, PEG-R1antTNF clearly improved the incidence, and the clinical score of arthritis was less than half of that of the control group due to its long plasma half-life. Next, we compared the effects of PEG-R1antTNF and existing TNF-blocking drug Etañecet on antiviral immunity using a luciferase-expressing recombinant adenovirus vector. In the Etañecet-treated group, luciferase activity was significantly higher than in the control group, suggesting that the drug delayed clearance of the virus. In contrast, PEG-R1antTNF-treated group did not reactivate viral infection. These results indicate that PEG-R1antTNF might lack one of the serious side effects of existing anti-TNF drug because of its TNFR1 selectivity.

1883 GENDER-DIMORPHIC INFLAMMATORY RESPONSE IN HALOHITE HEPATITIS IN MICE.

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The once widely used anesthetic halothane induces a mild hepatotoxicity in 1 in 5 patients and idiosyncratic fulminate liver injury, or halothane hepatitis, in approximately 1 in 30,000. The mechanism of the severe form of halothane hepatic injury
is thought to involve the adducts of halothane’s reactive metabolite, trifluoroacetyl-(TFA) chloride. Female gender is a risk factor for halothane hepatotoxicity in humans. We tested the hypothesis that the sensitivity to halothane hepatotoxicity conveyed by the female gender is due to an enhanced inflammatory response in mice. Female Balb/c mice developed severe liver injury within 12 hrs when treated with 15mmol/kg halothane (ip) as indicated by increased serum alanine aminotransferase (ALT) activity (6,000 U/L). The histopathological lesions were consistent with those seen in postmortem liver specimens from halothane hepatitis patients. This is in contrast to males, which had no significant ALT activity and minimal liver pathologic lesions at this dose. There was equivalent immunohistochemical staining of TFA-added proteins in the livers of male and female halothane-treated mice, suggesting that a difference in bioactivation does not explain the lack of liver injury in males. Several tumor necrosis factor (TNF) concentration increased to 300 pg/ml by 12 hrs in halothane-treated female mice and remained elevated at 24 hrs. In contrast, males had no significant increase in serum TNFα. Hepatic necroinflammatory accumulation occurred sooner and to a greater magnitude in halothane-treated female mice compared to male mice. Halothane-induced hepatic injury was reduced (ALT -2000 U/L) at 12 hrs in females mice treated with CD18 antisera to inhibit transmigration and activation of neutrophils. These results suggest that the greater sensitivity of females to halothane hepatotoxicity may be due to a gender dimorphic innate immune response. (Supported by NIH grant GM075865.)
to LPS treatment, ameliorated the increase in CXCL-1 expression. ALXR-mediated pro-resolution pathways include induction of SOC-2. H441 cells treated with 5(S), 6(R)-lipoxygen A4 methyl demonstrated a 0.8 fold increase in SOCS-2 expression over basal levels. Conclusions: The data suggests that activation of ALXR pathways, by administration of 5(S), 6(R)-lipoxygen A4 methyl ester increases cytokines responses and induces expression of inflammatory resolution molecules in H441 cells.

1888 CORRELATION OF TOTAL ANTIOXIDANT CAPACITY WITH NITRIC OXIDE AND NEOPTERIN IN GASTROINTESTINAL CANCER PATIENTS.

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Reactive oxygen species participate in the progression of carcinogenesis by causing oxidative deterioration of membrane lipids, proteins and DNA, although, no specific biochemical marker has been identified yet. Total antioxidant capacity (TAS) and related substances, albumin, nitric oxide (NOx), neopterin, homocysteine (Hcy) are considered major circulating antioxidants which are exposed to continuous oxidative stress. High amount of NOx production is major cause of tissue damage in various gastrointestinal cancers. Increased plasma Hyc level along with NOx overproduction exerts deleterious effects by peroxynitrite formation. In parallel to the enhanced oxidative stress, immune activation marker, neopterin is raised. Thus, the aim of the present study was to assess the TAS and related substances, NOx, neopterin and Hcy, in gastrointestinal cancer cases. After ethical committee approval, blood samples were collected from 36 noncancerous volunteers (Group 1) and from 70 primary gastrointestinal cancer patients (II and III clinical stages) (Group 2). Serum albumin, "high sensitivity C-reactive protein" (Sensitivity of C-reactive protein), plasma cortisol, IL-6 and IL-10, (ELISA, Amb.R and D system), plasma NOx, serum TAS (Randox, UK), serum neopterin (ELISA, Demeditec) and serum Hcy measurements were done in addition to routine biochemical tests. Significantly decreased serum albumin and increased cortisol might be related to the increased oxidative stress in cancer patients. Although TAS, NOx, Neopterin and Hcy levels were similar in both groups, NOx and neopterin were shown to exert significant, linear control on TAS levels of group two. We concluded that current evidence partially support the hypothesis of oxidative stress in gastrointestinal cancer patients; however, in addition to albumin, NOx and neopterin seem to contribute to antioxidant pool in response to the oxidative stress. (Supported by TUBITAK, SBAG-HD-131, 106S116)

1889 REDUCTION IN THE ABILITY OF MACROPHAGES TO PHAGOCYTOSE APOPTOTIC NEUTROPHILS FOLLOWING EXPOSURE TO CRYSSTALLINE SILICA.

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The role of phagocytic apoptosis of neutrophils by macrophages in the resolution of inflammation in lung diseases, such as in lipopolysaccharide (LPS) induced neutrophilic pneumonia, is currently being investigated. Neutrophils and monocytes/macrophages interact with each other in a complex manner. silica is known as an effective adjuvant for the phagocytosis of apoptotic neutrophils. We have successfully been able to isolate apoptotic, necrotic, and viable control neutrophils. Neutrophils and U937 macrophages were treated for 2 hrs with 50 and 25 μg/ml silica, respectively. Control cells were cultured in medium alone. After each exposure the cells were harvested and assessed flow cytometrically for apoptosis using Annexin V and 7-AAD. To measure phagocytosis of apoptotic neutrophils by macrophages, two-parameter flow cytometry phagocytosis assay was used in which a Tracker Green labeled apoptotic, necrotic, and viable control neutrophils were incubated with U937 cells labeled with Cell Tracker Orange. Macrophages were treated with 50 μg/ml crystalline silica and the thereafter co-incubated with apoptotic neutrophils and the extent of phagocytosis was quantitated with flowcymeter using the FACScaliber and Cell Quest Software. The levels of nitric oxide and different cytokines during phagocytosis were also determined. Silica induced apoptosis in neutrophils and U937 macrophages. Control U937 macrophages were able to phagocytose at least 50% of apoptotic neutrophils. On the other hand, treatment of U937 macrophages with silica resulted in a significant (70%) decrease in their ability to phagocytose the apoptotic neutrophils. A concomitant increase in the levels of nitric oxide (NOx) and cytokines TNF-alpha produced. Therefore, it may be suggested that silica treatment of U937 macrophages may decrease the ability of these cells to remove apoptotic neutrophils as well as increase the levels of inflammatory molecules and thus contributing to the persistent inflammation seen following exposure to crystalline silica.

1890 ENHANCING ADCC IN HUMANS VIA NK CELLS.


Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) is one of the mechanisms through which antibodies, as part of the humoral immune response, can act to limit and contain infection. Classical ADCC is mediated by natural killer (NK) cells; monocytes and eosinophils. Natural Killer cells express membrane receptors for the Fc region of antibody molecules. The FcεRII receptor expressed by NK cells is responsible for antibody mediated recognition and killing of tumor and viral infected cells. This study examined the interactions of human IgG1 monoclonal antibody with enhanced Fc receptors on NK cells and the subsequent activation of ADCC. We utilized a monoclonal antibody to stimulate NK cells in normal human PBMCs to facilitate the examination of cytolytic and antibody dependent cellular toxicity. Peripheral blood mononuclear cells from several healthy donors were freshly isolated by density gradient centrifugation and cell suspensions were stimulated with a human IgG1 monoclonal antibody for 24 hours at 37°C. Post incubation cells were characterized by multi-parametric flow cytometry. The population of interest was CD3-CD56+ NK cells. This population was further examined for cell surface expression of lysoasomal associated membrane protein-1 (CD107a) and lymphocyte activation marker (CD69). Treatment of PBMCs with IgG1 mAb resulted in increased cell surface expression in CD69 and CD107a. A single dose treatment of 80μg/ml of IgG1 mAb increased the level of expression of CD56/CD69 from a baseline of 8.81% to 66.22%. In addition to NK cell activation we observed upregulation of CD107a on the surface of NK cells following stimulation. Observed expression from vehicle was 27.37% to 74.80%. The data suggests that in the presence of mAbs directed to NK cell activation receptors can potent activators of NK cell cytotoxicity. The engagement of Fc receptors of effector cells in initiating ADCC, may play a large role in anti-cancer therapies. Utilizing these antibodies to trigger early steps of innate immune response against tumor cells may facilitate a method of inducing target cell ADCC for improved treatment of cancer.

1891 CX3CR1 CONtributes TO OZONE-INDUCED PULMONARY INFLAMmATION IN THE MOUSE.

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Purpose: Previous studies have shown that expression of chemokines and their receptors is elevated in human inflammatory processes. Enhanced expression of the chemokine receptor CX3CR1 results in enhanced leukocyte adhesion. CX3CR1 is known to play a major role in chronic pulmonary inflammation; however very little is known concerning its role in response to sub-acute pulmonary injury. Interleukin-17 (IL-17) is linked to neutrophilic mobilization in chronic pulmonary inflammation. Previous research suggests that IL-17 is not expressed in CX3CR1-/- mice, suggesting that IL-17 and CX3CR1 work together in the inflammatory response. The purpose of this research was to determine the role of CX3CR1 in ozone-induced pulmonary inflammation, and the role IL-17 plays in this response. Methods: CX3CR1-/-, CX3CR1+/-, Il17r+/+ and Il17r-/- mice were exposed to air or 0.3 ppm ozone (O3) for 6, 24, 48 or 72 hours. Bronchoalveolar lavage fluid (BAL) was collected after exposure and analyzed for total protein and numbers of inflammatory cells. IL-17 expression in lung homogenate was analyzed by ELISA. Results: Relative to respective air controls, 48 and 72 hour exposure to O3 caused a significant increase in BALF total protein and numbers of inflammatory cells in both wild type and CX3CR1-/- mice. However, significantly greater levels of IL-17 were detected in CX3CR1-/- mice compared to CX3CR1+/- mice. Slight elevation of IL-17 was detected in CX3CR1+/- mice after exposure to O3, but IL-17 was not detected in CX3CR1-/- mice after exposure. Significantly greater numbers of neutrophils were found in Il17r+/+ mice compared to Il17r-/- mice after exposure to O3 for 48 hours. Conclusions: Ozone-induced inflammatory cell infiltration, change in lung permeability, and IL-17 production were significantly diminished in CX3CR1-/- mice relative to CX3CR1+/- mice. This suggests that CX3CR1 contributes to the pulmonary inflammatory response to O3 exposure, and it appears that IL-17 does play a role in this inflammatory response. NIH Training Grant T32-E507126.

1892 ROLE OF SURFACTANT PROTEIN-D IN OZONE-INDUCED INFLAMmATION, INJURY AND ALTERED LUNG FUNCTIONING.

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Ozone is a ubiquitous urban air pollutant known to induce lung damage. This is associated with macrophage accumulation in the tissue and the release of reactive nitrogen intermediates (RNIls) which we have shown contribute to tissue injury. In
these studies we analyzed mechanisms by which RNIs induce lung injury. Surfactant protein D (SPD), a pulmonary collectin, has both pro- and anti-inflammatory activities. SPD has been shown to be involved in acute lung injury and is a target for RNI. Acute exposure of mice to ozone resulted in RNI-mediated post-translational modification of SPD. This was associated with increased inflammatory activity, suggesting that nitrosylation of SPD may be one mechanism whereby RNIs induce pulmonary inflammation. To further investigate this, we analyzed the effects of ozone on SPD/- mice. Ozone inhalation (0.8 ppm, 3 hr) resulted in increased BAL protein levels and macrophage content, markers of lung inflammation and injury in both wild type and SPD/- mice 72 hr post exposure. These effects were more pronounced in SPD/- mice. Greater levels of NOx were also detected in BAL from SPD/- mice. Using a C57/6F3 FvXm, we analyzed the effects of loss of SPD on pulmonary function. Challenging mice with methacholine resulted in increased total and central airway resistance. Whereas total resistance was reduced in SPD/- mice relative to WT mice, central airway resistance increased. In both groups, total and central airway resistance were reduced after ozone exposure. The effects of ozone on central airway resistance were greater in SPD/- mice when compared to WT mice. Methacholine challenge also resulted in a dose dependent decrease in total, as well as static lung compliance in both WT and SPD/- mice, a response that was attenuated after ozone inhalation. These results suggest that SPD plays distinct roles in pulmonary inflammation and aberrations in lung function following ozone inhalation. Supported by NIH ES004738, ES050522, ES007148, AR055073, and HL074115.

1893 MACROPHAGE ACTIVATION BY FACTORS RELEASED FROM NECROTIC HEPATOCYTES IN AN ACATEMINOPHEN MODEL OF HEPATOTOXICITY. POTENTIAL ROLE OF HMGB1.
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Acateminophen (APAP) is a widely used analgesic known to cause hepatic necrosis at toxic doses. Evidence suggests that activated macrophages and inflammatory mediators play a key role in the pathogenic process. In the present studies we analyzed mechanisms mediating the release of inflammatory mediators by macrophages. HMGB1 is a DNA-binding protein that is passively released from necrotic cells and is known to stimulate macrophage production of proinflammatory mediators. Treatment of mice with APAP (300 mg/kg, i.p.) resulted in a time-dependent decrease of HMGB1 expression in hepatocytes, which coincided with the development of hepatic necrosis. We speculated that hepatocyte-derived HMGB1 plays an important role in macrophage activation during APAP hepatotoxicity. To test this, primary cultures of mouse hepatocytes were treated with 5mM APAP or control. Conditioned medium was collected 24 hr later and its effects on macrophage production of inflammatory mediators analyzed. Treatment of macrophages with conditioned medium from APAP-treated hepatocytes resulted in increased mRNA expression of the proinflammatory cytokines TNF-alpha, IL-1, and IL-6, as well as COX-2, a key enzyme in prostaglandin biosynthesis. Conditioned medium from APAP-treated hepatocytes also induced oxidative stress in macrophages, as indicated by increased mRNA expression of heme oxygenase-1, an enzyme involved in antioxidant defense, and our previous studies demonstrating increased production of reactive oxygen species. Uptregulation of proinflammatory and antioxidant genes in macrophages exposed to hepatocyte-conditioned medium correlated with reduced expression of HMGB1 protein. These data, together with reports that HMGB1 is actively secreted by macrophages subjected to oxidative stress, suggest that HMGB1 plays a role in macrophage activation during APAP-induced hepatotoxicity. (Supported by NIH GM034310, ES005022)

1894 IN VITRO PHOTOCHEMICAL FORMATION OF KOJIC ACID-DNA ADDUCTS.
J. Duan, G. M. Williams and A. M. Jeffery. Department of Pathology, New York Medical College, Valhalla, NY.
Kojic Acid (KA) is a natural product which inhibits melanin production. Topical application of KA alone, or in combination with other compounds, is effective in reducing pigmentation in melanoma patients. Based largely on a single study showing thyroid neoplasia KA was classified by the International Agency for Research on Cancer (IARC) as presenting limited evidence of carcinogenicity (group 3). More recently KA has been shown to have carcinogenic properties in rat liver, but not mouse skin. KA has produced positive and negative results in both in vivo and in vitro genotoxicity tests and was considered to be genotoxic in vivo by IARC. Only one report addressed potential phototoxicity. KA has long been known to be photochemically unstable causing in vivo breakage of calf thymus DNA.

1895 ROLE OF ESTROGEN RECEPTOR ALPHA IN DECREASED HEPATIC NUCLEOTIDE EXCISION REPAIR AFTER 17α-ETHINYLESTRADIOL EXPOSURE.
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Estrogen is a known human carcinogen and is implicated in the etiology of human hepatocellular carcinoma. Zebrafish exposed to the strong estrogen receptor agonist 17α-ethinylestradiol (EE2) displayed decreased mRNA expression of multiple nucleotide excision repair (NER) genes in vitro and in vivo. Additionally, liver derived zebrafish cell lines treated with estrogens were hindered in their ability to repair bulky DNA adducts. These data suggest that estrogen can act as a co-carcinogen with bulky adduct forming mutagens by hindering a cells ability to deal with DNA damage. The role of nuclear estrogen receptors in altered NER processes remains unclear. In vitro, co-exposure to EE2 and a complete estrogen receptor antagonist (ICI 182 780) also resulted in reduced NER capacity, whereas ICI 182 780 alone did not affect the ability of ZFL cells to repair UV damage. These results indicate that estrogen exposure can decrease cellular NER capacity and that this effect can occur in the presence of the complete estrogen receptor agonist, ICI 182 780. This suggests that EE2 can affect NER processes through mechanisms other than nuclear estrogen receptor activation.

1896 FAILED BASE EXCISION REPAIR LEADS TO NECROTIC CELL DEATH THROUGH NAD+ DEPLETION.
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In eukaryotic cells, DNA polymerase β (Pβ) is an essential component of the base excision repair (BER) machinery responsible for repairing DNA damage occurring from both endogenous and environmental sources. Failure to repair base damage may lead to cancer or tissue degeneration due to genomic instability or cell death; respectively. A deficiency in Pβ renders cells hypersensitive to DNA damage mediated by alkylolation, oxidation and radiation. Cellular hypersensitivity in Pβ deficient cells has been attributed to abrogated repair of the 5-deoxy ribose phosphate (5dRP) repair intermediate. We have recently propose that PARP1 acts as a sensor of abrogated repair, leading to rapid synthesis of poly(ADP)ribose (PAR) and subsequent necrotic cell death. We hypothesize that the cellular mechanism behind BER mediated necrotic cell death is NAD+ depletion and cellular energy failure, exclusive of a signaling cascade initiated by PAR. NAD+ is a critical substrate for the poly(ADP)riboseylation reaction; therefore activation of PARP1 could lead to depletion of NAD+. A nontoxic 24 hour pretreatment of BER deficient cells with the NAD+ biosynthesis inhibitor FK-866 before methyl methanesulfonate (MMS) exposure leads to reduction of PAR synthesis to levels undetectable by immunoblot; however it also leads to a 10 fold increase in cell death. Quantitative analysis of NAD+ content shows that reduction of NAD+ to 25% of control with FK-866 leads to a more rapid and increased reduction of NAD+ after exposure to MMS. Furthermore, immunofluorescence for AIF release from the mitochondria, reported to occur when PARP is effectively destroyed, is not observed. Taken together this data suggests that necrotic cell death due to incomplete BER is due primarily from NAD+ depletion and subsequent ATP loss, not from a PAR signaling event. It also highlights the critical role NAD+ levels and overall metabolic status has on the cellular response to base damage.
increased formation of glycidamide (GA)-HGB and GA-DNA adducts in diet-induced obese male C57Bl/6J mice.


Global projections of obesity indicate that > 1 billion adults (≥ age 15) are overweight, (BMI > 25 kg/m2) and at least 400 million are obese (BMI > 30 kg/m2). Chronic diseases such as type 2 diabetes have also been associated with elevated BMI. In general, the etiology of most human diseases can be linked to environmental pollutants and lifestyle. We hypothesized that obese individuals may exhibit varied metabolism and responses to environmental agents. Recently, we found acrylamide (AA) exhibited an exaggerated reproductive toxicity and germ cell mutagenicity in diet-induced obese vs. control mice. To assess AA mechanism of action, current studies compared AA metabolism in obese vs. control. Male C57Bl/6J mice were maintained on either a control or high fat (60% kcal fat) diet from 5 to 30 weeks of age. Mice fed the high fat diet for 30 weeks showed significantly higher body weight and % body fat. AA was administered (po) to groups of obese and control mice at 12.5, 25, or 50 mg/mg/kg. Six hours after dosing, mice were euthanized and blood was collected to detect AA and GA in the plasma and HGB adducts in RBCs. Liver, lung, and testes were removed for DNA adducts measurement. AA and GA HGB and DNA adducts were measured using LC-ESI/MS/MS analysis. In control and obese mice, AA and GA adducts increased in a dose-dependent manner. AA and GA adducts in liver, lung and testes were considerably higher in obese vs. controls. In conclusion, increased AA metabolism to the epoxide intermediate, GA and increased GA-DNA adducts may explain the elevated sensitivity of obese mice to the genotoxic effects of AA and support the premise that obese individuals are more susceptible to environmental exposures.


The recent discovery of acrylamide (AA), a potent carcinogen, in the frying or baking of a variety of foods raises human health concerns, in particular, for children, because AA is relatively highly contained in snacks, cereals, and baby foods. AA is known to be metabolized by CYP2E1 to glycidamide (GA) which is responsible for AA-inducing genotoxicity and carcinogenicity. The activity of CYP2E1 varies during postnatal development implying that the genotoxic and carcinogenic risk of AA may be different between adults and children. To elucidate in vivo genotoxicity of AA and its child-adult differences, we treated adult or neonatal male rats (gpt-delta strain) with or without metabolic activation for 5 weeks. The activity of CYP2E1 varied during postnatal development. In both above mentioned assays and the outcome was compared to the OECD 471 comet assay. The Ames micro-assay method uses 2 strains (TA98 and TA100) which are pre-incubated with the test compound before reversion induction. Plate and subsequent colony counting is performed like described in the OECD 471 assay. On the other hand, the Ames fluctuation method uses 2 strains (TA98 and TA100) which are pre-incubated with the test compound before reversion induction. Flow cytometric enumeration of GPI anchor deficient RBCs and/or RETs represents an effective in vivo mutation assay that is applicable across species of toxicological interest.

1999 Development of a cross-species mutation assay based on the pig-a gene.

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This laboratory has described a Pig-a gene mutation assay based on blood from Sprague Dawley rats (Bryce et al., Environ. Mol. Mutagen. 49 (2008) 256-264). The underlying premise is that lack of glycosylphosphatidylinositol (GPI) anchor proficiency and the presence of red blood cells (RBCs) as a reliable biomarker of pigmentary marker of Pig-a mutation. Here, we extend this work to include CD15 pig-a gene. After RBC enrichment, anti-CD24-PE was used to differentiate wildtype versus GPI anchor deficient cells (presumptive mutants). Cells were then incubated with SYTO 13 to discriminate mature erythrocytes from reticulocytes (RETs). Flow cytometry was used to score mutants in two RBC populations: RBCs irrespective of age, and RETs. Mutagen treatments (N-ethyl N-nitrosourea, 40 mg/kg/day, 3 days; or 7,12-dimethyl-1,2-benz[a]anthracene, 75 mg/kg/day, 3 days) increased the frequency of GPI anchor-deficient RBCs and RETs. Whereas the frequencies of mutants were significantly elevated 1 week after the last dose, the RBC population was unchanged until the second week. Thereafter, both populations exhibited persistent elevations for the duration of the experiment (mean frequency at termination = 510 ± 6 and 523 ± 6 for RBC and RET populations, respectively). These data provide evidence that Pig-a is a reliable biomarker of a positive and negative cell survival advantage to affected erythroid progenitors, although they suggest that affected RBCs have a reduced lifespan in circulation. Collectively, accumulated data support the hypothesis that flow cytometric enumeration of GPI deficient and normal RBCs and RETs represents an effective in vivo mutation assay that is applicable across species of toxicological interest.
Following 48 hours of incubation (37°C), revertant colonies were counted and the activities (revertants/cigarette) were determined. The resultant optimum conditions (GVP and WS) were 120 minute preincubation, 5.5X1010 CFU/mL, and 0.2 cig- arettes/mL PBS. Mutagenic activity was detected in GVP treated TA100 (S9-) only while WS elicited activity in both strains (S9-). Significant decrease in revertants/cigarette were seen with increasing cigarettes smoked per mL of PBS, indicating inefficient capture and/or saturation of the PBS with smoke components.

### 1902 DISPOSITION KINETICS OF BENZO(A)PYRENNE (BaP) METABOLITES AND BaP-DNA ADDUCTS IN OVARY OF F-344 RATS ORALLY EXPOSED TO BENZO(A)PYRENNE.

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Exposure to environmental toxicants has been implicated as one of the causative factors of infertility in mammals. Since the bioavailability of any xenobiotic compound is the prime driving force for toxicity, the objective of this study was to determine the amount of ingested benzo(a)pyrene (BaP), an environmental toxicant that reaches the reproductive tissues. Towards this end, the concentrations of BaP reactive metabolites and BaP-DNA adducts were measured throughout the course of BaP’s residence in the body. Eight-week-old female Fisher-344 rats were administered with 5 mg of BaP/kg body weight through oral gavage. Following, 1, 7, 14 and 21 days post-BaP exposure, BaP parent compound and major metabolites from plasma, ovary and liver tissues were extracted using water, methanol, and chloroform. The extracts were analyzed by a reverse phase HPLC equipped with a fluorescence detector. DNA was isolated and analyzed for BaP-induced DNA adducts by the postlabeling method using a four-directional thin-layer chromatography system. The BaP total metabolite concentrations in plasma, ovary and liver showed a gradual decrease from day 1 to day 21 post-BaP administration. The BaP-DNA adducts concentrations in ovary and liver tissues from the treatment group showed a trend similar to that observed for the metabolites. Ovary showed greater concentrations of DNA adducts compared to liver. However, with increase in post-cessation of exposure time, the adduct levels in liver tissue started declining rapidly, from day 1 to day 21. For ovary, the adduct levels showed an insignificant decline from day 1 to day 7 and showed a gradual decline thereafter. A concordance between BaP reactive metabolite concentrations and BaP-DNA adduct concentrations indicate that the bioavailability of reactive metabolites determine the binding with DNA and consequently the formation and persistence of adducts (research reported here is supported by the NIH grants G12 RR03032, S11ES014156 and RO3CA130112-01).

### 1903 REMOVAL OF BULKY MITOCHONDRIAL DNA ADDUCTS FOLLOWING ULTRAVIOLET RADIATION EXPOSURE INVOLVES MITOCHONDRIAL FUSION AND AUTOPHagy.

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Mitochondrial DNA (mtDNA) integrity is critical for human health; however, it is unclear how bulky mtDNA adducts formed after exposure to environmentally important genotoxins such as ultraviolet radiation and PAHs are handled. mtDNA may be particularly susceptible to these genotoxins due to the absence of nucleotide excision repair, the primary repair mechanism for bulky DNA adducts in nuclear DNA. We investigated the removal of bulky mtDNA adducts via mitochondrial fusion and autophagy in Caenorhabditis elegans. Larval fusion and autophagy mutant C. elegans were exposed to serial UVC doses over 48 hours. This exposure protocol allows for accumulation of mtDNA damage without lasting damage to nuclear DNA and results in measurable larval growth arrest. Strains carrying mutations in the autophagy gene atg-18 and fusion gene fzo-1 exhibited exacerbated larval growth arrest with little to no growth recovery after 72 hours. We concluded that the proteins responsible for DNA repair may drive UV-induced skin cancers by allowing subsequent mutations in other critical genes to be more readily acquired. One manifestation of differences in DNA repair capacities is altered UV-induced cytotoxicity. Ultraviolet-C (UVC) radiation-induced cytotoxicity was examined in different melanoma cell lines and in normal human fibroblast cells (NHHF1-hTERT). These cell lines displayed differential sensitivity to UVC-induced cytotoxicity, with A375 being the most sensitive (LD50=4.9 J/m2) and closest to NHHF1 (LD50=4.6 J/m2). WM266 and VM59 cell lines were less sensitive (LD50=7.4 and 7.8 J/m2 respectively), and SK-MEL-28 cells the most resistant (LD50=18.1 J/m2). Because melanocytic cells may differ in their production of UV absorbing pigments, cell lines used in this study were also examined for their relative density of cyclouate pyrimidine dimers (CPD) in DNA upon exposure to UVC (0, 2.5, 5, or 10 J/m2). The molecular dosimetry indicated that the CPD densities in these cell lines were not significantly different from one another (ANOVA p>0.2). Equivalent molecular dosimetry of CPD following UVC irradiation indicates that the differential cytotoxicity seen in these cell lines is due to factors other than the burden of CPD. Such factors could include alterations in downstream responses to UV, such as DNA repair, cell cycle checkpoints, or induction of apoptosis. Several of these responses are currently under investigation. Supported by NIH grants T32-ES07126 and ROI ES015856.

### 1904 IN VIVO MUTAGENESIS INDUCED BY DIESEL EXHAUST IN THE TESTIS OF GPT DELTA TRANSGENIC MICE.

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Diesel exhaust is a major airborne pollutant of urban areas. Previously, we have demonstrated that typical mutagens such as benzo[a]pyrene and 1,6-dinitropyrene, as well as inhalation of diesel exhaust, caused mutations in the lungs using transgenic rodents for analyzing in vivo mutagenesis. In this study, to estimate the systemic effect of diesel exhaust inhalation, mutations in extrapulmonary organs, such as testis and liver, were determined. Gpt delta transgenic mice, carrying the guanine phosphoribosyltransferase (gpt) gene as a transgene for the detection of mutations in genomic DNA, were exposed to 3 mg m-3 of diesel exhaust (as suspended particulate matter) via inhalation for 12 or 24 weeks. Compared to the control mice, this resulted in a 2.0 fold increase in mutation frequency in the testis of mice exposed for 24-week exposed mice (Inhalation group, 1.17 x 10-5; Control group 0.58 x 10-5), but not in the testis of the 12-week exposed mice (0.61 x 10-5). The mutation frequency became 2.6-fold higher than the control group in the lung, but was not elevated in the liver of the 24-week exposed mice. In testis, major mutations in the gpt gene were G:C to TA transversions, 1 base deletions, and G:C to AT transversions, while the only major mutations in the lung were G:C to AT transitions. Mutations at nucleotide 402, 406, 409 and 416-418 in the gpt gene in tests should be be characterized of DE inhalation. Our results suggest that inhalation of diesel exhaust is genotoxic for the testis as well as for respiratory organs.

### 1905 MOLECULAR DOSIMETRY OF UVC-INDUCED CYCLOUBA PYRIMIDINE DIMERS IN DNA OF MELANOMA CELL LINES.

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Ultraviolet radiation is an established environmental carcinogen that induces DNA lesions in melanocytes, potentially promoting cancer development. It is hypothesized that reduced expression or mutations in any of the proteins responsible for DNA repair may drive UV-induced skin cancers by allowing subsequent mutations in other critical genes to be more readily acquired. One manifestation of differences in DNA repair capacities is altered UV-induced cytotoxicity. Ultraviolet-C (UVC) radiation-induced cytotoxicity was examined in different melanoma cell lines and in normal human fibroblast cells (NHHF1-hTERT). These cell lines displayed differential sensitivity to UVC-induced cytotoxicity, with A375 being the most sensitive (LD50=4.9 J/m2) and closest to NHHF1 (LD50=4.6 J/m2). WM266 and VM59 cell lines were less sensitive (LD50=7.4 and 7.8 J/m2 respectively), and SK-MEL-28 cells the most resistant (LD50=18.1 J/m2). Because melanocytic cells may differ in their production of UV absorbing pigments, cell lines used in this study were also examined for their relative density of cyclouate pyrimidine dimers (CPD) in DNA upon exposure to UVC (0, 2.5, 5, or 10 J/m2). The molecular dosimetry indicated that the CPD densities in these cell lines were not significantly different from one another (ANOVA p>0.2). Equivalent molecular dosimetry of CPD following UVC irradiation indicates that the differential cytotoxicity seen in these cell lines is due to factors other than the burden of CPD. Such factors could include alterations in downstream responses to UV, such as DNA repair, cell cycle checkpoints, or induction of apoptosis. Several of these responses are currently under investigation. Supported by NIEHS T32-ES07126 and ROI ES015856.

### 1906 1, N6-ETHENO-2’-DEOXYADENOSINE (DA) IN WEANLING AND ADULT RATS EXPOSED TO 13C2-VINYL CHLORIDE BY INHALATION L. Gao, P.B. Upton, G. Boysen, and J.A. Swenberg. ENVIRONMENTAL SCIENCES AND ENGINEERING, UNC-CHAPEL HILL, CHAPEL HILL, NC.

L. Gao, P.B. Upton, G. Boysen and J.A. Swenberg. environmental sciences and engineering, UNC chapel hill, Chapel Hill, NC.

Vinyl chloride (VC) is classified as a human carcinogen by IARC. Through metabolic activation by CYP2E1, chloroethene oxide is formed which reacts with DNA. 1dA is one of the DNA adducts that formed following VC exposure but also...
formed endogenously by lipid peroxidation. In order to accurately determine the formation and repair of dεA, adult and weaning Sprague-Dawley rats were exposed to 1100 ppm 13C2-VC for 5d (6h/day). The weaning animals were sacrificed 2h post exposure. The adults were sacrificed 2h, 2, 4, and 8 weeks post exposure. A sensitive nanoUPLC-MS/MS assay was developed to detect both endogenous 12C2-dεA and exogenous 13C2-dεA in DNA. The results show that 13C2-dεA is detected in the exposed adult group and the exposed weaning group at the end of exposure, but that it was below detection levels in the exposed adult recovery groups. dεA is rapidly repaired in rat tissues and that the steady-state endogenous amount of dεA allows the clear distinction between endogenous and exogenous dεA. This permits accurate determination of dεA repair pathways. Our data demonstrate that dεA is readily repaired in rat tissues and that the steady-state endogenous amount of dεA is the result of daily formation and repair.

1997 ASSESSMENT OF ULTRAVIOLET LIGHT- AND CHEMICAL-INDUCED UDS IN VARIOUS CELL LINES USING FLOW CYTOMETRY.


UDS is a reliable assay to measure DNA damage/repair induced by genotoxins and ultraviolet (UV) radiation. Standard UDS assays detect radiolabeled nucleotides incorporated into repaired DNA. We have developed a Flow cytometry-based UDS assay (FLUDS) that measures biotin-dUTP incorporation into repaired DNA. DNA damaging agents, including direct, metabolically activated genotoxins and ultraviolet light (UVB and UVC) were exposed to cells in culture. Aphidicolin (Aph), a DNA replication inhibitor was included in cultures exposed to DNA damaging agents to temporarily inhibit repair and allow DNA damage to accumulate prior to the UDS step. The addition of Aph helps enhance the signal-to-noise (S/N) ratio. Using a human skin keratinoctye cell line, FLUDS exhibited 88% accuracy compared to the standard alkaline UDS assay. FLUDS was significantly higher in hepatic DNA of adult rats with or without VC exposure, with 1.08±0.17/108 adducts in exposed animals and 1.12±0.25/108 adducts in exposed animals. The use of stable isotope labeled VC allows the clear distinction between endogenous and exogenous dεA. This permits accurate determination of dεA repair pathways. Our data demonstrate that dεA is readily repaired in rat tissues and that the steady-state endogenous amount of dεA is the result of daily formation and repair.

1998 4-ETHYLCATECHOL CAUSES DNA DAMAGE RESPONSE AT LEVELS IN WINE AND CIGARETTE SMOKE.

M. R. Knight, M. D. Aitken and J. Nakamura. Environmental Science and Engineering, University of North Carolina-Chapel Hill, Chapel Hill, NC.

Cigarette smoke and Brettanomyces fermented wine both contain 4-ethylcatechol (4EC). Smoking one cigarette can introduce 4EC to the lung at 0.7 μmol 4EC/kg lung. Lower quality wines can also contain 1.2 μmol 4EC/L. In addition, 4EC is a product of ethylbenzene (EB) metabolism, which is classified as a possible human carcinogen and identified in almost half of the National Priorities List sites. Little, however, is known regarding DNA damage caused by 4EC in cells. DNA damaging mechanisms can include topoisomerase II inhibition, oxidative stress, and depurination or bulky adducts. We characterized the genotoxic profile of 4EC to elucidate types of DNA damage induced. We utilized wild type (WT) DT40 vertebrate cell line and ~40 isogenic mutants deficient in various DNA damage response pathways. After exposure to 4EC, differences in viability between WT and each mutant cell line indicate positive DNA damage response. Our results reveal two DNA repair pathways important for counteracting DNA damage induced by 4EC. Hypersensitivity of RAD54, Ku70, and LIGIV deficient cells suggests that double strand breaks (DSB) are possibly the most serious DNA damage. These results indicate both homologous recombination and non-homologous end-joining (NHEJ) pathways play essential roles in cell survival. Also two translesion synthesis (TLS) polymerases, POLκ and REV1, appear crucial. Sensitivity of NHEJ deficient cell lines suggests topoisomerase II can be inhibited by 4EC. DNA damage responses to 4EC show similar response to oxidative stress and DNA damage by 4EC may be explained oxidative stress. In contrast, POLκ, XPA, and XPG mutants are resistant to 4EC. Depurination or bulky adducts may cause negligible DNA damage. We have shown 4EC to diminish cell viability in concentrations as low as 1.7 μM. Our DNA damage assays suggest that significant implications of existing in cigarette smoke and low quality of wine on human health and implies that environmental EB may further poteniate these risks. (P30-ES10126/F24-ES05948)

1999 SUBOPTIMAL DNA REPAIR CAPACITY PREDISPOSES POLYCYCLIC AROMATIC HYDROCARBON EXPOSED WORKERS TO ACCUMULATE MORE CHROMOSOME DAMAGES IN PERIPHERAL LYMPHOCYTES.


DNA repair is an essential mechanism for cells to maintain the genomic integrity under the endogenous or exogenous assault. Reduced DNA repair capacity (DRC) is associated with increased risk for several environment-related cancers. The micronucleus in peripheral lymphocytes has been validated as a biomarker of chromosomal damage for cancer risk in human populations. We hypothesized that suboptimal DRC is associated with the increase of chromosomal damages among 94 polycyclic aromatic hydrocarbon (PAH) exposed workers and 64 controls. DRC was evaluated in isolated lymphocytes by comet assay. Chromosome damage in peripheral lymphocytes was detected by cytogenetics-block micronucleus assay. Four functional single nucleotides polymorphism in XRCC1 gene were genotyped by PCR-RFLP method. PAH exposed workers have significantly increased urinary 1-hydroxypyrene (9.0, 6.8-11.7 vs. 1.5, 1.3-1.7 μg/L; P<0.01) and micromolecular frequency (7.4±4.5% vs. 3.0±3.0%; P<0.01) and decreased DRC (55.9±16.4% vs. 63.6±18.5%; P<0.01) compared to controls. Significant correlations between DRC and micronuclear frequency were found in PAH exposed workers (r=-0.32, P<0.01, n=94) and all study subjects (r=-0.32, P<0.01, n=158), but not in controls (r=-0.21, P=0.11, n=64). Variants of Arg599Gln polymorphism was associated with a decreased DRC in both PAH exposed workers (51.6±16.1% vs. 60.6±15.7%; P=0.01) and controls (59.1±18.5% vs. 68.4±17.5%; P=0.04). The complicated interrelationship of these multiple biomarkers was also identified by the path analysis. These exciting findings will be of great importance for us to understand the mechanism of the lung carcinogenesis and facilitate developing a biomarker-based risk assessment model which includes both behavioral risk factors and multiple biomarkers for this fetal disease in this occupational population.

1910 EFFECTS OF CRYOPRESERVATION ON ALKALINE AND FPG-MODIFIED COMET ASSAY IN WHOLE HUMAN BLOOD AND ISOLATED LYMPHOCYTES.

X. Pu, L. M. Kamoundulis and L. E. Klaunig. Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN.

The Comet assay is a simple and sensitive technique for the detection of DNA damage in eukaryotic cells. Incorporation of lesion specific, oxidative DNA damage repair enzymes (i.e., Fpg, OGG1, EndoIII) in the standard alkaline Comet assay procedure provides a simple and sensitive method for the measurement of oxidative DNA damage. The Comet assay using human white blood cells (WBC) or isolated lymphocytes has proven useful in monitoring DNA damage from environmental agents in humans. A restriction in the use of this assay has been the need to perform the analysis immediately after sampling. In this study, we examined the effects of sample freezing and storage conditions on standard and Fpg-modified modified Comet assay using whole blood or isolated lymphocytes. Whole blood was stored in RPMI1640 media containing 10% FBS, 10% DMSO, and 1 mM deferoxamine at a sample to media ratio of 1:50. Samples were stored at -20°C and -80 °C for 1, 7, 14 and 28 days. Isolated lymphocytes from the same subjects were also stored under the same conditions. Direct DNA strand breakage and oxidative DNA damage in WBC and lymphocytes were analyzed using standard and Fpg-modified alkaline Comet assay and compared with freshly analyzed samples. No significant changes in either direct DNA strand breakage or oxidative DNA damage was seen in WBC and lymphocytes stored at -20°C for 1 and 7 days compared to fresh samples. However, significant increases in both direct and oxidative DNA damage were seen in samples stored at -20°C for 14 and 28 days. No changes in direct and oxidative DNA damage were observed in WBC and lymphocytes stored at -80°C for up to
Biomarker measurement allows better understanding of the factors that influence the health outcomes from air pollutant exposures. In this session, new biomarker strategies will be highlighted to show the use of biomarkers to study health effects derived from air pollution and to provide updates on the utility of new biomarker techniques including ‘omics-type of analyses. Our panel of experts will focus on improved use of biomarkers of exposure, effects, and susceptibility are included in this session. Session highlights will include the use of select urinary PAHs to reflect exposure to petroleum-derived emissions that show similar body burdens across occupational and controlled exposure studies followed by use of susceptibility factors such as obesity and employment status to demonstrate increased biological responses (e.g., heart rate variability) and health effects (e.g., mortality). Finally, our experts will cover the use of genomics to improve the understanding of the likelihood of the development of asthma as well as proteomics to identify unique exposure biomarkers and potentially the cell types producing the markers.

USE OF CARDIAC MARKERS AND SUSCEPTIBILITY FACTORS FOR EXAMINATION OF PARTICULATE MATTER INDUCED TOXICITY IN HUMANS.


We investigated whether PM2.5-mediated autonomic modulation depends on individual coronary risk profiles, using as outcomes five-minute average heart rate (HR) and heart rate variability measured from 24-hour ambulatory electrocardiograms, and personal PM2.5 exposures in a prospective study of 10 male boilermakers (aged 34.3 ± 8.1 years). We used the Framingham score to classify individuals into low (score = 1-3) and high (score ≥ 4) risk categories. Mixed-effect models revealed that each 1-mg/m3 increase in the preceding 4-hour moving average PM2.5 was associated with HR increase (5.3 beats/min) and HRV reduction (11.7%, confidence interval [CI] -6.2 to 17.1% for SDNN). Greater responses (2- to 4-fold) were observed in high-risk than in low-risk subjects. These findings suggest that adverse autonomic responses to particulate matter are aggravated in workers with higher coronary risk profiles. We followed this study with assessments of obesity as a potential susceptibility state to PM related autonomic (HRV) changes in 18 monitored boilermakers (39.5 ± 9.1 years of age). Subjects with body mass index >30 kg/m2 were classified as obese. Half (50%) of the study subjects were obese. After adjustment for confounders, each 1-mg/m3 increase in 4-hour moving average PM2.5 was associated with HR increase of 5.9 bpm [95% confidence interval (CI), 4.2 to 7.7] and with 5-min HRV reduction by 6.5% (95% CI, 1.9 to 11.3%) for SDNN. Obese individuals had greater PM2.5-mediated HRV reductions (2- to 3-fold) than non-obese individuals, and had more PM2.5-mediated HR increases (9-bpm vs. 4-bpm increase in HR for each 1-mg/m3 increase in PM2.5; p < 0.001). Our study revealed greater autonomic cardiac responses to metal particulates in obese workers, indicating that obesity imparts greater susceptibility to acute cardiovascular effects of fine particles. We conclude that cardiac risk factors and obesity modify PM-induced autonomic cardiac responses in asymptomatic individuals of working age.

USE OF GENE EXPRESSION CHANGES IN BLOOD TO ELUCIDATE MECHANISTIC INDICATORS OF CHILDHOOD ASTHMA (MICA).

S. Edwards1, D. Reif2, R. Heidenfelder1, E. Hudgens1, L. Neas1, E. Hubal1 and J. Gallagh1r1. NHEERL, U.S. EPA, Research Triangle Park, NC and NCCCT, U.S. EPA, Research Triangle Park, NC.

Risk assessment increasingly relies more heavily on mode of action, thus the identification of human biomarkers of disease becomes all the more important. Genomic methods represent a tool for both mode of action determination and biomarker identification. The Mechanistic Indicators of Childhood Asthma (MICA) Study incorporates genomics data from rodents and humans, together with phenotypic measurements, to predict the key events associated with asthma severity or symptomology and to identify human biomarkers for these key events. Gene expression was measured in blood collected from children participating in a case/control asthma study in Detroit. Transcriptional changes from blood were related to common environmental and clinical indicators to identify informative biomarkers to distinguish asthmatics with different etiologies. A parallel study using allergen sensitized Brown Norway rats included expression data from both blood and lung thereby establishing the relationship between transcriptional changes in the blood and corresponding changes in the target tissue. Systems approaches such as this are critical to realizing the National Research Council's 2007 vision of "Toxicity Testing in the 21st Century". [This abstract does not reflect EPA policy.]

COMPARING URINARY BIOMARKERS OF EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS.

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous products of combustion that are found in numerous workplaces, including those processing hot asphalt. Because some PAHs are potent carcinogens, specifically those bound to particulate matter, it is important to assess occupational exposures. Urinary PAH analytes including naphthalene (U-Nap) and hydroxynaphthalene (OH-Nap), phenanthrene (U-Phe) and hydroxyphenanthrene (OH-Phe), and hydroxypyrene (OH-Pyr) have been independently studied as biomarkers of asphalt exposure. Here, we compared these urinary analytes to determine the most suitable biomarker(s) of exposure to particulate asphalt emissions. Our study included 20 paving workers who were observed over three consecutive workdays. Daily breathing-zone air and dermal patch measurements of 4-6 ring polycyclic aromatic compounds (PACs) were evaluated along with postshift, bedtime, and morning levels of U-Nap, U-Phe, OH-Phe, and OH-Pyr. Linear mixed-effects models were used to evaluate exposure-biomarker relationships while controlling for other covariates. Measurements of PACs in the air and on the skin were significantly associated with U-Nap, U-Phe, OH-Phe, and OH-Pyr levels, with the strongest associations observed in postshift samples. Levels of OH-Nap were not associated with PAC measurements but were significantly correlated to the suppressed, age, day of sample collection, and urinary creatinine concentration. Overall the strongest associations were observed between PACs and OH-Phe. We conclude that OH-Phe, U-Nap, U-Phe, and OH-Pyr are useful biomarkers of exposure to particulate asphalt emissions, with OH-Phe being the most promising biomarker.
Biosensor, will provide validation of protein biomarkers as well as a clinic-deployed nanoparticle-based multiplexed Immunochromatographic / Electrochemical our ongoing development of ELISA microarray chips, in combination with a biomarkers in both mouse and humans. High resolution MS proteomic analyses of cytokine, and cellular levels provides fundamental biological insights regarding ex- response, altering both the number of genes and the specific molecular pathways in-duced or suppressed by smoke exposure. Thus, complementary knowledge at gene, cytokine, and cellular levels provides fundamental biological insights regarding ex-posure responses to each stressor that will facilitate reliable identification of protein biomarkers in both mouse and humans. High resolution MS proteomic analyses of plasma and tissue samples from both mice and humans are ongoing. In addition, our ongoing development of ELISA microarrays, in combination with a nanoparticle-based multiplexed Immunochromatographic / Electrochemical Biosensor, will provide validation of protein biomarkers as well as a clinic-deployable detector of individual exposure and response. Supported by U54 ES106015.

Identification of Oxidatively-Modified Proteins as Biomarkers of Chronic Inflammatory Stress.

J. G. Pounds and R. A. Corley Pacific Northwest National Laboratory, Richland, WA.

The ultimate goal of the NIH Exposure Biology Program is to understand the development and progression of complex diseases by precisely, accurately, and quantita-tively assessing the individual’s exposure and response to environmental stressors. Two important risk factors for human morbidity and mortality are exposure to cigarette smoke (CS) and obesity; both are associated with systemic chronic inflammation and oxidative stress. Thus, integrated high-throughput techniques are being used in both human and mouse studies to identify oxidatively modified proteins resulting from inflammatory stress. Exposures of normal and obese mice to defined conditions of CS and lipopolysaccharide (LPS) permit elucidation of bio-

logical responses to different inflammatory stresses. We find from both inflamma-
tory cytokine levels and immune cell populations in bronchoalveolar fluid demon-
strate distinct immune responses from these two stressors. In particular, obesity exacerbates the effects of CS exposure, but not of LPS. Similarly, microarray analy-
sis of murine lung reveals that obesity reprograms the lung’s transcriptional re-
sponse, altering both the number of genes and the specific molecular pathways in-
duced or suppressed by smoke exposure. Thus, complementary knowledge at gene, cytokine, and cellular levels provides fundamental biological insights regarding ex-
posure responses to each stressor that will facilitate reliable identification of protein biomarkers in both mouse and humans. High resolution MS proteomic analyses of plasma and tissue samples from both mice and humans are ongoing. In addition, our ongoing development of ELISA microarrays, in combination with a nanoparticle-based multiplexed Immunochromatographic / Electrochemical Biosensor, will provide validation of protein biomarkers as well as a clinic-deployable detector of individual exposure and response. Supported by U54 ES106015.

Mechanistic Analyses of Skin Homeostasis and Carcinogenesis.

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It is important to note the following as it relates to the significance of the studies fo-cused on understanding skin homeostasis and carcinogenesis. Researchers know that skin cancer is currently the most common type of human cancer and the skin cancer model is a well-studied model of multistage carcinogenesis that can provide mechanistic insights. Also, the common mechanisms elucidated from these studies are highly relevant to those involved in a number of chronic human diseases. Like other epithelial cancers, skin carcinogenesis involves initiation, promotion and pro-
gression that requires activation of oncogenes and inactivation of tumor suppressor genes. Activation of oncogenes, in particular Ras, often occurs following exposure to a number of chemicals and other agents, upregulated follow-
ing exposure to UV light, for example, include that of the ErbB receptors. Keratinocytes that are thus initiated acquire the capabilities to bypass normal cell death pathways, such as apoptosis and proliferate and ultimately form pre-malignant lesions. Recent studies have shown redox signaling plays a key role in modu-
ating oncogenic and tumor suppressive (i.e., p53) signals during skin carcinogene-
sis. In addition to p53, other proteins also thought to play important roles in suppressing epithelial tumorigenesis is C/EBP alpha that is involved in G1 check-
point. Genotoxic stress that can impinge on the regulation of skin homeostasis include dioxin, which likely exerts its tumor promoting activities, in part, via its ability to inhibit apoptosis and senescence. Thus, it is important to focus on the molecular and cellular mechanisms involved in maintaining proper skin homeostasis and how environmental factors may impinge on these mechanisms and contribute to the de-
velopment of not only skin cancer, but also to the progression of chronic disease states of tissues such as the heart, esophagus, and nervous system.

Activation of the AHR Alters Cell Fate Decisions and Skin Homeostasis.

H. Swanson, Molecular and Biomedical Pharmacology, University of Kentucky, Lexington, KY.

Considerable progress has made towards understanding how activation of the aryl hydrocarbon receptor by its agonists, such as dioxins, signal pathways that develop-
ment of chronic disease states in particular, cancers of epithelial cell origin. Using keratinocytes as a model of normal human epithelial cells, dioxin has been found to alter their ability of keratinocytes to proliferate, differentiate, apoptosis and senescence. These events observed in cultured keratinocytes are often in contrast to those re-

ported in other cell types. More recent in vivo studies indicate that within the con-
text of cutaneous wound healing, activation of the AHR by dioxin accelerates wound closure, but leads to an improperly healed wound. These dioxin-induced ac-
tions are accompanied by an acceleration in TGF beta signaling and appearance of active caspases and myofibroblasts within the wound bed. Our observa-
tions accrued using skin as a model tissue are consistent with those reporting dioxin-induced alterations in extracellular matrix remodeling and impaired mor-
phology of the heart and kidney. These findings also have implications to other chronic disease states.

ERBB2: A REGULATOR OF THE SKIN’S RESPONSE TO ULTRAVIOLET IRRADIATION.

L. A. Hansen. Biomedical Sciences, Creighton University, Omaha, NE. Sponsor: H. Swanson.

The cause of most non-melanoma skin cancer is prolonged exposure to ultraviolet (UV) irradiation, which activates cell cycle checkpoints and causes both DNA dam-
age and mutations. UV-induced DNA damage activates the ATR checkpoint lead-
ting to degradation of Cd25a and cell cycle arrest, which allows time for the repair of DNA damage. UV irradiation also activates members of the epidermal growth factor receptor family such as Erb2/HER2 through an indirect mechanism involv-
ing the inactivation of protein tyrosine phosphatases. This presentation will focus on the effects of ErbB activation on cell cycle arrest, DNA repair and skin carcinogene-
sis in response to UV irradiation. UV-induced ErbB activation permits S-
phase progression by suppressing Chk1 activation and consequent Cd25a degra-
dation through a phosphatidylinositol-3-kinase (Pi3K)/Akt-dependent mecha-
nism. Erb2 also delays the repair of DNA damage and increases mutagen-
ity of the Trp53 tumor suppressor gene in response to UV irradiation. Blockade of ErbB2 signaling in a mouse model suppresses skin carcinogenesis in response to UV irradiation, suggesting the receptor may be an appropriate therapeutic target for the treatment or prevention of skin cancer. Observations about the role of ErbB2 in the skin have important implications for the treatment of breast and other adenocarcino-

Molecular and Biomedical Pharmacology, University of Kentucky, Lexington, KY.

Multifaceted Roles for C/EBPs in the DNA Damage Response Network and Skin Tumorigenesis.

R. C. Smart, Department of Environmental and Molecular Toxicology, North Carolina State University, Raleigh, NC.

This presentation will focus on the known and emerging roles of the basic leucine zipper (bZIP) transcription factors, C/EBPα and C/EBPβ in epidermal homeostasis and skin tumorigenesis. While C/EBPα and C/EBPβ have similar DNA binding domains and bind to the same DNA consensus sequence, they have very different roles in keratinocytes and skin tumorigenesis. C/EBPα opposes oncogenic Ras via direct repression of E2F, C/EBPβ has a role in the G1/S transition as well as the DNA damage induced G1 checkpoint and functions as a tumor suppressor gene in skin. In contrast, C/EBPβ is a mediator of keratinocyte survival and tumorigenesis. When C/EBPβ-/ mice are treated with carcinogens that produce oncogenic Ras mutations in keratinocytes, they respond with abnormally elevated keratinocyte apoptosis and a block in skin tumorigenesis. While this aberrant carcinogen-in-
duced apoptosis results from abnormal up-regulation of p53, it was not known whether up-regulated p53 results from oncogenic Ras and its ability to induce p19ARF and/or activate DNA damage response pathways or from direct carcinogen-

Mitochondria and Skin Cancer: New Facet in an Old Partnership.

D. K. St. Clair, Graduate Center for Toxicology, University of Kentucky, Lexington, KY.

Mitochondria plays an important role as a source of ROS generation as well as a source of ROS removal. The pro- or anti-oxidant function of mitochondria is regu-
lated by the activity of the primary antioxidant enzyme, manganese superoxide dismutase (MnSOD), which is a nuclear-encoded antioxidant enzyme that localizes to the mito-

MnSOD modulates tumor formation caused by the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) by regulation of cellular proliferation pathways. However, MnSOD deficiency lead to an enhanced apoptosis because oxidative stress in the mitochondria can modulate the p53 mediated apoptosis pathways. In
MnSOD deficient mice. TPA induces p53 translocation to mitochondria and nucleus, a response, which eventually leads to apoptosis. Translocation of p53 to mitochondria precedes its nuclear translocation after TPA application. In the mitochondrial, p53 interacts with MnSOD, and reduces its superoxide scavenging activity, contributing to a transcription independent apoptosis action of p53. In addition to the immediate action on mitochondria, p53 translocation to the nucleus is accompanied by a decrease in the levels of its target gene, MnSOD, thereby amplifying the pro-oxidant conditions in mitochondria. Thus, p53 translocation to mitochondria and subsequent suppression of MnSOD explains the observed pro-oxidant function of mitochondria that leads to transcription-dependent mechanisms of p53-induced apoptosis. This work will allow for potential mechanistic based interventions of multi-stage chemical-induced skin carcinogenesis and can also be applied to a chemical-induced cardiac injury model.

S 1922 MULTIFACETED ROLES FOR C/EBPβ IN THE DNA DAMAGE RESPONSE NETWORK AND SKIN TUMORIGENESIS.

R. C. Smart, North Carolina State University, Raleigh, NC.

This presentation focuses on the known and emerging roles of the basic leucine zipper (bZIP) transcription factors CCAAT/enhancer binding protein alpha (C/EBPα) and C/EBPβ in epidermal homeostasis and skin tumorigenesis. While C/EBPβ and C/EBPβ have similar DNA binding domains and bind to the same DNA consensus sequence, they have vastly different roles in keratinocytes and skin tumorigenesis. C/EBPβ has a role in the G1/S transition, is highly inducible by UVB radiation and functions in the DNA damage-induced G1 checkpoint. C/EBPα is a tumor suppressor gene in skin. In contrast, C/EBPβ is a mediator of keratinocyte survival and skin tumorigenesis. When C/EBPβ-/- mice are treated with carcinogens that produce oncogenic Ras mutations in keratinocytes, they respond with abnormally elevated keratinocyte apoptosis and a block in skin tumorigenesis. This aberrant carcinoma-induced apoptosis results from abnormal up-regulation of p53 indicating C/EBPβ is a negative regulator of p53 levels and function. Recently, we have demonstrated that C/EBPβ represses p53 levels and functions to promote cell survival downstream of DNA damage independent of oncogenic Ras and p19ARF. Lastly, these findings and mechanisms will be discussed with respect to their relevance to other epithelial tissues.

S 1923 PULMONARY EFFECTS OF IN UTERO AND EARLY POSTNATAL EXPOSURE TO ARSENIC.

R. Lantz, Cell Biology and Anatomy, University of Arizona, Tucson, AZ.

Arsenic has long been recognized as a human lung carcinogen. In addition, the health effects of arsenic ingestion in the drinking water have also been associated with significant non-cancerous chronic pulmonary disease. It has been postulated that a significant proportion of adult lung disease originates in utero or in early infancy. Growth and development requires the temporal and spatial coordinated expression of genes and gene products. During this critical time, in utero and early postnatal exposure to toxics has the potential to affect gene expression, altering organ structure and physiological function which can lead to adult disease. The effect of in utero and early postnatal arsenic exposure on lung disease and the effects of arsenic exposure during lung development on human cancer and noncancerous lung disease in adults will be presented. The adverse health outcomes associated with in utero and postnatal exposures and will demonstrate the importance of understanding the mechanisms and targets of arsenic during these developmental time points will be provided as an overview. Further discussions will focus on gene-environment interactions in arsenic metabolism, metabolism and distribution of arsenic during fetal development and cancerous and noncancerous animal models of in utero and early postnatal exposures. In order to fully understand the issues presented, researchers will provide attendees with excellent examples and information from both population and laboratory based research that will indicate the importance of exposures during these sensitive developmental times. This symposium will be of interest to those involved in metallo/toxicology, developmental toxicology, public health, risk assessment, and regulatory management.

S 1924 IMPACT OF IN UTERO AND CHILDHOOD EXPOSURE TO ARSENIC IN DRINKING WATER ON MORTALITY IN YOUNG ADULTS.

A. Smith. School of Public Health, University of California, Berkeley, CA. Sponsor: R. Lantz.

Arsenic in drinking water is an established cause of lung cancer, and preliminary evidence suggests that ingested arsenic may also cause nonmalignant lung disease. A unique exposure scenario provided a rare opportunity to investigate the long-term mortality impact of early-life arsenic exposure. This study focused on subjects who were born during or just before a peak arsenic exposure period and who were 30–49 years of age at the time of death. For the birth cohort born just before the high-exposure period (1950–1957) and exposed in early childhood, the standardized mortality ratio (SMR) for lung cancer was 7.0 (p < 0.001) and the SMR for bronchietasis was 12.4 (p < 0.001). For those born during the high-exposure period (1958–1970) with probable exposure in utero and early childhood, the corresponding SMRs were 6.1 (p < 0.001) for lung cancer and 46.2 (p < 0.001) for bronchiectasis. These findings suggest that exposure to arsenic in drinking water during early childhood or in utero has pronounced pulmonary effects, greatly increasing subsequent mortality in young adults from both malignant and nonmalignant lung disease.

S 1925 GENETICS, INTRINSIC ENVIRONMENT AND EXTRINSIC ENVIRONMENT INFLUENCE HUMAN VARIABILITY OF ARSENIC METABOLISM.

W. Klimmeki, Pharmacology and Toxicology, University of Arizona, Tucson, AZ.

Studies characterizing human individual variation in arsenic metabolism, reflected in the speciation profile of urinary arsenic and its metabolites, suggest that variability in arsenic metabolism has a complex set of determinants that include age or developmental stage, sex, nutritional status and genetic variation. Recent studies have been repeatedly shown to methylate arsenic more efficiently than do men. Variants in genes involved with arsenic metabolism (AS3MT) and one-carbon biochemistry (MTHFR) have likewise been associated in replicated studies with the efficiency of arsenic methyltransferase. An additional layer of complexity exists in published evidence that suggests these factors can interact with each other, so that the effect of a genetic variant may be different in different age groups, or in different sexes. Exemplary in this regard is a population of arsenic-exposed individuals in Sonora, Mexico in whom we have measured urinary arsenic species as well as DNA variations in several candidate genes, testing for association with urinary arsenic species levels. In adults (>17 years) studied, females had mean urinary arsenic as monomethylated arsenic, valence V (%MMA) than did males (4.2% Vs. 6.7%, P=0.018). Children (7-11 years) had lower mean %MMA than did adults (3.7% Vs. 4.9%, P=0.001). Polymorphisms in AS3MT were significantly associated with %MMA, but interacted with age. To complicate matters further, ancestry in this population (largely a genetic admixture between European and indigenous American ancestry) is a potential confounding factor in this study. Considering that measurements of individual variability in arsenic metabolism have provided among the best biomarkers of disease risk, it is likely that these determinants of arsenic metabolic variability have important correlates in disease likelihood. Thus, a clear understanding of how these factors collectively contribute to variability in arsenic metabolism is critical to explain their contribution to arsenic-associated disease.

S 1926 ARSENIC METABOLISM AND DISTRIBUTION IN DEVELOPING ORGANISMS.

D. Thomas, U.S. EPA, Research Triangle Park, NC.

A growing body of evidence suggests that exposure to inorganic arsenic during early life has long term adverse effects. The biotransformation and excretion of methylated metabolites in utero is determined not only by the rates of formation and transfer of arsenicals across the placenta but also by ontology of arsenic metabolism in the developing organism. For example, in a mouse model for translational carcinogenesis of inorganic arsenic, fetuses at gestational day 18 contain inorganic, methylated, and dimethylated arsenic in placenta, liver, lung, and blood. The methylated arsenicals in fetal tissues could be transferred from the mother or formed in situ. Because concentrations of these arsenicals in fetal tissues were lower than those found in corresponding maternal tissues, there is likely a placental barrier to the transfer of arsenicals from mother to fetus or a maternal-fetal difference in the uptake, production, or retention of these arsenicals. Arsenic (+3 oxidation state) methyltransferase which catalyzes the formation of methylated arsenicals is detectable in fetal tissues by mid-gestation. However, it is unknown whether the protein is catalytically active in utero. For example, the activity of arsenic (+3 oxidation state) methyltransferase is dependent on the presence of dihydro reductants (thiourea, glutaredoxin) and is modulated by glutathione. Availability of these factors during development may control the ontology of the activity of arsenic (+3 oxidation state) methyltransferase and may affect the levels of methylated arsenicals found in fetal tissues. In addition, developmental changes in the levels of transporters that mediate the uptake and loss of arsenicals from cells could affect accumulation in fetal tissues. Elucidating the role of transport and metabolism in the kinetic behavior of inorganic arsenic in fetal tissues could contribute to understanding genetic and epigenetic consequences of early life exposure to this metalloid. (This abstract does not reflect US EPA policy.)
Inorganic arsenic has long been known as a known human carcinogen and can target the lung. More recently it has been established as a multi-site, transplacental carcinogen in mice, producing cancer in adulthood after brief fetal exposure via maternal drinking water. As a transplacental carcinogen inorganic arsenic often targets the lung as either an initiator or complete carcinogen and has shown activity in a variety of mouse strains. Arsenic-induced molecular changes in fetal lung included increased estrogen receptor-alpha (ER-alpha), and overexpression of estrogen-regulated genes (trefoil factor-3, etc.) and increases in the steroid metabolism genes such as aromatase. The IGF system, which is influenced by ER and has been implicated lung cancer, and several other genes (alpha-fetoprotein, epidermal growth factor receptor, and L-mycol) associated with lung cancer were overexpressed. In adult females exposed to arsenic in utero, lung adenoma and adenocarcinoma showed widespread, intense nuclear ER-alpha expression. In contrast, normal adult lung and diethylstilbestrol-induced lung adenocarcinoma showed little evidence of ER-alpha expression. Thus, transplacental arsenic exposure at a carcinogenic dose produced aberrant estrogen-linked pulmonary gene expression. ER-alpha activation was specifically associated with arsenic-induced lung adenocarcinoma and adenoma. These arsenic-induced aberrant steroid signaling could disrupt early life stage genetic programming in the lung leading eventually to lung tumor formation much later in adulthood. These data help provide a further example of the fetal basis of adulthood disease with arsenic exposure.

Liver diseases with different pathogeneses share common pathways of immune-mediated injury. Autoreactive T cells destroy hepatocytes or cholangiocytes in autoimmune liver disease and virus-specific T cells destroy infected hepatocytes in viral hepatitis. In these conditions antigen-specific mechanisms can be implicated but immune-mediated injury is central to many diseases including drug toxicities where there is a less defined role for specific antigens. In all these diseases "bystander" cells activated by the local microenvironment rather than a specific antigen are major players and amplify injury by recruiting effector cells from the circulation. Thus immune-mediated liver injury is driven by repeated cycles of inflammation and repair initiated by injury not sustained by the immune system. A major question that continues to be debated is the precise role of these immune cells to liver damage. Liver injury mediated by neutrophils has been reported in a number of animal models such as ischemia-reperfusion injury, endometriosis, alcoholic hepatitis, obstructive cholestasis and drug-induced liver damage such as by Acetaminophen (APAP), a widely used analgesic, is generally considered a safe drug at therapeutic levels but can induce centrilobular necrosis and even liver failure at higher doses. Although most studies still focus mainly on the intracellular signaling.
mechanisms of cell death in hepatocytes, there is an increasing interest in the role of hepatic nonparenchymal cells (Kupffer cells, NK- and NKT cells) and infiltrating leukocytes (neutrophils, monocytes) in the pathophysiology. For each cell type there is experimental evidence in the literature suggesting a contribution to the mechanisms of toxicity. However, the validity of several of these conclusions has been questioned recently. The focus of our investigations is to elucidate the role of neutrophils in APAP-induced liver injury and regeneration. The presentation will address basic mechanisms of neutrophil recruitment and activation into the liver during regeneration and the signaling mechanisms that determine cytotoxicity, the mechanisms of neutrophil-induced liver injury and potential differences in neutrophil activity during regeneration. A better understanding of the role of neutrophils in host defense, aggravation of tissue injury and promotion of tissue regeneration will allow the identification of strategies that prevent the detrimental effects of APAP and potentially predict liver injury and hepatic inflammation.

1934 THE CONTRIBUTION OF OSTEOPONTIN TO HEPATIC INFLAMMATION AND LIVER INJURY.

S. K. Ramaiah

Drug Safety Research and Development, Pfizer, St. Louis, MO.

Osteopontin (OPN) produced by cells of the immune system, epithelial tissue, smooth muscle cells, osteoblasts and tumor cells has been implicated in various pathophysiological functions such as cell binding, spreading and migration, and tumor metastasis. OPN is known to block integrins expressed on macrophages through the RCG motif and promote migration of cells resulting in granuloma formation. In the liver, it has been reported that hepatic Kupffer cells secrete OPN facilitating macrophage infiltration in necrotic areas following carbon tetrachloride liver toxicity. Recent work has underlined the importance of OPN as a pivotal cytokine/chemokine in the generated hepatic neutrophil response during early phase alcoholic liver injury. Increased hepatoellular OPN expression correlated well with higher neutrophil infiltration in a rat model of alcoholic steatohepatitis (ASH). In the same model of ASH, higher hepatic expression of OPN in females was attributed to the higher neutrophil infiltration and consequent higher female sensitivity to liver damage. OPN as a potential biomarker for inflammatory liver disease has also been assessed recently. In this presentation, the speaker will review some of the previous findings in addition to providing new in vitro data on the role of osteopontin and the ability of other hepatotoxic chemicals to induce this protein and potentially predict liver injury and hepatic inflammation.

1935 FOOD ALLERGY - BASIC MECHANISMS AND APPLICATIONS TO IDENTIFYING RISKS ASSOCIATED WITH PLANT INCORPORATED PESTICIDES AND OTHER GENETICALLY MODIFIED CROPS.


Food allergy is a relatively new concern for toxicologists as a result of the incorporation of novel proteins into food crops in order to promote resistance to pests and other stresses, improve nutrition, or otherwise modify the phenotype. Food allergy can manifest as inflammation of the skin (hives), gut, and/or lung and in the most extreme cases can result in anaphylactic shock and death. Thus, although the technology to modify crops genetically has many advantages over more conventional approaches, there is some concern that introduction of a novel protein into the food supply could result in unintentional introduction of a new allergen and could pose a risk to susceptible individuals. A number of potential strategies have been proposed to assess this risk, but many questions regarding basic mechanisms underlying food allergy limit our ability to provide the public with information not only about potential allergenicity of transgenic proteins, Food allergy is a relatively new concern for toxicologists as a result of the incorporation of novel proteins into food crops in order to promote resistance to pests and other stresses, improve nutrition, or otherwise modify the phenotype. Food allergy can manifest as inflammation of the skin (hives), gut, and/or lung and in the most extreme cases can result in anaphylactic shock and death. Thus, although the technology to modify crops genetically has many advantages over more conventional approaches, there is some concern that introduction of a novel protein into the food supply could result in unintentional introduction of a new allergen and could pose a risk to susceptible individuals. 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Funding: US EPA STAR Grant#R833133.

Human non-allergenic food proteins (kidney bean, pinto bean) were non-allergenic cow’s milk and chicken egg) produce all three readouts of allergenicity; (iii) two the mice to obtain significant readouts of systemic anaphylaxis and hypothermia to ensures to the allergenic food protein at 1000 ug/mouse dose is required to sensitize

transdermal food protein exposure, clinical signs of systemic anaphylaxis and hy-

in the mouse model for testing allergenicity of dietary proteins. The data demon-

The mouse-model; and 2) Determine the negative predictive value (i.e.,specificity)

(Birmingham et al., Int Arch Allergy Immunol. 2007, 144, 3:203-210). We have

adjuvant-free mouse model of food allergy that we have recently reported

lagenic potential of GE foods—a major challenge facing the regulatory agencies and

2Pediatrics, UTMB, Galveston, TX.

This model may be a useful tool to identify allergenic food proteins and to study the mechanisms of food allergy. Supported by EPA STAR R833134.

1939 SAFETY ASSESSMENT OF DIETARY PROTEINS FOR ALLERGENICITY USING AN ADJUVANT-FREE


Currently there is no validated animal model available for the assessment of all-

genetic potential of GE foods—a major challenge facing the regulatory agencies and

the agro-biotech industry. The goal of this project is to test the validity of a novel

adjuvant-free mouse model of food allergy responses (Birmingham et al., Int Arch Allergy Immunol. 2007, 144, 3:203-210). We have been testing the hypothesis that this mouse model, which involves transdermal sen-

sitization to food proteins followed by oral food challenge, will be highly reliable in discriminating food proteins that have or do not have intrinsic allergic sensitivity potential in humans. The two specific objectives for this project are: 1)Determine the positive predictive value (i.e., sensitivity) of the mouse-model; and 2) Determine the negative predictive value (i.e.,specificity) of the mouse model for testing allergenicity of dietary proteins. The data demon-

strates that:(i) the allergenicity readouts (food specific IgE antibody response to transdermal food protein exposure, clinical signs of systemic anaphylaxis and hy-

pothemia to oral food challenge) in this mouse model are dependent on the num-

ber of exposures to the allergenic protein; a minimum of four transdermal expo-

sures to the allergenic food protein at 1000 ug/mouse dose is required to sensitize the mice to obtain significant readouts of systemic anaphylaxis and hypothermia to oral food challenge; ii) four human allergenic food proteins (hazelnut,cashew nut, cow’s milk and chicken egg) produce all three readouts of allergenicity; (iii) two human non-allergenic food proteins (kidney bean, pinto bean) were non-allergenic based on clinical scores and hypothermia readouts but not IgE data; interestingly, Amaranth seed protein, presumably non-allergenic in humans, produced all three readouts of allergenicity in this model. Currently we are testing additional dietary proteins for allergenicity using this adjuvant-free mouse model. Funding: US EPA STAR Grant R8351353.

1940 PREDICTION AND DETECTION OF CONFORMATIONAL EPITOPES OF IgE ANTIBODIES.


We created the Structural Database of Allergenic Proteins (SDAP, http://fermi.utmb.edu/SDAP/) to archive data on allergens and to provide unique bioinformatics tools to assess the potential risk of genetically modified food pro-

ducts for allergenicity. In this talk we demonstrate the use of 3D structures of aller-

gen to characterize the potency of allergens, address the experimental validation of the property distance (PD) index and present a novel approach to determine con-

formational IgE epitopes from phage display experiments. As an example for a highly potent allergen we examined the sequence similarities and 3D structural lo-

cations of IgE epitopes from peanut and related nut allergens. Our findings indicate that the potency of peanut allergens might be due to the proteins’ arrays of similar antigenic regions on opposite sides of a single protein structure. To quantitatively assess the quality of the PD index we used the previously identified epitopes of the Jun a 1 allergen from cedar pollen as a model system. Immuno dot blot assays showed that peptides with low PD indices, i.e. less than 6, relative to a given epi-

tope are more likely to bind IgE from sera of pollen sensitive patients than those with high PD indices. We also demonstrate how 3D structures of allergens can be used to gain insights on the physical-chemical nature of conformational epitopes. We generated more than 400 reliable 3D models of allergens, considerably extend-

ing our knowledge from the 45 currently known 3D structures of allergens. Statistical analysis of surface exposed parts of experimentally determined IgE ep-

topes revealed a distinct distribution of residues different from that in typical protein-protein interfaces. Finally we present a new automated search method, EpiSearch that predicts the 3D location of conformational epitopes on the surface of an allergen from peptides, selected by phage display technology. We show that EpiSearch is capable to find the correct epitope locations in several cases, including one blind test. Funding: US EPA STAR grant RD 833137

1941 HUMAN SERUM IgE SCREENING: IDENTIFICATION OF ALLERGENS AND POTENTIALLY CROSS-REACTIVE PROTEINS.


Based on US and CODEX guidelines for assessing the potential allergenicity of foods from genetically modified (GM) crops, serum IgE binding tests are required if the gene is from a commonly allergenic source or the newly introduced protein shares greater than 35% sequence identity match over any 80 or more amino acid segment with a known allergen. Since allergy to any specific source is relatively rare and IgE binding affinity, specificity and abundance varies between subjects, serum donor selection of test and control subjects must be based on a clear history of al-

ergy and diagnostic testing. Since IgE binding to sequential or conformational epi-

topes or likely irrelevant cross-reactive carbohydrate determinants (CCD) is vari-

able, laboratory methods must be more stringent than those used in academic research. We propose a highly validated method for commercial diagnostic assays. Examples of clinical history forms and ethical issues from a study of legume cross-reactivity (US, EU and India) will be presented. Direct IgE binding methods with native protein (dot-blot, ELISA, IgE:immunoblot of native gel electrophore-

sis) and model of food allergen (SDN-PAGE, reducing/non-reducing blots) and inhibition and verification to differentiate CCD binding and strong vs weak cross-

reactivity will be presented with preliminary data evaluating IgE binding to 14 common food-legumes (peanut, chickpea, lupin, pigeon pea, soybean, lima bean, kidney bean, navy bean, black lentil, cowpea, fava bean, lentil and pea) from five tribes of the family Fabaceae, which demonstrate that substantial cross-

reactivity is rare. Methods for verification of protein identity and clinical relevance will be mentioned. Funding: US EPA STAR grant RD83513501 and the Food Allergy Research and Resource Program (FARRP, Unv. of Nebraska).

1942 ON THE HORIZON – EXPLORATORY RESEARCH INITIATIVE IN FOOD ALLERGY.

S. Landsig. U.S. Environmental Protection Agency, Washington, DC.

The U.S. Environmental Protection Agency (EPA), the National Institute of Allergy and Infectious Diseases (NIAID), the Food Allergy and Anaphylaxis Network (FAAN) and the Food Allergy Project (FAP) are partnering on an initia-
tive to stimulate innovative research in food allergy and advance the current under-

standing of mechanisms of action, risk factors, and safety assessment of food allergy. The program objective is to spark new interest in food allergy research and ap-

proaches for safety assessment of potential dietary allergens including proteins in-

troduced into food through genetic engineering. An additional major goal of the program is to encourage new investigators to move into the field of food allergy re-

search. The initiative supports basic research on factors that contribute to the de-

velopment of food allergies, the relationship between food allergies and other im-

mune disorders, and the epidemiology and genetics of food allergy, as well as

methods to predict the allergenicity of novel proteins in food. Under the initiative, EPA recently funded four projects that will develop computational models, in vitro tests, and transgenic mice to improve understanding of the initiation and suscepti-

bility of people to dietary allergens, the importance of protein cross-reactivity, the involvement of cytokines, and the role of cell signaling. Eleven projects funded by NIAID, FAAN, and FAP are addressing key questions aimed at improving treat-

ment, preventing food allergies, defining the genetics of human food allergy, and understanding the interactions between genes and the environment in food allergy pathogenesis. Epidemiological studies on the prenatal and maternal factors in-

volved in oral tolerance and sensitization will help delineate sensitive populations and the probability for development of food allergy. An improved understanding of the basic immunology of food sensitization should illuminate the most important factors to consider for ensuring the safety of new bioengineered proteins or new ex-

poures to proteins in food. This abstract does not reflect EPA policy.

1943 THE IMPACT OF TRANSCRIPT PROFILING IN DRUG SAFETY ASSESSMENT.


Transcript profiling technology has been around for a decade and is arguably the most developed systems biology approach that has been applied to drug safety as-

essment. Although there are examples where transcriptomics has been applied to
safety assessment of drug candidates, much of the data remains unreported and re-
ports are anecdotal. In part, the lack of publication leads to the impression that transcriptomics has had little impact. Specific examples where transcriptomics are being applied to preclinical drug safety assessment will be highlighted as will the areas of success in addition to areas where transcriptomics has had less impact. The emphasis will be on cases studies outlining applications that are being integrated into safety assessment and where the technology is being reduced to practice. The impact of the technology among scientists in industry and the regulatory commu-
nity will be illustrated by sharing both positive and negative experiences. Industry
scientists will present case studies that illustrate the use of transcriptomics to under-
stand issues or risks that emerge in preclinical development. Finally, the opportuni-
ties and challenges for applying transcriptomics to drug safety assessment will be summarized.

W 1944 IMPACT OF ILSI-HESI IN RESOLVING ISSUES IN DRUG
SAFETY ASSESSMENT USING TRANSCRIPTOMICS – CASE REVIEW.

C. Afshari, Amgen, Thousand Oaks, CA.
The rapid development and evolution of genomic, proteomic, and metabolomic
based technologies has led to the development of the field of “toxicogenomics.”
Application of these technologies is directed toward improving the efficiency of
safety and risk assessments. This is being accomplished by facilitating better under-
standing of the mechanisms by which compounds induce injury or coupled
with the potential for identification of early, sensitive biomarkers of toxicity. The
scientific community, academic, industry and regulatory groups have all put forth
together efforts targeted at evaluating the development and use of genomics technologies in tox-
ics. Recently, a collaborative review of this effort was reviewed in a case study
workshop coordinated by the International Life Sciences Institute (ILSI). Members
of the ILSI consortium include a large representation of pharmaceutical companies,
as well as membership from academic and government institutions including the
FDA. A number of industry-related case studies around predictive toxicology and
issue resolution were reviewed in the context of scientific and regulatory impact/perspective. This presentation will review the efforts of ILSI-HESI in coor-
dinating a global, cross-industry/regulatory effort to establish genomics in safety re-
lated applications and will present highlights from the recent ‘state of the science
survey’ and workshop that ILSI hosted.

W 1945 CASE STUDY: APPLYING TRANSCRIPTOMICS IN
VITRO AND IN VIVO.

I. Stevens, Toxicology, Lilly Research Laboratory, A Division of Eli Lilly and Co.,
Indianapolis, IN.
Application of transcript profiling in the earliest stages of drug discovery creates op-
portunity to advance molecules with acceptable preclinical safety profiles into de-
velopment. Using a contextual genomics database, DrugMatrix®, and a battery of
gene signatures derived from both a training set and from internal compounds, we
have integrated transcript profiling into a cell-based surrogate screening strategy for
liver injury. Primarily cultures of rat hepatocytes (RPH) were used to characterize
the cytolethality of individual compounds. Transcript profiles were also collected from RPH
treated with the test compounds. The combination of cytolethality data and
gene signatures scores were used to predict the potential to cause liver injury in vivo
in short term (4 day) rodent repeat dose studies. An initial set of performance met-
rRICS suggested that both the RPH cytolethality and the gene signature scores pre-
dict in vivo outcomes with acceptable accuracy, sensitivity and specificity. Using
this strategy, compounds synthesized in the Hit to Lead and early Lead
Optimization phases were screened for hepatotoxic potential. Selected examples
from project teams illustrate that early safety assessment based on in vitro cyto-
lethality and gene expression results allows selection of Lead compounds with an
improved safety profile.

W 1946 PREDICTING DRUG SAFETY USING
TRANSCRIPTOMICS AND SYSTEM BIOLOGY APPROACHES.

L. Lehman-McKeeman, Bristol-Myers Squibb, Princeton, NJ.
Retrospective analysis of transcriptomic data collected over approximately the last 5
years has confirmed its utility as a predictive and diagnostic tool in drug discovery
and development toxicology. Furthermore, there is an increasing body of literature
summarizing transcriptional changes following drug or chemical insult, and data-
bases of transcriptional profiles that can be queried as a potential tool for predicting
toxic potential have been developed. In general, these efforts have focused on the

W 1947 EVALUATING SAFETY ISSUES IN EARLY DRUG
DEVELOPMENT WITH TRANSCRIPTOMICS.

E. Waring, Cellular, Molecular and Exploratory Toxicology, Abbott Laboratories,
Abbott Park, IL. Sponsor: S. Perini.
In the pharmaceutical industry, toxicity represents a major cause of failure at the
costly stages of development, and reducing late-stage attrition due to toxicity can
decrease overall research and development costs. The use of transcriptomics in tox-
icology, also known as toxicogenomics, represents one of the few recent technolog-
ical advances that has improved the detection and mechanistic characterization of
molecular changes in preclinical species. Published reports and our internal data have
provided strong evidence that toxicogenomics is a viable and useful methodology to
evaluate compounds in discovery and to develop a mechanistic understanding of
toxic events at later stages in development. In the industry, toxicology has tradi-
tionally been an observational discipline that uses limited numbers of endpoints
coupled with mechanistic value. In contrast, global evaluation of transcriptomic changes
can occur early to detect abnormalities in subcellular compartments or deregulation of
biological pathways. Accurate analysis and understanding of these complex data sets is largely facilitated by the use of pathway analysis software pack-
ages and by the access to repositories of reference gene expression profiles. This
higher level of clarity represents a robust and efficient approach for toxicologists to
rapidly formulate data-driven hypotheses regarding the mechanisms of toxic
tissue changes. These hypotheses typically need to be confirmed or refuted in follow-up
functional experiments using appropriate complementary technologies. In our or-
ganization, toxicogenomics is an integrated part of the battery of toxicological end-
points used to profile or evaluate compounds in discovery and preclinical develop-
ment. This presentation will provide an overview of the use of toxicogenomics at
Abbott with a particular emphasis on its application to address toxicity issues in
early development. Specific examples will be used to illustrate how this technology can
be used to develop an understanding of the mechanisms underlying toxic
changes in various tissues, such as liver, kidney, heart or testis.

W 1948 SUMMARY: OPPORTUNITIES AND CHALLENGES IN
APPLYING TRANSCRIPTOMICS TO DRUG SAFETY
ASSESSMENT.

F. D. Szatmary, Merck and Co., Inc., West Point, PA.
Scientists in the pharmaceutical industry have ready access to samples from animal
toxicology studies carefully designed to test the safety characteristics of a steady
pipeline of agents advancing toward clinical testing. Advancing applications of tox-
icogenomics to the evaluation of cardiovascular risk in the clinical setting is best real-
ized if this promising technology could be implemented in these studies fully
anchored in the traditional study endpoints currently used to characterize pheno-
typic outcome and to support the safe conduct of clinical testing. Regulatory au-
thorities worldwide have declared their support for toxicogenomics and related
technological tools to positively impact drug development and guidance has been
published. However, applications of exploratory “-omics” technologies to com-
pounds undergoing safety testing remain inhibited. Two core data submission re-
 sponsibilities implicit in these technologies are contributing: 1) constraints arising
from continual literature surveillance and data reanalysis burdens, under the
shadow of looming subsequent reporting requirements to regulatory authorities as
gene expression endpoints loosely linked to safety gain attention in the published
literature, and 2) ambiguities in interpretation of validation stature remain between
EXPLORATORY, PROBABLE, VALID, AND KNOWN SAFE BIOMARKERS. REGULATORY AUTHORITIES HAVE BEEN ENGAGED AND A PROPOSAL HAS BEEN OFFERED WITH PROMISING FEEDBACK TO ADDRESS THESE REGULATORY IMPLEMENTATION BARRIERS AND OPEN UP ACCESS FOR EXPLORING THIS TECHNOLOGY IN PROSPECTIVE DRUG DEVELOPMENT ANIMAL TOXICOLOGY STUDIES.

W1949 THE ROAD TO PERSONALIZED MEDICINE.
D. Mendrick1 and V. S. Vaidya.1,2 Division of Systems Toxicology, NCTR/FDA, Jefferson, AR and Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

To improve the safety of marketed drugs and chemicals, new biomarkers are needed to identify unsafe compounds earlier, discover patients who are at risk of adverse events to specific drugs and chemicals prior to exposure, and provide tools for the management of patients who are or will undergo adverse events. Single nucleotide polymorphisms (SNPs) and gene expression alterations provide clues into a person's response to xenobiotics enabling personalized medicine. Our panel of experts will highlight new approaches being used in preclinical species to understand individuals' responses and improve preclinical detection of idiosyncratic drugs, the use of translational biomarkers to enable better correlation between animals and humans, and the status of monitoring SNPs in the clinic to avoid adverse events.

W1950 MODELING CHEMICAL TOXICITY IN THE POPULATION: MOUSE TO THE RESCUE!
J. Rusyn, Department of Environmental Sciences and Engineering, UNC, Chapel Hill, NC.

Rodent models have been used extensively to assess safety and investigate the mechanisms of toxicity of drugs and chemicals yet seldom do we think about a choice of a particular strain for our experiments. Significant recent breakthroughs in understanding the extent of the genetic diversity in the humans and mice show that mouse may be used to determine what genetic variants correlate with susceptibility or resistance to toxicity and disease, thus potentially helping to identify susceptible sub-populations. By using a panel of mouse inbred strains it is possible to model genetic diversity in a population which will likely provide a rationale for "individualized" assessment of risk and understanding of the variability in dose-response and mechanism. Specifically, we propose and validate a strategy using a Mouse Model of the Human Population (MMHP) to identify key mechanisms contributing to xenobiotic-induced liver injury. Whole-genome haplotype association analysis in the MMHP allows for a discovery of the candidate "susceptibility" genes which then may be validated in humans.

W1951 TOWARDS IMPROVEMENTS IN TOXICOGENOMICS FOR UNDERSTANDING HEPATOTOXICITY.

Hepatotoxicity is a major reason for clinical failures and drugs being withdrawn from the market. The FDA's Voluntary Genomics Data Submission (VGDS) program has observed considerable efforts made by sponsors in exploring biomarker identification technologies to detect drug-induced liver toxicity. This presentation will discuss two FDA projects and VGDS experience on improvements of experimental design in toxicoogenomics for hepatotoxicity. The FDA's Liver Toxicity Knowledge Base (LTKB) is a liver specific knowledge base which provides a resource to discover the relationship between liver toxicity, pathways/network, genes/proteins and drugs based on literature information and other public resources as well as in house experimental data. The Liver Toxicity Biomarker Study (LTBS) is a collaborative project between the FDA and BG Medicine that is utilizing transcriptomic, proteomic, and metabolomic platforms to investigate markers of idiosyncratic hepatotoxicity. These projects and the VGDS program form the scientific basis to fill the gap in FDA regulation for hepatotoxicity.

W1952 THE TRANSITION FROM PRE-ClinICAL TO CLINICAL SAFETY RELATED GENOMICS.
F. Feuch, PhD, Medco Health Solutions, Inc., Darnestown, MD. Sponsor: D. Mendrick.

Preclinical safety biomarkers have traditionally been "qualified" over a period of many years and the accumulation of such data occurred in an uncoordinated, ill-defined manner. Hence, many of the traditional markers we are using today do not have a clearly demonstrated sensitivity or specificity and lack a true definition of context for their usefulness of safety assessment. There is a need for better, well-characterized markers enabled through the availability of novel technologies and we have the opportunity to develop more sensitive and specific markers for preclinical safety evaluation studies, and to translate these markers into clinical settings. It is therefore reasonable to anticipate that these markers might be true bridging markers that will enable drug developers to move compounds into phase 1 studies in situations where the program may have been stopped otherwise. If indeed these new markers deliver on their promise, we will be able to use such a "tested risk" approach as long as the toxicity is reversible and justified. Theoretical approaches of qualifying such new biomarkers will yield important clues as to the appropriate methodologies.

W1953 TRANSLATIONAL BIOMARKERS FOR KIDNEY TOXICITY.
V. S. Vaidya. Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

The public health impact of kidney disease is significantly high with about 20 million Americans having chronic kidney disease with a gross mortality rate for patients on dialysis being ~50-70%. Drug-induced nephrotoxicity plays a major role in the high incidence and prevalence of kidney injury which may be prevented or at least minimized by effective predictive toxicity screening in preclinical drug development studies. The absence of sensitive, specific, reliable and reproducible renal injury biomarkers affects the evaluation of response to therapy and individual patient safety, especially dose monitoring decisions for important life saving drugs with potential inherent kidney toxicity risk. For the last 100 years there have been no accepted kidney injury biomarkers. The standard metrics such as serum creatinine and blood urea nitrogen are very insensitive and non-specific functional biomarkers. The urine has yielded the most promising markers for the early detection of acute kidney injury (AKI) and further characterization may qualify these markers as useful tools for earlier diagnosis, identification of mechanism of injury, and assessment of site and severity of injury. Hopefully, these biomarkers will facilitate early diagnosis, guide targeted intervention and monitoring of disease progression and resolution. Translational biomarkers have the potential to not only transform the way we do predictive nephrotoxicity.

W1954 PREDICTING TOXICITY IN PEOPLE.

The goal of personalized medicine is to deliver the right drug to the right person and at the right dose. Biomarkers may emerge as clinical tests to predict a patient's response to treatment. Among the best studied cases is warfarin. Warfarin is the most extensively utilized oral anticoagulant yet has a narrow therapeutic range with pronounced interindividual variation in response. Variants in gene underlying metabolism (CYP2C9) and the target of the therapy (VKORC1) have been shown to contribute to the unpredictability in dosing. However, it is not yet known if genetic tests could lessen the high number of potentially fatal adverse events such as hemorrhage, embolism and stroke. Several smaller scale clinical trials have been conducted to address this question, but the endpoints have differed and conclusions are mixed. C-Path is working with NHLBI on a definitive clinical trial to determine if there is clinical benefit to routine genotyping in determining warfarin dose. A 1,965 participant multicenter, double-blind, randomized, trial is in development comparing two possible approaches to guiding warfarin therapy initiation: 1) initiation of warfarin therapy based on an algorithm using clinical information and the individual's genotype relative to two genes known to influence warfarin metabolism (CYP2C9 and VKORC1 genes); 2) initiation of warfarin therapy based on an algorithm using only clinical information. Results from this trial are expected to elucidate the utility of genotyping in guiding dosing of warfarin to reduce adverse events and improve clinical outcome.

W1955 PHYSIOLOGICAL AND TOXICOLOGICAL TRANSCRIPTOME CHANGES IN HEPG2 CELLS EXPOSED TO COPPER.
M. Sang1, J. L.1 and J. L. Freydmann1,2,1 LMHT, NIEHS, Research Triangle Park, NC and 2 Biostatistics Branch, NIEHS, Research Triangle Park, NC. Copper is an essential trace element, however, at supraphysiological levels it can be extremely toxic. Principal components, K-means and hierarchical clustering, interactome, and pathway mapping analyses of transcriptomes of HepG2 cells exposed
to 100, 200, 400 and 600 μM copper for 4, 8, 12 and 24 h suggest that these exposure conditions induce physiological and toxicological changes. As a general trend, at the level of toxicity increases, the number and diversity of affected genes, Gene Ontology categories, regulatory pathways, and complexity of interactomes increases. Physiological responses to copper include transition metal ion binding and response to stress/stimulus; while toxicological responses include apoptosis, morphogenesis and negative regulation of biomolecule metabolism. Pathway mapping analysis indicates that copper modulates signal transduction pathways associated with MAPK, NF-κB, death receptor, IGF-1, hypoxia, IL-10, IL-2, IL-6, EGF, Toll-like receptor, protein ubiquitination, xenobiotic metabolism, leukocyte extravasation, complement and coagulation, and Sonic Hedgehog signaling. Interactome analysis using CytoScape suggested that HNF4α (hepatocyte nuclear factor 4 α) may be a major regulator of copper-responsive transcription. We investigated the expression changes of HNF4α after exposure to copper. Our data showed that copper exposure downregulated HNF4α expression at both the mRNA and protein levels. In addition, qRT-PCR and RNAi results demonstrated that the changes in HNF4α expression, in turn, modulated expression of target genes (UCHL1, TXNRD1, JUNB, LIPA, APOM and APOC1). We examined the changes in HNF4α expression after p53 knock down, which clearly showed the increase in the mRNA level of HNF4α. This data suggested that p53 may have a role in copper-induced downregulation of HNF4α expression. These results may provide insights into the global and molecular mechanisms regulating the response to copper exposure.

1956 CHEMICAL GENOMICS OF CANCER CHEMOPREVENTIVE DITHIOLETHIONES.

Q. T Tran, 1-4, L. Xu, 1-4, V. Phan, 1-4, S. Goodwin, 1-4, M. Rahman, 1-4, C. H. Sutter1, 4, B. Roebuck1, T. K. Kessler1, E. George1, 2 and T. R. Sutter1

Biology, The University of Memphis, Memphis, TN, 1Computer Science, The University of Memphis, Memphis, TN, 2Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH, 3Environmental Health Sciences, Johns Hopkins University Bloomberg School of Public Health, Hanover, MD and 4W. Harry Proctor Center for Genomic Research, The University of Memphis, Memphis, TN.

3H-1,2-Dithiole-3-thione (DST) and its analogs 4-methyl-5-pyrazinyl-3H-1,2-dithiole-3-thione (OLT, oltipraz) and 5-tert-butyl-3H-1,2-dithiole-3-thione (TBD) are chemopreventive agents that block or diminish early stages of carcinogenesis by inducing activities of conjugating and other detoxication enzymes. While OLT has been used in clinical trials, TBD has been shown to be more efficacious and possibly less toxic than OLT in animals. Here we describe and validate a novel, robust, and high-resolution chemical genomics procedure by examining the pharmacological structure-activity relationships of these compounds in livers of male Fischer F344 rats by microarray analyses. We identified 220 differentially expressed genes that were common to all treatments. Functional analysis identified the relation of these genes to glutathione metabolism and the NrF2 pathway that is known to regulate many of the protective actions of dithiolothiones. OLT and TBD were shown to have similar efficacies and were both weaker than DST. In addition, we identified 40 genes whose responses were common to OLT and TBD, yet distinct from DST. As inhibition of cytochrome P450 has been associated with the effects of OLT on cytochrome P450 expression, we determined the IC50 values for inhibition of cytochrome P450 1A2. The rank order of inhibitor potency was OLT >> TBD >> DST, with IC50 values estimated as 0.2, 12.8 and >100 μM, respectively. Functional analysis revealed that OLT and TBD, in addition to their effects on cytochrome P450, modulate liver lipid metabolism, especially fatty acids. Together these findings provide new insight into the actions of clinically relevant and lead dithiolothione analogs.

1957 INCORPORATION OF BIOCHEMICAL INTERACTION NETWORK INFORMATION HELPS IN IDENTIFICATION OF BIOLOGICALLY-RELEVANT PATHWAYS AND IMPROVES THE TOXICITY CLASSIFICATION OF CHEMICALS.

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With the increasing availability of molecular data collected from living systems under conditions of insult, it is imperative to focus on a smaller domain of the organism in order to understand the mechanisms by which these insults affect the organism. Biochemical pathways are an obvious target for studying molecular data. We propose a method for finding enriched pathways relevant to a studied condition that, in addition to using the measured molecular data, would use the structural information of the pathway viewed as a network of nodes and edges. Our results demonstrate a clear improvement in the identification of biologically-relevant pathways. In the context of chemical toxicity classification, we extend the above ideas (which apply to individual pathways) to the network of pathways in the organism. Two pathways are connected to one another in the pathway network if a signal from one of the pathways initiates activity in the other pathway. Genomic data is measured at snapshots in time. We consider a pair of chemicals to have a similar toxicity profile if the pathways that they affect are mostly the same. We can take this idea a step further by assuming that chemicals have similar toxicity effects if the pathways that one of them effect are known to initiate the observed affected pathways in the others. This motivates the underlying measure of closeness between a pair of chemicals as a distance their networks of enriched pathways (derived from their genomic data). We demonstrate the utility of these ideas in carcinogenicity classification and prediction using two sets of known liver carcinogens and non-carcinogens.

1958 DEVELOPMENT OF A GENE SIGNATURE FOR NON-GENOTOXIC CARCINOGENICITY.

N. Raghavan, D. Amarantunga, A. Nie and M. McMillian

Johnson & Johnson, Raritan, NJ.

Genomic drug safety screens will accelerate the process for developing safer drugs and limit the failure of drugs in late stage development due to toxicity issues, by identifying potential toxicity issues early in the development process. As a result, there is now heightened interest in developing biomarkers for predictive toxicity and risk assessment using toxicogenomic technologies within pharmaceutical companies as well as from governmental agencies worldwide. A widely-recognized challenge in using high-throughput technologies, such as microarrays, has been the problem of over-fitting the data, and consequently, the irreproducibility of results. In this talk, we present a novel and carefully crafted methodology for developing a toxicogenomic signature for non-genotoxic carcinogenicity using gene expression data from 24 hour microarray experiments on rats. This is of particular interest since short-term assays for non-genotoxic carcinogenicity, which is commonly observed in long-term rodent studies, have proven difficult to develop. A performance assessment demonstrates that the gene signature has superior predictive performance compared to classic algorithms. In addition, it is also compact and interpretable. We also discuss the need for validating and standardizing gene signatures, to make them portable to other platforms and technologies.

1959 COMPUTATIONAL MOLECULAR MODELING METHODS APPLIED TO TOXICITY SCREENING.

J. R. Rabiniowitcz1, S. B. Little1 and M. Goldsmith1


The risk to human health and the environment of chemicals that result from human activity often must be evaluated when relevant elements of the preferred data are unavailable. Therefore, strategies are needed that distinguish action and prioritize the outstanding data requirements. The rate determining step of many relevant mechanisms of action requires the chemical or one of its biotransformation products (the toxicant) to interact with a specific biological macromolecule (the target). A library of potential protein targets for chemical toxicity has been developed from the Protein Data Bank (www.rcsb.org/pdb). Molecular modeling methods have been used to determine the interaction between these targets and small libraries of environmental chemicals, the Laws 281 and the Forcast 320 (www.epa.gov/nct/dutos). The Laws 281 contains small organic chemicals. Each chemical has been tested under the same experimental conditions for competitive binding to rat estrogen receptors. Only 15 of these chemicals bind to the estrogen receptors, 3 – 5 orders of magnitude more weakly than estrogen. Computational docking methods that would be applicable to larger chemical data bases interacting with larger numbers of targets were employed to investigate the same library of chemicals interacting with the agonist and antagonist modes of both the alpha and beta rat estrogen receptor. These methods consider the potential ligands as flexible but not the target. All active chemicals were in the top 20% of those screened. Our analysis of these results considers the importance of decreasing the false positive rate while eliminating false negatives. When a pharmacophore filter based on two hydrophobic patches is employed to restrict the space explored, a significant decrease in the false positive rate was observed. All actives are found in the top 11% of the chemicals. This approach is applicable to many of the proteins in our library of potential targets. [This work was reviewed by EPA and approved for publication but does not necessarily reflect official Agency policy.]
1960 AN INTEGRATED IN VITRO AND COMPUTATIONAL APPROACH TO DEFINE THE EXPOSURE–DOSE–TOXICITY RELATIONSHIPS IN HIGH–THROUGHPUT SCREENS.

H. L. Clewell1, F. Boelmann1, C. Smith1, L. Tsai1, D. M. Rotroff1, D. J. Dix1, M. E. Andersen1, S. S. Ferguson2, E. LeChuyse2 and R. S. Thomas3, The Hammer Institutes for Health Sciences, Research Triangle Park, NC. 1CellzDirect, Invitrogen Corporation, Research Triangle Park, NC. and 3U.S. Environmental Protection Agency, Research Triangle Triangle Park, NC.

Research efforts by the US Environmental Protection Agency have set out to develop alternative testing programs to prioritize limited resources toward chemicals that likely represent the greatest hazard to human health and the environment. Efforts such as EPA’s ToxCast research program use high–throughput screening to evaluate large numbers of chemicals across a variety of in vitro assays. One challenge has been to provide a human exposure–dose context to the concentration values obtained in the in vitro assays. Organ slice cultures were established for rat liver, lung, and kidney. Cultures were exposed to 140 chemicals, a subset of the ToxCast 320, in a 5–point dose response (0.8–100 μM). Cytotoxicity was measured as the endpoint. Only 48 chemicals had measurable LC50 in at least one tissue, with kidney as the most sensitive organ (38 chemicals), followed by lung (10 chemicals), and liver (8 chemicals). For the 48 cytoxic chemicals, intrinsically clear–ance was measured using primary human hepatocytes and protein binding was measured in human plasma. Using these two pharmacokinetic parameters, equivalent oral exposures were estimated using SimCyp® software that would result in steady state tissue concentrations comparable to the LC50 values. This integrated approach promises to provide refined exposure–dose–toxicity evaluations that will aid in interpretation of high–throughput screening results. Such context will be essential for identifying appropriate priorities for follow–up testing and risk evaluation exercises. Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.

1961 MODULATION OF XENOBIOTIC METABOLIZING ENZYME AND TRANSPORTER GENE EXPRESSION IN PRIMARY CULTURES OF HUMAN HEPATOCYTES BY TOXCAST CHEMICALS.

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ToxCast chemicals were assessed for induction or suppression of xenobiotic metabolizing enzyme and transporter gene expression using primary human hepatocytes. The mRNA levels of 14 target and 2 control genes were measured: ABCB1, ABCB11, ABCG2, SLC01B1, CYP1A1, CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP3A4, UGT1A1, GSTA2, SULT2A1, HMGC2S, and control genes ACTB, GAPDH. These genes represent 5 nuclear receptor signaling pathways: AHR, CAR, PXR, PPARa and FXR. Gene expression was quantitatively measured by nuclear protection assays at 5 concentrations, 3 time points (6, 24, 48 hr), in 4 replicate wells. Hepatocytes from 2 male donors were isolated and cultured with 6 reference chemicals and 320 ToxCast phase I chemicals. Only a limited number of these assays have metabolic capacity, and it is not feasible to test all potential metabolites in the over 600 assays of ToxCast. In order to assess the magnitude of this issue and explore practical solutions, the ToxCast Phase I chemicals include 12 parent–metabolite pairs that allow comparisons of in vitro assay results between the parents and metabolites. Parent–metabolite differences were not seen when these same parent and metabolite cytoxicities were evaluated independently, indicating the limitations of these cell–based assays. Biochemical assay results also yielded distinct activities for certain chemicals, relative to the complementary parent chemical, emphasizing the need for development of biochemical assays with biotransformation capacity or to account for biotransformation through other approaches. A practical solution may include metabolic simulation or prediction, along with targeted testing of metabolites. Results from the wide range of assays in ToxCast, with and without metabolic capacity, are being incorporated into the data mining and interpretive processes. The bioactivity profiles of parent–metabolite pairs allowed us to identify assays with the potential to recognize chemical–specific biotransformation. Such potential assay panels may be further developed by the selection of assays, and parent and metabolite chemicals for Phase II of ToxCast. Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.

1962 PATHWAY–BASED CONCENTRATION RESPONSE PROFILES FROM TOXICOGENOMICS DATA.

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Microarray analysis of gene expression of in vitro systems could be a powerful tool for assessing chemical hazard. Differentially expressed genes specific to cells, chemicals, and concentrations can be organized into molecular pathways that inform mode of action. An important parameter in this hazard assessment is the lowest observed concentration (LOC) at which a particular pathway is perturbed. We present a method to derive LOC using microarray–based pathway analysis, and assess the reproducibility of results across biological replicates. Concentration response of pathways was modeled by clustering genes across concentrations, and then calculating enrichment across pathways. Because clustering continuous gene expression data can make comparisons between replicates difficult, we discretized fold–change to one of 3 values: 0 (no change), + (increase), or - (decrease). Genes with the same order of discretized values across concentrations were grouped together for pathway enrichment analysis. We applied this approach to data from a concentration–response study of rat primary hepatocytes treated with Phenobarbital, RU–486, or WY–14643, which are inducers of xenobiotic metabolism pathway via nuclear receptor. Cells were exposed to 4 different concentrations of each chemical, with 5 biological replicates per concentration–chemical combination. Although a total of 3^N (N–concentrations) unique signatures was possible, the observed number of pathways and concentration–response signatures observed was much lower. Affine–invariant expected results on xenobiotic metabolism, and the signatures gave a direct reading of the LOC at which gene expression in these pathways was significantly perturbed. Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.

1963 MODELING BIOTRANSFORMATION USING IN VITRO DATA ON PARENT–METABOLITE PAIRS WITHIN THE TOXICAST PHASE I CHEMICAL SET.


A major focus in toxicology research is the development of in vitro methods to predict in vivo chemical toxicity. Within the EPA ToxCast program, a broad range of in vitro biochemical and cellular assays have been deployed to profile the biological activity of 320 ToxCast Phase I chemicals. Only a limited number of these assays have metabolic capacity, and it is not feasible to test all potential metabolites in the over 600 assays of ToxCast. In order to assess the magnitude of this issue and explore practical solutions, the ToxCast Phase I chemicals include 12 parent–metabolite pairs that allow comparisons of in vitro assay results between the parents and metabolites. Parent–metabolite differences were not seen when these same parent and metabolite cytoxicities were evaluated independently, indicating the limitations of these cell–based assays. Biochemical assay results also yielded distinct activities for certain chemicals, relative to the complementary parent chemical, emphasizing the need for development of biochemical assays with biotransformation capacity or to account for biotransformation through other approaches. A practical solution may include metabolic simulation or prediction, along with targeted testing of metabolites. Results from the wide range of assays in ToxCast, with and without metabolic capacity, are being incorporated into the data mining and interpretive processes. The bioactivity profiles of parent–metabolite pairs allowed us to identify assays with the potential to recognize chemical–specific biotransformation. Such potential assay panels may be further developed by the selection of assays, and parent and metabolite chemicals for Phase II of ToxCast. Although this work was reviewed by EPA and approved for publication, it may not necessarily reflect official Agency policy.

1964 CLEAR EVIDENCE OF AHR RECEPTOR MEDIATED THRESHOLDS FOR CYP 1A1 AND 1A2 INDUCTION BY TCDD, TCDF AND 4-PCEDF IN RAT AND HUMAN.

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CYP1A1 and 1A2 induction are hallmark biomarkers of the aryl hydrocarbon receptor (AHR) activation by a host of ligands with 2,3,7,8–tetrachlorodibenzo–p–dioxin (TCDD) being the most well–known and studied. The existence of response thresholds and rodent/human differences for TCDD mediated effects are key questions for TCDD risk assessment, yet systematic studies exploring these questions in rat and human hepatocytes, a key target tissue for effects, are limited. CYP1A1 & 1A2 induction (mRNA and protein) was measured in vitro in rat and human primary hepatocytes exposed to 0.0001–100 nM TCDD, 2,3,4,7,8–Pentachlorodibenzofuran (4–PcCDF) or 2,3,7,8–tetrachlorodibenzofuran (TCDF). The large dose range, doses (11) and replicates (N=5 donors) were selected to provide robust data set for statistical analysis and modeling. CYP 1A1 mRNA induction response thresholds for human hepatocytes identified by extrapolation from
the EC50 (Silkworth et al., 2005) were between 10 and 50 pM for TCDD; the corresponding values in the rat were -one order of magnitude lower, demonstrating a significant reduced sensitivity in human relative to a common test species. A novel modification of the Silkworth approach that extrapolates from the significant response dose identified by a step-down trend test was developed and also applied to the induction data. The threshold for TCDD induced CYP 1A1 expression in human hepatocytes was somewhat lower using this method (2-6 pM), as were the rat thresholds (0.06-2 pM). The lower potency TCDD analogs 3CDD and TCDF each showed thresholds much higher than TCDD, as expected. These data confirm the existence of response thresholds for early biochemical responses to TCDD and species differences in sensitivity to TCDD-like compounds.

**1965** ATTENUATION OF HYPEROXYGENIC LUNG INJURY IN NEWBORN WILD TYPE AND CYTOCHROME P450 (CYP)1A2-NULL MICE EXPOSED PRENATALLY TO THE CYP1A INDUCER, BETA-NAPHTHOFAVOLONE (BNF).

X. J. Courouci, Y. W. Liang, W. Jiang, L. Wang and B. Moorthy. Pediatrics, Baylor College of Medicine, Houston, TX.

Supplemental oxygen administration is frequently administered to preterm and term infants having pulmonary insufficiency. However, prolonged hyperoxia contributes to the development of bronchopulmonary dysplasia (BPD) in human infants. In this study, we tested the hypothesis that pre-treatment of pregnant WT or Cyp1a2-null mice with BNF, followed by exposure to hyperoxia, would alleviate lung injury by mechanisms involving CYP1A2-mediated detoxification of F2-isoprostanes. Pregnant WT or Cyp1a2-null mice were treated, i.p., with the vehicle corn oil (CO) or BNF (20 mg/kg) on gestational days 16, 17, and 18, and newborn mice delivered from these mothers (on gestational day 22) were exposed to hyperoxia (> 95% O2) for up to 7 days. The WT or Cyp1a2-null newborn mice treated prenatally with CO showed increased lung injury, as determined by histology, at 4 and 7 days after hyperoxia, with Cyp1a2-null mice displaying increased susceptibility to lung injury compared to WT mice. Lung injury was significantly attenuated in WT as well as Cyp1a2-null mice that were treated prenatally with BNF. The BNF-treated mice showed increased expression of hepatic and pulmonary CYP1A1 and 1A2 protein (fourmulary/Western blotting) and mRNA (real time RT-PCR) expression, compared to those exposed to CO. These results suggest that in utero exposure to BNF induces CYP1A enzymes in the newborn mice, and that these mice are less susceptible to lung injury induced by oxygen. The extent of lung injury positively correlated with levels of F2-isoprostanes, as determined by GC/MS. Our observations support the hypothesis that BNF protects against hyperoxic lung injury by inducing CYP1A enzymes, which may act via detoxifying F2-isoprostanes. Future studies on the role of CYP1A enzymes in oxygen-mediated lung injury could lead to the development of rational strategies in the prevention/treatment of BPD in premature infants.

**1966** PERSISTENT INDUCTION OF CYP1A1 GENE EXPRESSION BY 3-METHYLCYANTHRENE (MC) IN HUMAN HEPATOMA CELLS IS MEDIATED BY SUSTAINED TRANSCRIPTIONAL ACTIVATION OF THE CYP1A1 PROMOTER.

F. S. Inayat, W. Jiang, L. Wang and B. Moorthy. Pediatrics, Baylor College of Medicine, Houston, TX.

3-Methylcholanthrene (MC) is one of the most potent polycyclic aromatic hydrocarbon carcinogens known to date. Metabolism of MC by cytochrome P450 1A (CYP1A) enzymes leads to the formation of chemically reactive intermediates that bind covalently to DNA, and initiate carcinogenesis. We reported previously that MC elicits a persistent induction of hepatic microsomal cytochrome P450 (CYP) 1A enzymes in rodents in vivo for several weeks after MC withdrawal. In this study, we tested the hypothesis that sustained induction of CYP1A1 gene expression by MC is mediated by persistent transcriptional activation of the CYP1A1 promoter in human hepatoma cells (HepG2). A plasmid containing the human CYP1A1 promoter (pGL3-1A1) and reporter (luciferase) gene was transiently transfected into HepG2 cells for 16 h, followed by MC (25 μM) or dimethylsulfoxide (DMSO) (solvent control) treatment, and luciferase expression was determined at 6, 12, 24, 48, 72 and 96 hours. MC elicited a 16-fold increase in luciferase activity at 24 hours time point in transfected cells compared to DMSO-treated controls. There was significant induction of luciferase expression even at the 96 hour time point. MC also caused sustained induction in the endogenous catalytic activity, apoprotein content and mRNA levels of CYP1A1 for up to 96 hours time point in treated cells. Electrophotoic mobility shift assays revealed that persistent CYP1A1 induction by MC may have, in part, involved Ah receptor-independent mechanisms. Pharmacokinetic experiments with radiolabeled [3H]MC revealed that the persistent induction of CYP1A1 in HepG2 cells was not due to persistence of the parent MC in the medium. In conclusion, our results support the hypothesis that persistent induction of CYP1A1 expression by MC is mediated by sustained transcriptional regulation of the CYP1A1 promoter. These observations have important implications for human carcinogenesis. (Supported by NIH grant ES09152.)

**1967** CAR-NULL MICE ARE SENSITIVE TO PARATHION.

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The Constitutive Androstane Receptor (CAR) is a key regulator of Phase I-III detoxification genes. Parathion is one of the two most potent environmentally relevant activators of CAR based on our transactivation assays. Animal studies were conducted to determine if CAR was activated by parathion in vivo. Male and female, wild-type and CAR-null mice were treated i.p. with 100μl of 10% DMSO/corn oil, 3mg/kg/day TCPQOBP or parathion at either 5 or 20mg/kg/day for two consecutive days. CAR-null mice showed greater sensitivity to parathion compared to wild-type mice as determined by tear formation, lethargy, and perturbation in gross motor skills. Parathion did not induce Cyp2b expression as determined by Q PCR or Western blot, but did induce CAR expression due to its short half-life. However, untreated CAR-null mice demonstrated lower expression of Cyp2b10 and Cyp2c29 than wild-type mice, indicating that CAR is important in the basal regulation of the Cyp2s. Previously published reports indicate that Cyp2b10 preferentially forms the non-toxic metabolite p-nitrophenol instead of paraaxon (toxic metabolite). Parathion incubation of micromes from untreated wild-type and CAR-null mice revealed differential metabolism between the genotypes. The paraaxon/p-nitrophenol ratio was higher in male CAR-null mice, indicating that parathion was preferentially metabolized to paraaxon, while the formation of p-nitrophenol was much greater in the wild-type mice. This is consistent with greater sensitivity in the CAR-null mice. We hypothesize that the drop in expression of Cyp2b10 in the CAR-null mice decreased the formation of p-nitrophenol. The formation of both paraaxon and p-nitrophenol was faster in female wild-type mice than female CAR-null mice, indicating that female wild-type mice show a slightly different strategy for protecting themselves from parathion. Because we are importing a greater percentage of our vegetable and fruit crops from Central and South America, places that still use parathion, we may be placing individuals who show repressed CAR expression, such as newborn children, at increased risk.

**1968** TETRAMETHOXYSTILBENE (TMS), AN INHIBITOR OF CYP1B1, DELAYS BENZO/A PYRENE (BP) METABOLISM AND DOES NOT PREVENT BP-INDUCED DNA DAMAGE IN MCF-7 CELLS.

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Exposure to carcinogenic polycyclic aromatic hydrocarbons (PAHs) induces cytochrome P450 (CYP) 1A1 and 1B1, which biotransform PAHs, including BP, into metabolites that form DNA adducts. We hypothesized that TMS, an analogue of resveratrol and a potent CYP1B1 inhibitor, may modulate BP-DNA adduct levels. At 2-12 hr intervals during 96 hr of exposure, we measured BP-DNA adducts by chemiluminescence immunoassay (CIA). CYP1A1 and 1B1 gene expression changes by relative real-time PCR, and CYP1A1/1B1 enzyme activity by ethoxyresoru-fin-O-deethylase (EROD) assay in MCF-7 breast cancer cells exposed to 1μM BP with or without 1μM or 4μM TMS. Peak BP-DNA adduct levels for BP alone, BP + 1μM TMS, and BP + 4μM TMS were 1572, 1658, and 1718 adducts/108 nucleotides, respectively, and were at 24, 48, and 60 hr, respectively. Similarly, fold-increase values for peak CYP1A1 expression were 758, 1713, and 2995 for BP alone, BP + 1μM TMS, and BP + 4μM TMS, respectively, with higher values (p<0.05) found in TMS-exposed groups. Fold-increase values for peak CYP1B1 expression were 50, 69, and 83 for BP alone, BP + 1μM TMS, and BP + 4μM TMS, respectively, with peaks occurring at the same time points as maximum CYP1A1 expression. Peak EROD activities for BP alone, BP + 1μM TMS, and BP + 4μM TMS were 2443, 3596, and 6987 fmoles/min/mg protein, respectively, and were at 24, 48, and 60 hr, respectively. Overall, addition of TMS to BP-exposed MCF-7 cells caused a delay in induction of BP-DNA adducts, CYP1A1 and CYP1B1 gene expression, and EROD activity. Furthermore, CIA, EROD, and luciferase values were increased for all endpoints in groups exposed to TMS. Therefore, CYP1B1 inhibition by TMS may not reduce PAH genotoxicity in vivo, and TMS may not be a good chemopreventive agent.
1969 CYTOCHROME P450B1 PROVIDES A NEW LINK BETWEEN LEPTIN AND OBESITY.

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Obesity enhances Type 2 diabetes and numerous cancers. The expression of cytochrome P450B1 (Cyp1b1), which is unique to mesenchymal cells, increases early in adipogenesis, prior to the adipogenic mediator, PPARγ (1). Cyp1b1 deletion increases Wnt-derived intestinal adenomas (2), alters neural patterning (3), and disrupts eye development (4). Here, we report that Cyp1b1-/- mice maintain basal adipose tissue levels when fed a high fat (60%) diet. MRI imaging (5) indicated uniform suppression of fat deposition. Dietary intake was unaffected in these mice, indicating enhanced fat metabolism. Improved glucose tolerance of Cyp1b1-/- mice suggested minimal oxidative damage (6). By contrast, fat deposition was not attenuated by Cyp1b1 depletion in obese leptin-deficient ob/ob C57BL/6j mice. Systemic leptin activity (7) may, therefore, be required for these Cyp1b1-mediated effects. Cultured Cyp1b1-/- pre-adipocytes differentiate norma-

1970 3-METHYLINDOLE-MEDIATED CYTOCHROME P450 INDUCTION IN PRIMARY NORMAL HUMAN BRONCHIAL EPITHELIAL CELLS.


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3-Methylindole (3MI) is a highly selective pneumotoxicant present in cigarette smoke. Several human cytochrome P450 (CYP450) enzymes, including 1A1 (CYP1A1), catalyze the dehydrogenation of 3MI to the reactive intermediate 3-methyleneindolenine (3MEIN). This metabolite has been shown to alkylate DNA and may induce apoptosis. In addition, 3MI has been observed to induce a dose de-

1971 A NEW VERTEBRATE CYTOCHROME P450 1 SUBFAMILY, CYP1D, AND CONSTITUTIVE EXPRESSION OF CYP1D1 IN ZEBRAFISH.

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Enzymes in the cytochrome P450 1A (CYP1A) subfamily oxidize polynuclear and halogenated aromatic hydrocarbons that are common environmental toxics. We identified a new CYP1 subfamily in zebrafish, CYP1D1, and found that this enzyme expresses 42 human CYP1 subfamilies, indicating that the induction was AhR dependent. These results indicate that low concentra-

1972 CYTOCHROME P450 GENES IN ZEBRAFISH DEVELOPMENT.

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This presentation summarizes observations on the full complement of CYP genes in zebrafish, a non-mammalian model for pharmacological, toxicological and car-

1973 IMPACT OF THE BIOLOGICAL ENVIRONMENT ON NANOPARTICLE COATING AND CYTOTOXICITY.


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Many studies have suggested that coating nanomaterials helps to protect cells from cytotoxicity; however, no long term exposure studies have shown the impact of the biological environment on coating stability. Previous studies from our laboratory have shown that polysaccharide coated silver nanoparticles (PS-Ag) were more compatible than hydrocarbon silver nanoparticles (HC-Ag) following a 24 h incubation in vitro. However, when the PS-Ag was kept in culture for up to one week, this biocompatibility was lost. The goal of this study was to evaluate the effects of the biological environment on the physico-chemical properties of nanomaterials. The PS-Ag 25nm and the HC-Ag 25nm were exposed to artificial alveolar, inter-

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difference in agglomeration between the two nanoparticle types. In addition, the
PS-Ag is more biocompatible than the HC-Ag however exposure to the alveolar,
intestinal, and lysosomal fluid had a stimulatory effect on the cells with an in-
crease in viability observed. In contrast, over time exposure to the gastric fluid
made the nanoparticles more toxic in a dose-dependent manner. This study
demonstrated when addressing nanotoxicity, the effect of the aqueous biological
environment not only effects the dynamics of aggregation and agglomeration but
also modulates the presentation and exposure properties of nanomaterials on cellular
and tissue systems.

**1974 DOSE AND RESPONSE METRICS IN ASSESSING IN VITRO AND IN VIVO NANO Particule TOXICITY.**

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The increasing number and variety of new nanoparticles (NPs) calls for the de-
velopment of low-cost in vitro screening assays for ranking NP toxicity. Unlike chem-
icals which toxicity can be measured by mass dose, the variety of physicochemical
properties of NPs (size, shape, crystallinity, etc) means that other parameters need
to be scrutinized in order to examine how useful these in vitro assays are in predict-
ing the in vivo toxicities. We explored several dose and response metrics with the
objective to predict in vivo toxicity from in vitro data. Critical to this effort are the
choices of biological endpoints and cellular systems that are relevant targets in vivo.
We assessed toxicity of several TiO2 NPs of sizes 3-100 nm. A rat lung Type 1 ep-
ithelial cell line (RI/1) was used for in vitro study; in vivo study involved intrastra-
chial instillation of NPs to rats. In vitro endpoints were lactate dehydrogenase
(LDH) release, protein carbonylation, and heme oxyge nase-1 (HO-1). Number of
PMNs in bronchoalveolar lavage fluid (BALF) was used as endpoint in vivo. Several
metrics were used to rank the toxicity of these NPs, including LD50 and the steep-
est slopes in the dose-response curve calculated using two different methods. We
then compared the correlations between the in vitro and in vivo ranking results.
Carbonylation and HO-1 assays were good markers of oxidative stress related toxi-
city. The steepest slope in the dose-response curve was the best response metric for
ranking NP toxicity when both dose and response were expressed in unit area.
It was concluded that toxicity rankings by certain in vitro assays were consis-
tent with the in vivo toxicity rankings when proper dose and response metrics were
utilized.

**1975 MECHANISMS OF INHALED MULTIWALLED CARBON NANOTUBE-INDUCED IMMUNOSUPPRESSION.**

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Inhalation of multiwalled carbon nanotubes (MWCNT) decreased showed sys-
temic immune function with is modulated by the COX-2 pathway. These studies report
on elucidation of this pathway. Male mice were exposed to atmospheres con-
taining 0.3 or 1 mg/m3 MWCNT for 6 hours/day for 14 consecutive days in whole
body inhalation exposure chambers. Approximately 18 hours after exposure mice
were assayed for systemic immune function in the spleen. Systemic immune func-
tion was compromised after 1mg/m3 MWCNT exposure. Spleocytes from ex-
posed animals were less able to produce antibody in response to antigenic stimuli
and exhibited decreased T cell proliferation when co-cultured with a mitogen
(Concanavalin A). Furthermore, spleocytes from exposed animals exhibited in-
creased gene expression of prostaglandin synthase enzymes. Prostaglandin synthase
enzymes catalyze the metabolism of arachidonic acid to prostaglandins known T
 cell suppressors. Therefore an additional inhalation exposure was conducted with
mice that received a Prostaglandin Synthase 2 (PTGS2 or COX-2) antagonist
(ibuprofen) in their drinking water. Ibuprofen treated animals exhibited significant
responses in rodents with very early termination of the acute phase and rapid onset of
chronic fibrosis. Timely elimination of the MWCNT from the lungs, through apopto-
sis and their subsequent clearance by macrophages is a necessary stage in the resolution of pulmonary inflammation whereby NADPH oxidase con-
tributes to control of apoptotic cell death and clearance of PMNs. Thus, we hy-
potthesized that after NADPH oxidase nonsense mice exposed to MWCNT from the acute inflammation to the chronic fibrotic stage in response to SWCNT.
To experimentally address the hypothesis, we employed NADPH oxidase-deficient mice which lack the cytochrome b subunit of the enzymatic complex. We found that
NADPH oxidase null mice responded to SWCNT exposure with a marked accu-
mulation of PMNs and elevated levels of apoptotic cells in the lungs, production of
pro-inflammatory cytokines, decreased production of the anti-inflammatory and pro-fibrotic cytokine, TGF-β, and significantly lower levels of collagen deposition,
as compared to C57BL/6 control mice. These results demonstrate a role for
NADPH oxidase-derived reactive oxygen species in determining course of pul-
monary response to SWCNT.

**1978 DIFFERENTIAL GENOTOXIC EFFECTS OF TRANSITION METAL NANOPARTICLES: THE ROLE OF OXIDATIVE STRESS.**

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Nanotechnology is considered to be one the world’s most promising new techno-
gologies. It directly improves our lives in areas as diverse as engineering, information
technology, and diagnostics. Our current knowledge of the health effects of metal

**1976 NADPH OXIDASE REGulates NeUTrophil AND FIBROSIS IN C57BL/6 MICE EXPOSED TO CARBON NANOTUBES.**

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Airborne exposure to environmental particulates is associated with inflammation and adverse health effects, in particular increased pulmonary and cardiovascular morbidity and mortality. Scavenger receptors, expressed on the cell surface of
macrophages, have been implicated as responsible for recognition and internaliza-
tion of micron-sized environmental particles. However, the molecular mechanism of engineered nanoparticles recognition and uptake has not been addressed. Recently, the three-dimensional structure of the cysteine-rich domain (SRCR) of
the macrophage receptor with collagenous structure (MARCO) has been deter-
mined by X-ray crystallography. The SRCR domain of MARCO was shown to be
involved in cell interactions with LTA, LPS, intact bacteria, and some metal oxide particulates. In this work, the binding of silicates to MARCO is characterized
through computer simulations involving its SRCR domain and a number of sili-
cates. Molecular docking of a variety of nanowire silicates were used as screening
probes to identify the putative nanosilicate binding sites on MARCO surface.
Molecular dynamics simulations reveal that the binding is an energetically favorable
process and involves the arginine cluster at the -sheet outer surface. Although the
secondary structure of MARCO is maintained, significant realignment at the
dimer interface was observed upon substrate adhesion slightly altering MARCO
electrostatic surface potential signature. These results highlight, for the first time,
potential differences between MARCO interactions between micron and nano-
scaple particles, and suggest potential receptor structural realignments that may
facilitate nanoparticle induced signaling in macrophages. Supported by R01
ES016212 and the Environmental Biomarkers Initiative.
nanoparticles (such as nano-size nickel, cobalt and titanium dioxide) is limited but suggests that metal nanoparticles may exert more adverse effects than standard-sized particles. This study addresses the overall hypothesis that transition metal nanoparticles exert genotoxic effects via alternation of cell homeostasis through a mechanism mediated by oxidative stress. In this study, we used an intracellular insultation model, and in vitro system, to study the potential genotoxic effects of transition metal nanoparticles. Our results show that exposure to Nano-Co and Nano-Ni caused reactive species species (ROS) generation in vitro and in vivo. Exposure of lung epithelial A549 cells to Nano-Co or Nano-Ni resulted in p53 protein expression, modification and down-regulation of Rad-51 protein expression, and increased formation of H2AX foci which were abolished by pretreatment of cells with ROS scavangers or inhibitors. The results were further confirmed by in vivo studies; the concentration of 8-OhdG in mouse lungs exposed to Nano-Co and Nano-Ni significantly increased over time after exposure. Furthermore, proliferating cell nuclear antigen (PCNA) staining in mice lungs exposed to Nano-Co and Nano-Ni significantly increased over time and were greater than that caused by Nano-TiO2. Our results suggest that metal nanoparticles cause oxidative stress which is involved in nanoparticle-induced DNA damage and genotoxic effects. These findings have important implications for understanding the potential health effects of nanoparticle exposure.

**PL 1979** **INDUCTION OF ANEUPLOIDY BY SINGLE WALLED CARBON NANOTUBES.**


Engineered carbon nanoparticles are newly emerging manufactured particles with potential applications in multiple fields, including electronics, computers, and aerospace. The low density and small size of these particles makes respiratory exposures to workers likely during the production or use of various commercial products. To examine the potential of nanotubes to induce genetic damage in normal lung cells, primary and immortalized human small airway epithelial cells were cultured and exposed to single walled carbon nanotubes (SWCNTs) or a positive control vanadium pentoxide. The nanotubes had an average diameter of 1.1 nanometer and a length of 50-100 microns. Celluar tubulin, mitotic spindle integrity and centriole number were determined by immunofluorescence for beta-tubulin and centrin and photographed using fluorescent and confocal laser scanning microscopy. The chromosome number was examined by fluorescent in situ hybridization. After 24 hours of exposure to either SWCNT or vanadium pentoxide, binucleate cells, bundled tubulin and fragmented centrosomes were present. Abnormalities included changes in mitotic spindles, including multiple poles that resulted in aneuploid chromosome number. Confocal microscopy demonstrated nanotubes within the nucleus that were in association with cellular and mitotic tubulin as well as the chromatin. These findings indicate that these SWCNTs can enter the nucleus, inducing mitotic spindle disruption and abnormal chromosome number. Thus, our study indicates that direct interaction between chromatin and SWCNTs may contribute to genetic changes in somatic cells. Exposure to agents that interfere with the formation and movement of the mitotic spindle apparatus and cause abnormalities in chromosome number result in a greater risk of cancer.

**PL 1980** **QUANTITATIVE STRUCTURE ACTIVITY MODELING OF GOLD NANOPARTICLE TOXICITY IN A ZEBRAFISH DEVELOPMENTAL SYSTEM.**

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Understanding how the surface physiochemical properties of nanomaterials dictate biocompatibility and potential toxicity is a central challenge in the emerging field of nanotoxicology. Although Quantitative Structure Activity Relationship (QSAR) modeling has been useful for deriving predictive relationships between the properties of chemicals and their biological effects, there has been little effort to evaluate the accuracy of QSAR methods in predicting biological response from nanomaterials. In this work, experimental results were employed to develop a generalized linear model to predict the probability of developmental toxicity in zebrafish embryos 24 hours post-fertilization. The experiment consisted of exposing zebrafish embryos to eight different concentrations of gold nanoparticles, with three different core sizes and four different surface modifications with chemical ligands. The toxicity model was statistically developed using physical and structural characteristics of the gold nanoparticles, including nine different chemical property descriptors as predictor variables. Beyond particle size and concentration, two of the QSAR variables investigated (polar surface and solvent accessible surface area) were found to be statistically significant predictors of toxicity. The model exhibits good performance, and because all the predictor variables are continuous, it can be used to point to regions in the experimental space with reduced toxicity and aid to further the understanding of the mechanisms that influence it.

**PL 1981** **THE EFFECT OF CADMIUM EXPOSURE ON CALCIUM HOMEOSTASIS AND SIGNALING PATHWAYS.**

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Cadmium constitutes a threat to human health. Yet the exact molecular mechanisms involved in mediating transcriptional responses to cadmium remain unresolved. Cadmium has been shown to elevate intracellular calcium levels, which could activate multiple signaling cascades. Here we report the functional analysis of two cadmium responsive genes in the nematode Caenorhabditis elegans, numr-1 and numr-2, whose expression, in part, is regulated by alterations in intracellular calcium ((Ca2+))i) levels. In the absence of metal, constitutive expression of numr-1/2 is developmentally regulated with maximal expression in intestinal cells during the L1 larval stage and minimal expression in adults. When adult nematodes are exposed to metal, numr-1/2 expression increases dramatically in intestinal cells. In C. elegans the intestinal cells experience endogenous endoplasmic reticulum (ER) stress during development and cadmium is a toxicant that induces ER stress. It has been proposed that cadmium can increase intracellular calcium levels through the release of calcium from ER stores. We found that when adult nematodes carrying a NUMR-1::GFP translational fusion are exposed to thapsigargin or a calcium ionophore, numr-1 expression dramatically increases throughout the intestine, suggesting alterations in (Ca2+)i levels may regulate numr-1/2 expression. To gain a better understanding of the effects of cadmium on (Ca2+)i we used a protein based calcium ion sensor,cameleon YC 3.60, expressed in HEK 293 cells. We found that exposing HEK 293 cells to 30μM cadmium decreased the release of intracellular calcium ions by treatment with ionomycin; suggesting that ER calcium stores had been depleted. Taken together, our data suggests that cadmium affects intracellular calcium levels, which may ultimately regulate the transcriptional response of cadmium inducible genes. These results offer insights into the effect of cadmium exposure on calcium homeostasis and signaling pathways.

**PL 1982** **MANGANESE TOXICITY IN CELLS THAT HYPERACCUMULATE PHOSPHATE INVOLVES PROTEIN TURNOVER EFFECTS OF THE PROTEOSOME.**

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Manganese ions are known to form tight complexes with phosphate in vitro, and we have investigated the effects of intracellular phosphate on manganese using the bacteria yeast *S. cerevisae.* Phosphate accumulation and metabolism in *Saccharomyces cerevisae* is regulated by the cyclin dependent kinase Pho85 and the cyclin Pho80. When phosphate is abundant, they negatively regulate the transcription of phosphate transport and metabolism genes. Cells with a genetic deletion of pho80 Δ accumulate increased amounts of multiple species of phosphate. The pho80 Δ genetic deletion strain also accumulates increased levels of manganese when exposed to high manganese and is very sensitive to manganese toxicity. To investigate the mechanism of manganese sensitivity in the high manganese and hyperphosphate *S. cerevisae* strain, we compared the transcriptional profile of pho80 Δ vs WT strains exposed to manganese. As expected, a number of phosphate metabolism genes were up-regulated, including the metal-phosphate transporter PHO84. However, up-regulation of the metal-phosphate transporter only partially explains the sensitivity towards manganese and both PHO84 dependent and independent pathways are responsible for manganese sensitivity. In a screen for multi-copy suppressors of the pho80 Δ manganese sensitivity, we isolated RAD23, which encodes a proteasome-associated protein. RAD23 does not act at the level of transcription and instead works downstream in a PHO84 independent fashion to reverse manganese toxicity. The reduction in manganese sensitivity is dependent on the N-terminal UBL (‘ubiquitin-like’) domain of Rad23 that interacts with the proteosome. We conclude that manganese toxicity in cells that hyperaccumulate phosphate involves protein turnover effects of the proteosome.

**PL 1983** **BIS(MALTOLATO)OXOVANADIUM ACTIVATES STAT-1 IN HUMAN LUNG FIBROBLASTS AND ANTAGONIZES IL-13-INDUCED STAT-6 SIGNALING.**

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Bis(maltolato)oxovanadium(IV) (BMOV) is a bioavailable vanadium-based compound that has potential therapeutic value for the treatment of diabetes. We have previously shown that vanadium analogs activate signal transducer and activator of
transcription-1 (STAT-1) which is protective against fibrosis of the lungs after injury with bleomycin. Interleukin-13 (IL-13), which is a potent activator of STAT-6, is a major mediator in the progression of pulmonary fibrosis. We hypothesize that BMV causes the causative elements of lung fibrosis by activating STAT-1, which antagonizes IL-13 induced STAT-6 binding. To address this hypothesis, we confluent cultures of human lung fibroblasts (HLF) were rendered quiescent in serum-free medium and then dosed with both BMV and IL-13 alone or in combination. We then collected cell lysates for Western blot analysis and cell supernatants for ELISA assays at various time points to measure STAT phosphorylation and levels of the STAT-1-regulated chemokine CXCL10, respectively. In this study we show that BMV induced STAT-1 phosphorylation at 6 hrs post-exposure, but did not activate STAT-6. In contrast, IL-13 activated STAT-6 phosphorylation within 5 min but did not activate STAT-1. Interestingly, BMV significantly reduced IL-13-induced STAT-6 phosphorylation. BMV also stimulated fibroblasts to secrete CXCL10, a chemokine that inhibits angiogenesis and promotes epithelial repair. Our data suggest that BMV inhibits pro-fibrogenic signaling pathways through a STAT-1-dependent mechanism. We propose that activated STAT-1 could reduce fibrosis in two ways; first, by antagonizing IL-13-induced STAT-6 signaling and second, by increasing CXCL10. Our data suggest that dietary BMV could serve as a potential treatment for pulmonary fibrosis, a fatal disease for which there is currently no effective treatment strategy. Further study will test the therapeutic value of BMV in a mouse model of pulmonary fibrosis. (Funded by NIH R21-ES015801-01).

1984 SYSTEMS BIOLOGY-DEFINED CROSSTALK BETWEEN P53 AND NFκB SIGNALING AND MODULATION BY ARSENIC.

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Arsenic (As) is a well-known environmental toxicant and carcinogen as well as an effective chemotherapeutic agent. The underlying mechanism of this dual capability, however, is not fully understood. In our previous study, our microarray-based gene expression analysis demonstrated that arsenic-induced cell cycle arrest and apoptosis in p53-proficient and p53-deficient cells occurred through differential gene pathways, suggesting a potential role of NFκB pathway in As-induced apoptosis in p53−/− cells. In this study, we examined the crosstalk between the p53 and NFκB pathway by applying a systems-biology-based approach. These results were verified by comparing the activation of NFκB by western blot analysis of phosphorylated NFκB p65 (p-p65) and IκBα (p-IκBα) and cytokines by cytokine protein array. In the canonical pathway-based analysis between the gene expression from p53−/− and p53+/+ cells, we found that deletion of the p53 pathway resulted in significant up-regulation of NFκB related signaling pathways, suggesting a possible inhibitory effect of p53 on NFκB. A transcriptional regulatory network analysis revealed that As treatment resulted in activation of transcription regulatory elements CAAT and NRSF in the p53+/+ cells while in the p53−/− cell As treatment seemed to reduce p-p65 and p-IκBα transcription activity. We observed significant higher level of p-p65 and p-IκBα in p53−/− cells as compared to p53+/+ cells. Treatment of As reduced p-p65 and p-IκBα in a dose-dependent manner in p53+/− whereas increases of the p-p65 and p-IκBα were observed in p53+/+ cells. Our study provides strong support for the existence of cross-talk between p53 and NFκB signaling pathways, and demonstrates that As induces apoptotic processes in p53−/− cells occur through the down-regulation of NFκB, a p53-independent pathway (EPA R826862 NIEHS PO1ES09601, R01-ES15063, P30 ES07033, UW Royal Research Fund and CAAT Award)

1985 LEAD INHIBITS BONE FORMATION THROUGH A SCLEROSTIN-DEPENDENT MECHANISM.

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Lead exposure can adversely affect the skeleton by reducing bone formation, compromising fracture healing and decreasing bone density. This last finding portends lead exposure as a risk factor for osteoporosis. Unfortunately, the mechanisms by which lead has its effect have not been well characterized. Sclerostin is an actively produced protein that plays a role in the control of osteogenesis. Its inhibitory effect is mediated through a dose dependent clear-cut stimulation of sclerostin levels in osteocytes and osteoblasts. We have also identified a putative Smad repressor element in the promoter for sclerostin. Upon basal stimulation of the TGFbeta pathway, sclerostin expression is normally low. However upon inhibition of TGFbeta and sclerostin signaling, sclerostin increases. Therefore, we investigated the effects of lead on Smad phosphorylation and found a strong blockage of the kinase responsible for its phosphorylation. We hypothesize that lead depresses bone formation through an up regulation of sclerostin and that this regulation is mediated, in part, by blocking Smad activation. The significance of this research lies in the possibility of treating patients with anti sclerostin therapies for the prevention of lead-induced osteoporosis.

1986 EPIDERMAL GROWTH FACTOR RECEPTOR DEPENDENT HYPOXIA INDUCIBLE FACTOR-1α EXPRESSION BY MONOMETHYLARSONOUS ACID (MMA III) IN HUMAN BLADDER CELLS.

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Arsenic is a human bladder carcinogen. Recent evidence showed that human bladder cells (UROtsa) are capable of bioconverting arsenic to monomethylarsonous acid (MMA[III]) and can also be transformed following low, long-term, low-level exposure of MMA(III). Expression of hyoxia inducible factor-1α (HIF-1α), a major regulatory protein of cellular response to hypoxia, correlates with angiogenesis and an unfavorable prognosis in bladder cancer. Acute exposure of MMA(III) [10nM, 50nM and 100nM; 6 h] induced a concentration-response HIF-1α protein expression in UROtsa cells. Inhibitors of the mitogen-activated protein kinase pathway, epidermal growth factor receptor (EGFR), and reactive oxygen species (ROS) led to attenuated expression of HIF-1α. Inhibitors of Src and ROS reduced the phosphorylation of EGFR induced by 50nM of MMA III. Elevation in HIF-1α was also observed following 52 weeks of 50 nM MMA(III) exposure. These results indicate that MMA(III) can induce HIF-1α expression by a mechanism involving Src and ROS dependent EGFR activation. This finding may provide more understanding of arsenic induced angiogenesis and its promotion of bladder tumor progression. Supported by Training Grant (ES 07091) and the Superfund (ES 04940)

1987 ARSENIC PERSURURATION OF ALLERGY/ASTHMA SIGNAL TRANSDUCTION IN MAST CELLS.

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Millions of people worldwide are exposed to arsenic (As) via drinking water, various foods, and industrial and mining sites. Ingestion of As has been associated with increased risk of various cancers, cardiovascular disease, and reproductive abnormalities. As has been well-described as potent endocrine disruptor, which may underlie many of its toxic effects. Also, recent epidemiological studies in Bangladesh suggest that As exposure elevates asthma incidence, but the underlying mechanism(s) of action are unknown. The most common form of asthma is allergic asthma, in which mast cells are major effector cells. Antigen (Ag) or allergen crosslinking of IgE-bound receptors on mast cells leads to signaling, beginning with tyrosine phosphorylation and ending with degranulation, the release of histamine and other allergy asthma mediators. It was recently shown that environmental exogenous xenobiotics can exacerbate degranulation, thereby potentiating the allergic and asthmatic response. Thus, we have tested whether As affects degranulation. Using the rat basophilic leukemia (RBL) mast cell model, we have measured degranulation in a fluorescence microplate assay based on release of beta-hexosaminidase. In assays in which RBL cells were exposed short-term to sodium arsenite, plus or minus Ag addi- tion, we have found that As-alone has no effect on degranulation but As plus Ag perturb degranulation. We have observed a potentiation of degranulation due to very low dose As (~2.5 ppb). Conversely, at higher As doses, we have observed inhi- bition of degranulation (without cell death): ~15% at 100 ppb, ~25% at 250 ppb, and ~40% at 500 ppb. We have confirmed that the interaction of beta-hexosa- minidase with its substrate is unaffected by As, showing that the As effects are on mast cell signaling. Inhibition at high doses may be a mechanism underlying the traditional Chinese medicine use of As to treat asthma. As’s potentiation of degranulation at low, environmentally-relevant doses may be a mechanism by which As el- evates asthma incidence.
PL 1988  DISRUPTED POLYAMINE SYNTHESIS AND COBALAMIN TRANSPORT CONTRIBUTES TO ABERANT METHYL METABOLISM DURING CHRONIC ARSENIC EXPOSURE.

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Arsenic, a human carcinogen that may target the prostate, can be enzymatically methylated using S-adenosylmethionine (SAM) as the methyl donor. After chronic low-level arsenic exposure, the human prostate cell line RWPE-1, which cannot methylate arsenic, acquires a malignant phenotype with DNA hypomethylation. These cells show disrupted methyl metabolism with increased homocysteine (Hcy), transsulfuration pathway activation, glutathione over-production and decreased SAM as part of a complex acquired adaptive tolerance to arsenic concurrent with malignant transformation. Because cofactor deficiencies and the polyamine synthesis can cause similar changes in SAM and Hcy, we studied the effect of acquired adaptation to arsenic (5 μM arsenic ≤ 16 weeks) on polyamine synthesis/regulation genes and cobalamin transporters. The polyamine synthesis genes ornithine decarboxylase (ODC1) and spermidine synthase, which use a SAM donor to synthesize spermidine, showed elevated expression with arsenic. Expression of c-myc, a positive regulator of ODC1, was also elevated by arsenic while the transcription factor E2F1, which regulates the SAM synthesizing enzyme methionine adenosyltransferase, was drastically reduced. Thus, arsenic stimulates the SAM consuming polyamine synthesis pathway. Hcy remethylation depends on the cofactor cobalamin which replenishes the methionine pool required for SAM synthesis. Cobalamin deficiency causes Hcy accumulation and may play a role in carcinogenesis. Transcript levels of the cobalamin transporters, transcobalamin (TCN) 1 and 2 were either abolished (1) or strongly reduced (2) by arsenic. Cobalamin deficiency could lead to Hcy accumulation and thereby indirectly inhibit methylene transferase activity and, ultimately, DNA methylation, while activation of polyamine synthesis, often observed in cancer, could consume SAM and directly impact DNA methylation. These changes could ultimately contribute to arsenic-induced malignant transformation.

PL 1989  EFFECTS OF TUNGSTEN ON PHOSPHATE-DEPENDENT BIOCHEMICAL PROCESSES.

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Tungstate, the oxidized form of tungsten (W) metal, is known to polymerize with phosphates, resulting in disruption of phosphate reaction in tissues. A series of studies were conducted to evaluate the effect of tungsten on several phosphate-dependent intracellular functions, including energy cycling (ATP production), regulation of enzyme activity (protein tyrosine kinase (PTK)), and intracellular secondary messengers (cyclic adenosine monophosphate [cAMP]). Rat non-cancerous (Clone-9), rat cancerous (H4IIE), and human cancerous (HeP2) liver cells were used to compare rat vs. human cell response as well as cancerous vs. non-cancerous cell responses. Cells were exposed to 0, 1, 10, 100, 1000 mg/L tungsten, in the form of sodium tungstate, then analyzed for cell viability, ATP concentration, tyrosine kinase activity, and cAMP concentration. In general, tungsten significantly reduced cell viability in all three cell lines at concentrations greater than 100 mg/L; H4IIE cells were most sensitive to tungsten cytotoxicity. Tungsten did not affect ATP concentrations in all three cell types. Tungsten decreased PTK activity by 30% in Clone-9 cells but dramatically increased PTK activity by 70% in HeP2 cells. A horseradish peroxidase in PTK activity was also seen in HeP2 cells after a 24 hr exposure to tungsten, but the horseradish response was not observed after a 48 hr exposure to tungsten. Tungsten increased cAMP by 60% in Clone-9 cells. Tungsten also increased cAMP in H4IIE cells at 100 mg/L and in HeP2 cells at 10-100 mg/L, though at much lower levels than in Clone-9 cells. In conclusion, tungsten appears to increase cellular kinase activity and secondary messenger concentrations, yet the responses were species-specific and cancer-cell specific. Thus, these data indicate that tungsten produces complex results that must be carefully interpreted in the context of the respective animal model used.

R 1990  WHAT IS AN ADVERSE EFFECT IN THE AGE OF OMICS?


Research in biology has recently been characterized by a switch in emphasis from reductionist studies that describe an organism based upon its biological components to more integrative approaches that emphasize the processes through which these parts interact to produce the complex systems that form an organism. As it is applied to toxicology, this development has provided the opportunity to understand how perturbations of molecular-level signaling and regulatory pathways elicit apical toxic responses. In accord with these developments, the NRC (Toxicity Testing in the 21st Century: A Vision and a Strategy) has proposed a major paradigm shift. They believe that toxicity testing and risk assessment should involve characterization, with an emphasis on relevant levels of exposure and dose-time response surfaces for in vitro perturbation of toxicity pathways. Before this approach can become a standard practice in risk assessment, and potentially, in biomonitoring, substantial research and development is needed to enhance data generation and analysis capabilities for characterization and prediction of both exposure and tissue dosimetry. Thus, a comprehensive set of toxicity pathways and cell culture systems that embody these pathways, delineation of adaptive responses from frankly toxic responses, and correlation of the in vitro dose-time response surfaces for perturbation of toxicity pathways with apical responses measured in vivo (i.e. responses that are either adverse or that represent critical steps on the route to adverse responses) should be specified. Finally, computational models of in vivo biology that will integrate toxicity pathway data obtained in vitro to predict in vivo dose-time response should be developed. The materials presented should enable us to consider a subset of these issues, highlighting some of the practical challenges posed by the NRC recommendation.

R 1991  CAREER OPPORTUNITIES AND TRANSITIONS IN TOXICOLOGY.

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It’s never too early, or too late, to think about where your career in toxicology will lead you next. Whereas students and postdocs are typically familiar with the ins and outs of pursuing an academic research career, opportunities to investigate non-academic careers in toxicology can be few and far between. Early-career scientists often ask “What careers are available to toxicologists?” What skills and experiences do I need in order to be competitive for these positions? Such questions are also relevant to established toxicologists looking to expand their work experiences or embark upon a new career path. Toxicologists that practice in various work sectors are faced with the difficult and sometimes painful task of transitioning from one sector to another, as each sector often demands unique skills. Most often, guides on career transitions are not readily available for these toxicologists. The material presented will provide participants with insight into toxicology careers in diverse settings, including industry, government, consulting groups, and nonprofit organizations, and provide information about career transitions across the various sectors. Our panel of experienced toxicologists will describe the paths that their careers have taken, intentional or otherwise. Both practical and applicable advice will be offered for those participants interested in pursuing similar avenues, or for those just wishing to step off the beaten path. The presenters will highlight their motivations, challenges, success stories, and lessons learned. Be sure to bring questions to ask our panel of seasoned toxicologists during the interactive question and answer period. Whether you are a graduate student ready to jump into a job search, or an established scientist looking to move your career in an unexpected direction, join us for an interactive and informative discussion designed to expand your awareness of unique and exciting scientific career opportunities for toxicologists and more importantly, how to successfully transition between sectors.

R 1992  PHOTOTOXICOLOGY: A PASSING FANCY OR ENDURING CONCERN.

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Light is a necessary part of our existence as both air and water. Light is central to our interactions with both natural and artificial environments. Therefore we face a constant need to assess both risks and benefits, and to establish balanced guidelines for exposure in discussing this aspect of phototoxicology, which involves the influence of medications on biological responses to light (principally visible and ultraviolet radiation). Chronic phototoxicity is a proven precursor to skin cancer in human populations. Following up on earlier reports by R. Stern and others, a recent publication by Karagas and colleagues provides epidemiological evidence that reported use of photosensitizing medicine was associated with an increased risk of both basal cell carcinoma and squamous cell carcinoma. The widespread use of photostimulating drugs and chemicals mandates adequate characterization of drug interactions and heightened counseling on sun exposure. In 2002 and 2003, the Committee for Proprietary Medicinal Products (CPMP) and the U.S. FDA published guidance documents describing their respective regulatory preferences for the testing of substances for phototoxicity and photo co-carcinogenicity. The EU has adopted an in vitro test method for photocarcinogenicity testing. Technical specialists generally, and the pharmaceutical industry in particular, have described problems with this regulatory guidance, particularly with regard to the over-prediction of in vitro tests and the duration of the in vivo tests. In the U.S. and other regions, the outcome of
these tests may help to determine more appropriate wording that may be used in product labels to adequately characterize the risks of excess sun exposures. Thus, it is important that strategies for providing relevant safety information and novel testing approaches to provide useful short-term safety data to predict photoxicities and photo co-carcinogenicity be explored.

1993 LEAD: CHILDREN'S EXPOSURES AND CURRENT REGULATORY STANDARDS.


Recent news reports raise concerns about children's exposure to lead in toys, paint, candy, and other everyday materials. While the toxicity of lead has been well studied and the use of lead in many materials has been reduced or eliminated over the past 30 years, the possibility of lead exposure in children from both past and current uses of lead remains a public health priority. While a major contributor to lead exposure in children continues to be from the home, 30% or more of children aged 0-6 years with lead poisoning are exposed to lead through sources other than residential lead paint such as cosmetics, folk and traditional medications, painted and metallic toys and trinkets, and ceramic food ware. Examples regarding lead exposure and risk assessments from sources such as lead based ceramics in schools and bioassessability of lead exposure in children's products will be presented. Numerous agencies are involved in controlling lead exposure and enforcing regulations including the Centers for Disease Control and Prevention (CDC), U.S. Consumer Product Safety Commission (CPSC) and the U.S. FDA. CPSC regulates more than 15,000 types of consumer products including the lead content of numerous products including paint and similar surface coatings on products and furniture intended for consumer use, including toys. The FDA is responsible for ensuring the safety of products as authorized in the Federal Food, Drug and Cosmetic Act. FDA has addressed lead issues for diverse products such as pesticides, metal food cans, lipstick and crystal baby bottles for infants. An overview of known mechanisms of lead toxicity as well as newer research on the long term effects of lead will be presented.

1994 MULTIPLE GENES EXHIBIT PHENOBARBITAL (PB)-INDUCED CONSTITUTIVE ACTIVE/ANDROSTANE RECEPTOR (CAR)-MEDIATED DNA METHYLATION CHANGES DURING LIVER TUMORIGENESIS AND IN LIVER TUMORS.

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The constitutive active/androstane receptor (CAR) mediates responses to the nonsteroidal rodent liver tumor promoter phenobarbital (PB), including certain gene expression changes, hepatomegaly, and tumor formation. Abrupt DNA methylation represents epigenetic events that can play multiple roles in tumorigenesis. Previously, 146 unique PB-induced regions of altered DNA methylation (RAMs) were observed in liver-tumor susceptible CAR wildtype (WT) mice (in 23 weeks, precancerous tissue and 32 weeks, tumor tissue), as compared to the resistant knockout (KO). We believe that at least some of these might be key for tumorigenesis (Phillips et al., 2007). In this study, cloning and annotation of a subset (82%) of the unique RAMs revealed 47 genes exhibiting altered methylation; 17 are already implicated in cancer or related processes. Thus, we have identified 30 “new” candidate genes that might be involved in carcinogenesis due to an epigenetic alteration. These may contribute to tumor development through their involvement in angiogenesis, apoptosis, epithelial-mesenchymal cell transition, growth/survival, and invasion/migration/metastasis. We have also, previously, discovered unique PB-elicted RAMs in liver tumor-prone B6C3F1 mice, as compared to the relatively resistant C57BL/6 strain, at 2 or 4 weeks (Bachman et al., 2006), and identified 51 genes exhibiting altered methylation (Phillips and Goodman, 2008). Importantly, 11 of these genes were identified from identical, unique RAMs discerned in both the sensitive B6C3F1 and CAR WT mice, thus representing an initial, potential candidate “fingerprint” which might serve as a biomarker for PB-induced tumorigenesis. These 2 studies reveal “new” genes whose epigenetic statuses changed uniquely in liver-tumor susceptible mice (B6C3F1 and CAR WT), compared to their resistant counterparts (C57BL/6 and CAR KO, respectively), within a continuum of PB-induced tumorigenesis.

1995 EPIGENETIC ANALYSIS OF ALDH1A2 GENE IN JAPANESE MEDAKA DEVELOPMENTALLY EXPOSED TO ETHANOL.

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We have demonstrated that medaka embryos exposed to ethanol developed pronounced features in craniofacial, cardiovascular and skeletal organs which are homologous to the FAS observed in human. We hypothesized that ethanol metabolism during development induces oxidative stress which is responsible for epigenetic modification in several developmentally regulated genes, in turn, promoting FAS. In this regard, we have analyzed the promoter region of aldehyde dehydrogenase 1A2 (Aldh1a2) gene, which encodes an enzyme responsible for the synthesis of retinoic acid. The information about the nucleotide (nt) sequences of 5’ UTR and first exon of Aldh1a2 (nt 705 to +117, ATG +1) of medaka were obtained from the web site (http://medaka.utmorgan.org/) and were verified by PCR. After bisulfitetreatment of the genomic DNA, a fragment of 735 nt (1-668 to +117 nt) was amplified which contained 27 CpG dinucleotides. There were 175 cytosine nucleotides present in the amplified region, however, none were methylated. Medaka embryos exposed to ethanol (100-400 mM) for 48 hours post fertilization (hpf) also showed no methylation in the promoter region of Aldh1a2. Some of the ethanol treated embryos (300 mM, 0-48 hpf) were raised to adults (breeding) and the Aldh1a2 promoter methylation was analyzed in liver, brain, heart, and eye tissues. The methylation pattern of Aldh1a2 also remained unaltered in these organs. Therefore, it was concluded that ethanol did not alter the Cpg methylation pattern of Aldh1a2 gene of medaka during development [supported by NIH RO3AA016915; COBRE-P20RR021929].

1996 ROLE OF EPIGENETICS IN DIXION-INDUCED DIFFERENTIAL REGULATION OF THE HUMAN CYP1A1 AND CYP1B1 GENES.

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The Aryl Hydrocarbon Receptor/ARNT heterodimer mediates carcinogenesis by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin) and certain polycyclic aromatic hydrocarbons (PAHs) by activating gene transcription. Metabolism of PAHs by CYP1A1 and CYP1B1 plays a major role in carcinogenesis mediated by these compounds. The overall goal of this study is to understand the mechanisms involved in differential regulation of the human CYP1A1 and CYP1B1 genes. We are studying the regulation of these genes in three different human cell lines (MCF-7, MDA-MB-231, and HepG2) as the endogenous expression and induction levels of these genes upon treatment with TCDD differ significantly between these cell lines. To elucidate the mechanisms involved in the differential regulation of these genes, we studied the recruitment of AHR and RNA PolII at their enhancers and promoters, respectively. The data indicate that RNA PolII, but not AHR recruitment, correlates with CYP1A1 and CYP1B1 expression. Subsequently, we characterized the different dioxin-inducible chromatin modifications (i.e. acetylation, methylation, and phosphorylation) that occur across the CYP1A1 and CYP1B1 enhancer and promoter regions using the chromatin immunoprecipitation (ChIP) assay. The results show H3K9Ac, H3K14Ac, H3K4me3 and H4Ac chromatin modifications at the CYP1A1 and CYP1B1 promoter regions strongly correlate with the TCDD-induction of the CYP1A1 and CYP1B1 genes in different cell lines. These modifications, at least in part, are likely to contribute to the differential induction of these genes upon treatment with TCDD. Knock-down studies using siRNA experiments also show that the coactivator p300, but not CBP and SRC-1, play a role in the differential regulation of these genes.

1997 MOUSE EPIGENOME DEFINES THE STRAIN-SPECIFIC DIFFERENCES IN SUSCEPTIBILITY TO NON-ALCOHOLIC STEATOHEPATITIS.

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Current trends in cancer research are moving toward the uncovering of molecular mechanisms, cellular pathways, networks, processes, and related disease states that underlie tumorigenesis. Specifically, interest in recent years has been devoted toward uncovering the fundamental mechanisms of individual cancer susceptibilities. This has a great significance in identifying vulnerable subpopulations susceptible to cancer and for establishing new cancer prevention strategies. In light of these considerations, the present study was undertaken to determine whether or not the
mouse strain-specific susceptibility to non-alcoholic steatohepatitis (NASH), a pathological state that predisposes individuals to the development of hepatocellular carcinoma (HCC), is associated with differences in the epigenetic status. The initial analysis of the epigenetic status in the livers of BALB/c, C57/Hii, A/J, AKRJ, C57BL/6J, and C57BL/10ScCr mice revealed the substantial strain-specific differences in the degree of cytosine methylation and histone modification patterns. The most distinctive strain-specific epigenetic differences were detected in DBA/2J and C57BL/6J strains. Next, we used a mouse nutritional model of NASH induced by a methyl-deficient diet that is relevant to NASH pathogenesis in humans, to study whether or not the status of the liver epigenetic phenotype indeed mediates the individual sensitivity to NASH. Indeed, male C57BL/6J and DBA/2J mice were fed a methyl-adequate (control) or a methyl-deficient diet for 6, 12, and 18 weeks. Both the C57BL/6J and DBA/2J mice maintained a methyl-deficient diet exhibited significant hepatosteatosis; however, the DBA/2J mice that were characterized by lesser amounts of histone H3 lysine 9 and H3 lysine 27 trimethylation and a lower extent of methylation of repetitive DNA elements, as compared to those in the livers of C57BL/6J mice, developed milder severe hepatosteatosis. These data indicated the importance of epigenetic background in individual susceptibilities to disease.

**2000**

**EPIGENETIC MECHANISM FOR POTENTIATION (“PRIMING”) OF PREGNANE X RECEPTOR-REGULATED GENE EXPRESSION BY DIMETHYL SULFOXIDE.**

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Prior exposure to chemicals/agents may alter epigenome in such a way that subsequent exposure to the same or a different agent would produce different responses. This hypothesis obviously has important implications in xenobiotic/drug metabolism and detoxification, drug-drug interactions as well as therapeutic application of drug combinations. To test this hypothesis, we utilized the pregnane x receptor (PXR)-regulated luciferase reporter cell line (PXR-HepG2) to analyze the pretreatment (priming) effect of dimethyl sulfoxide (DMSO) on the rifampicin-induced PXR transcriptional activity. DMSO has extremely wide applications in medicine. It is a cryoprotectant for preserving organs, tissues and embryos. It has therapeutic applications for many inflammatory conditions, such as treatment for interstitial cystitis approved by FDA. We demonstrated that by pretreatment (priming) with 1.25±0.25% DMSO, PXR-regulated luciferase reporter gene induction was drastically elevated in PXR-HepG2. The DMSO priming effect on CYP3A4 induction was confirmed in human primary hepatocytes by RT-PCR. The priming effect persisted over 48 hours after removal of DMSO. DMSO treatment impacted epigenome by selectively changing the acetylation of histone H4 lysine 8. Pretreatment of methyltransferase inhibitor ADOX or histone deacetylase inhibitor TSA abolished the DMSO priming effect. However, treatment of ADOX after DMSO has not impact on DMSO priming effect. In contrary, treatment of TSA after DMSO priming enhanced the priming effect. Based on these observations and literatures (Science 293(5531):853-7; Genes Dev. 19(16):1885-93), we proposed a model of “Relay between histone methylation and acetylation on the transcription cycle” which posits transcription cycle begins with methylation of histone (e.g. H4R3) “a Ready State” which facilitates the histone acetylation, i.e. “Activation State”. To turn off an active gene the acetylated histone needs to be deacetylated which leads to the “Inactive State” which then allows methylation of H4R3 (“Ready State”). (The research is supported in part by ES 09859).

**1999**

**LOW-DOSE IONIZING RADIATION ALTERS THE FETAL EPIGENOME.**

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Radiation-induced bystander effect is a phenomenon where cells not directly exposed to ionizing radiation display a marked enhancement in chromosomal and genomic instability, which is thought to result in part from epigenetic changes (Kovachuck & Baulch, 2008). There is now accumulating evidence that epigenetic dysregulation during early development is also mechanistically linked to the pathogenesis of adult-onset diseases. We sought to determine whether low doses of ionizing radiation during early gestation affect susceptibility to adult-onset diseases by deregulating DNA methylation and/or chromatin structure of the genome. Viable yellow agouti (Ay/y) mice were used to test this novel postulate because they are extremely sensitive bioassays for environmental agents that alter the epigenome (Waterland and Jirtle, 2003; Dolinoy et al. 2006; Dolinoy et al. 2007). In this study, viable yellow agouti Ay/y offspring were exposed in utero to a total dose of 8.4 cGy of low LET radiation delivered at 1.2 cGy/day from gestational day 1.5 to gestational day 8.5 using the Imtek small animal MicroCAT X-ray/CAT scanner. A second set of Ay/y offspring was sham-irradiated in utero for 7 consecutive days. The results demonstrate that maternal exposure to this low dose of fractionated radiation shifts the coat color distribution of Ay/y offspring toward the brown pseudoagouti coat color phenotype (p=0.026). Additionally, preliminary DNA methyltransferase analysis demonstrated increased methylation at several Cpg sites up-stream of the Ay/v promoter. These data indicate that the protective effect frequently observed following low dose radiation exposure may be mediated by epigenetic mechanisms that lead to increased DNA methylation at critical sites in the genome. To test this intriguing postulate, additional exposure groups will be added to this study.
2002  AN LC-MS/MS BASED METHOD TO QUANTIFY N\textsuperscript{6}-FORMYLATION OF LYSINE, A PATHOLOGICAL FORMATION OF HISTONE PROTEINS.

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DNA oxidation by endogenous and exogenous agents affects both base sugar and moieties of DNA, with the latter leading to formation of reactive electrophiles capable of forming adducts with neighboring nucleobases and proteins. We recently discovered an abundant endogenous modification of histones, N\textsuperscript{6}-formylation of lysine, that arises by transfer of the formyl moiety from a 3\textsuperscript{-}formylphosphate residue (a product of 5\textsuperscript{-}oxidation of deoxyribose in DNA). This adduct is a chemical analog of lysine N-acetylation that has important regulatory roles in gene expression. On the basis of this evidence, we aim to characterize N\textsuperscript{6}-formylation in terms of abundance and locality and investigate its possible role in interfering with acetylation signaling in cells. We now present an ultrasensitive and specific LC-MS/MS based method for quantifying N\textsuperscript{6}-formyl-lysine residues and report its application for measuring the adduct in different classes of histones and in response to various DNA oxidizing agents. N-formylation of lysine in histones may be an important epigenetic determinant of gene expression.

2003  MOLECULAR MECHANISM OF BENZO(A)PYRENE-INDUCED GENETIC AND EPIGENETIC DAMAGES IN MAMMARY GLANDS.

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Polycyclic aromatic hydrocarbons, such as benzo[a]pyrene (BaP), are known mammary carcinogens in rodents and may be involved in the etiological factor of human breast cancer. In this study, time and concentration-dependent gene expression profiling induced by BaP exposure was assessed to investigate the novel gene/pathways involved in the cellular response to BaP. To examine the precise mechanism for genetic alterations, we measured BPDE-I-protein adduct and oxidative DNA damage (8-OHdG) in the mammary glands. Taken together, expression levels of several genes involved in cell proliferation, cell cycle regulation and apoptotic pathway were measured in mammary tissues. Moreover, it is not known whether BaP play any role in the methylation or demethylation of these genes. Using methylation specific RT-PCR methods, CpG island hypermethylation and histone H1 expression levels are also measured in mammary tissues. As expected, significant time- and concentration-dependent alteration in genetic biomarkers (BPDE-I-protein adduct and 8-OHdG) were observed in mammary tissues of ICR mice. BaP significantly inhibited the development of mammary glands following acute exposure of BaP with high dose. Expression changes of these genes observed in this study were identified as xenobiotic metabolism, cell cycle regulation, apoptosis and chromatin remodeling. The recovery of gene expression identified in mammary glands is likely to be associated with levels of DNA damage because these gene expression occurred only after BaP exposure with high concentration. These results are relevant to biomarker screening approaches involving environmental exposures in which DNA adduct formation at specific tissues and compromise the correct identification of epigenetically responsive genes.

2004  BIOLUMINESCENT BACTERIA AS BIOSENSORS FOR POLYCYCLIC AROMATIC HYDROCARBONS.

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Polycyclic aromatic hydrocarbons (PAH) are a group of carcinogenic persistent organic pollutants that are products of incomplete combustion reactions. Their toxicity is of concern to human health due to their bioaccumulation in the food chain and persistence in the environment. Current research is focused on developing highly sensitive analytical techniques to detect PAHs in the environment. It has been previously shown that bioluminescent bacteria serve as adequate biosensors for different contaminants. The goal of this project is to determine if bioluminescent bacteria can be used as an inexpensive, fast and highly sensitive biosensor for specific PAHs. In this study, a bioluminescent bacterium was exposed to four different PAHs at various concentrations to determine sensitivity and EC\textsubscript{50}. Luminescence intensity was analyzed to measure sensitivity using a Luminoskan. Preliminary kinetic results show the decay of luminescence for most concentrations are likely to be \textsuperscript{2}k order reactions and dosage response curves suggest a decrease in luminescence as PAH concentration increases. Future directions include reporting the EC\textsubscript{50} for benzo[a]pyrene, phanethrene, acyclanethracene and pyrene and a comparison of sensitivity among different bioluminescent strains. These results will be used to investigate the possible use of bioluminescent bacteria as biosensors for specific PAHs in the environment.

2005  PERFLUORINATED CHEMICALS IN MUNICIPAL DRINKING WATER FROM CATALONIA, SPAIN: PUBLIC HEALTH IMPLICATIONS.

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Perfluorinated chemicals (PFCs) are used in a wide variety of consumer products. Due to their persistence and bioaccumulative properties they are taken up by the general population from different sources. In this study, we analyzed (UPLC-MS/MS) the concentrations of 13 PFCs (PFBS, PFHxS, PFOS, TFPFOS, PFHxA, PFOA, PFNA, PFDa, PFUnDA, PFDoDA, PFTDA and PFOSA) in municipal drinking water samples collected from 40 different locations in Catalonia Spain. Detection limits ranged between 0.02 (PFHxS) and 0.85 ng/L (PFOA). PFHxS and PFOS were detected in 35 samples, with maximum concentrations of 5.30 and 58.1 ng/L, respectively, found in sample No. 14 collected near Barcelona City. PFBS, PFHxS and PFOA were also frequently detected (31, 28 and 26 samples, respectively), with maximum levels of 69.4, 8.55 and 57.4 ng/L. However, PFDoDA and PFTDA could not be detected in any sample. The most contaminated samples were No. 6, 9 and 14 (Barcelona Province), with 11 compounds detected. In contrast, not any of the analyzed PFCs could be detected in samples No. 19 (Banyoles) and 22 (Iclea), while only one PFC was found in samples No. 5, 18, 30 and 37. Although currently there is no drinking water quality criteria established for PFCs in the European Union, recently the European Safety Authority established TDIs for PFOS and PFOA: 150 and 1,500 ng/kg/day, respectively. Assuming a human water consumption of 2 L/day, the maximum daily intakes of PFOA and PFOS from municipal drinking water would be, for a subject of 60-kg b.wt., 1.94 and 1.91 ng/kg/day, values clearly lower than their TDIs. All PFOS and PFOA levels were also lower that the values of 1,000 ng PFOS/L (DWHA) and 500 ng PFOA/L (US EPA) potentially used for guidance purposes. Although PFOS and PFOA concentrations found in drinking water in Catalonia are not expected to pose health risks, safety limits for exposure to the remaining PFCs are clearly necessary.

2006  PERFLUOROOCANESULFONATE (PFOS) INCREASES DE NOVO TESTOSTERONE SYNTHESIS IN JUVENILE XENOPUS (SILURANA) TROPICALIS GONADS.

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The impact of the perfluorochemical, perfluorooctanesulfonate (PFOS), on gonadal steroidogenesis during sexual differentiation in Xenopus tropicalis was examined because of its ubiquity in the environment, bioaccumulative nature, and potential to disturb endocrine activity. In previous studies, exposure of Xenopus tropicalis to PFOS during development increased the proportion of phenotypic males, decreased mean plasma E\textsubscript{2} levels, and increased mean plasma DHT levels compared to controls. Further, mean gonadal aromatase activity in X. tropicalis exposed to PFOS was increased compared to control frogs. To further examine the apparent anti-estrogenic effects of PFOS, de novo testosterone synthesis in differentiating gonads was examined. Samples from the gonads were collected for testosterone synthesis measurements from juvenile control specimens and organisms exposed to PFOS (control, 0.05, 0.12, 0.29, 0.62, and 1.1 mg PFOS/L) from early embryo through metamorphosis. Increased testosterone synthesis was found in X. tropicalis exposed to 0.62 and 1.1 mg PFOS treatments compared to the control. Overall, the increase in testosterone synthesis was concentration-dependent. The increase in the proportion of phenotypic male frogs in our previous studies historically appears to be the result of reduced aromatase, increased St-17 reductase and CYP17 activities, and increased testosterone synthesis resulting from PFOS exposure. Overall, these results demonstrate the anti-estrogenic effects of PFOS in X. tropicalis.

2007  CROSS-SPECIES GC-MS BASED METABOLIC ANALYSIS OF HEPATIC LIPID COMPOSITION IN TCD3-TREATED C57BL/6 MICE AND SPRAGUE-DAWLEY RATS.

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Mice and rats exhibit different genomic and phenotypic responses to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), including mouse-specific steatohepatitis. To investigate changes in lipid composition and gene expression, livers from immature, 30-g male rats and mice were exposed to TCDD (TCD3) at various concentrations for 21 days and were sacrificed. The liver lipid compositions were analyzed using GC-MS and metabolic profiles were compared to identify pathways responsive to chemical treatment. 2-D principal component analysis was used to reduce the dimensionality of the data and identify classes of metabolites that are responding to treatment. Probing individual pathways such as cholesterol metabolism, fatty acid biosynthesis, and phospholipid metabolism identified highly significant changes in the TCDD-treated animals.
ovariectomized C57BL/6 mice and Sprague Dawley rats were harvested 2, 4, 8, 12, 18, 24, 72 and 168 hrs following gavage with 30 μg/kg or 10 μg/kg TCDD, respectively. Fatty acid methyl esters derivatives from 24 and 72 hr treated liver lipid extracts were profiled by gas chromatography-mass spectrometry (GC-MS). Significant increase in unsaturated fatty acids (16:1n7, 18:3n3, 20:1n9, 18:2n6, 20:2n6, 18:3n3 at 24 and 72 hrs; 24:1n5 and 20:3n6 at 72 hrs) were detected in mice, while only 20 carbon fatty acids (20:1n9 at 24 and 72 hrs, 20:2n6 and 20:3n6 at 72 hrs) in rats. Quantitative real-time (QRT) PCR of lipid metabolism genes found Elovl, Fads1, Fas, Vldlr were differentially regulated in the mouse, but not in the rat. Elovl5, a fatty acid elongase, exhibited a different expression profile in mouse compared to the rat. In addition, Scl1, a fatty acid desaturase was only induced in the rat. The late induction of these genes suggests a compensatory mechanism in gene regulation rather than direct AhR regulation, although putative dioxin response elements (DREs) were identified in the regulatory region as determined using computational searches and a position weight matrix derived from bona fide DREs. Collectively, these results suggest that species-specific changes in lipid metabolism and transport genes may be an important factor for the incidence of steatohepatitis in mice. This work was supported by NIH/NIEHS grant R01ES013927.

**2008 CONCURRENT EXPOSURE OF PREGNANT MICE TO PFOS AND RESTRAINT STRESS: EFFECTS ON CORTICOSTEROIDE LEVELS IN THE OFFSPRING.**

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**Toxicology, Universitat de les Illes Balears, Palma de Mallorca, Spain.**

Stress can enhance the developmental toxicity of certain chemicals. Perfluorooctane sulfonate (PFOS), an organic persistent pollutant, is present in human tissues and wildlife samples on a global scale. Although prenatal PFOS exposure can cause developmental adverse effects, disruptive PFOS disorders on hormonal systems are still controversial. Nowadays, no studies concerning the effects of PFOS on stress response and plasma corticosterone levels have been reported. The aim of the present study was to assess the combined effects of maternal restraint stress and PFOS on HPA axis function in the offspring of mice. Twenty plug-positive females were divided in two groups. Animals received po 0 and 6 mg PFOS/kg/day on gestation days 12-18. One-half of the animals in each group were subjected to restraint stress (30 min/session, 3 sessions/day) during the same period. Five plug-positive females were also included as non-manipulated controls. At 3 months of age, the stress response was evaluated by analyzing the offspring by exposing them to restraint stress (30 min/session, 3 sessions/day) molecular signals by computational searches and a position weight matrix derived from bona fide DREs. Collectively, these results suggest that species-specific changes in lipid metabolism and transport genes may be an important factor for the incidence of steatohepatitis in mice. This work was supported by NIH/NIEHS grant R01ES013927.

**2009 DEVELOPMENTAL TOXICITY OF PERFLUORONONANOIC ACID IN THE MOUSE.**

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Perfluorononanoic acid (PFNA) is a persistent environmental contaminant. Although its levels in the environment are lower than those of perfluorooctanoic sulfonate (PFOS) or perfluorooctanoic acid (PFOA), its presence in humans appears to be on the rise. Previous studies have indicated developmental toxicity of PFOS and PFOA in the laboratory rodent models, the current study examines whether similar effects are extended to PFNA. PFNA was given to timed-pregnant CD-1 mice by oral gavage daily from GD 1-17 at 1, 3, and 5 mg/kg/day for 30 min/session, 3 sessions/day during the same period. Five plug-positive females were also included as non-manipulated controls. At 3 months of age, the stress response was evaluated by analyzing the offspring by exposing them to restraint stress (30 min/session, 3 sessions/day) molecular signals by computational searches and a position weight matrix derived from bona fide DREs. Collectively, these results suggest that species-specific changes in lipid metabolism and transport genes may be an important factor for the incidence of steatohepatitis in mice. This work was supported by NIH/NIEHS grant R01ES013927.

**2010 SERUM LEVELS OF DIOXIN AND DIOXIN-LIKE COMPOUNDS IN MOTHERS LIVING IN A HIGHLY INDUSTRIALIZED AREA OF WEST VIRGINIA.**


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Historical releases of dioxin and dioxin-like congeners with subsequent impacts to environmental media in the Kanawha River Valley (KV) of West Virginia have been well documented. This study evaluated the impact of potentially high background dioxin exposure to body burdens in women of reproductive age (ranging from 25 to 46) and living in or near KV. This cohort of women was derived from a larger study investigating the relationship between the women’s dioxin levels and body burdens. The study was conducted at Marshall University and all relevant procedures were performed under Marshall’s IRB in accordance with 45 CFR 46.110(A)(8)(ii). Serum was analyzed by high-resolution gas chromatography/mass spectrometry for dioxin-like compounds. Results were reported on a whole-body and lipid-adjusted basis and as international toxicity equivalents (WHO2005 TEQs). The major constituents of the total serum TEQ were 1,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD); 1,3,6,8-tetrachlorodibenzo-p-dioxid (TCDD); 2,3,4,7,8-pentachlorodibenzo-p-dioxid (PCDF); 1,2,3,4,7,8-HxCDF; PCB126; 2,3,4,7,8-pentachlorodibenzo-p-dioxid (PCDD); 1,2,3,4,7,8-HxCDF; and 1,2,3,4,6,7,8-HpCDD. The TEQ min/max and median/mean based on pg/g lipid were PCDD (0.229 & 3.5/3.5); HxCDF (3.5/4.5 & 221.5); 3.5 & 221.5); PCB126 (3.5/6 & 121 & 12143); TCDD (0.186.7 & 1211); PCD (0.25/10 & 3.5/4.5); HxCDF (3.5/4.5 & 3.2 & 3.54); and HpCDD (7/110 & 25/29), respectively. This study demonstrates that body burdens of these congeners in this age group of women are similar to levels recently reported in the general United States population and that living in an area of high levels of contamination is not necessarily reflected in the body burden for this class of chemicals. This abstract does not reflect USEPA policy.

**2011 GENE PROFILING IN WILD-TYPE AND PPARα-NULL MICE EXPOSED TO PERFLUOROOCANCE SULFONATE.**

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Perfluorooctane sulfonate (PFOS), a perfluoroalkyl acid (PFAA), is a persistent environmental contaminant found in the tissues of humans and wildlife. Health concerns have been raised over the last decade, in part, because of the long half-life of PFOS and other PFAAs in humans, although serum levels of PFOS have begun to decrease since its phase out in 2002 by its major manufacturer. PFOS is a weak activator of peroxisome proliferator-activated receptor-alpha (PPARα) and exhibits hepatocarcinogenic potential in rats. PFOS is also a developmental toxicant in rodents, where differences have been observed between its mode of action and that of perfluorooctanoic acid (PFOA). A related PFAA, Wild-type (WT) and PPARα-null (KO) mice were dosed by oral gavage with PFOS (3 or 10 mg/kg/day), or vehicle for 7 days. Animals were euthanized, livers weighed, and liver samples collected for preparation of total RNA. Gene profiling was conducted on 5 mice per group using Affymetrix 430_2 GeneChips. In WT mice, PFOS induced changes characteristic of PPARα activation including regulation of genes associated with fatty acid metabolism, peroxisome biosynthesis, and proteasome biosynthesis. The expression of PPARα nuclear genes such as Cyp4a14, Ehhadh, and Pdk4 was up-regulated. In KO mice, PFOS altered the expression of a subset of genes related to lipid metabolism and, in contrast to data previously evaluated for PFOA, induced unique changes to genes associated with oxidative phosphorylation/electron transport and cholesterol biosynthesis. The constitutive androstane receptor (CAR) marker gene, Cyp2b10, was up-regulated across all treatment groups while Cyp2j1, a gene known to be regulated by liver X receptor (LXR) and retinoid X receptor (RXR), was up-regulated only in KO mice. These data suggest that PFOS may have the potential to activate nuclear receptors other than PPARα, such as CAR, LX, or RXR. This abstract does not necessarily reflect EPA policy.
PCBs are persistent organic pollutants that were once widely applied industrially until their production was banned in the 1970s. PCB47 (2,2',4,4'-tetrabromodiphenyl ether) is a substantial constituent of former commercial PCB mixtures and has recently been reported to be present in Chicago air. Because of its ortho-para-chlorination pattern, PCB47 is expected to exert toxicity through activation of the nuclear receptor CAR. However, few studies have been carried out to test this. The objective of this study was to compare the global gene expression in PCB47-treated rats with classical inducers-treated ones to explore the mechanisms of PCB47 toxicity. Sprague Dawley rats received short-term i.p. exposure to corn oil, phenobarbital (PB), 3-methylcholanthrene, β-naphthoflavone, dexamethasone, and PCB47. Liver RNA was extracted, pooled in each group and hybridized against a reference on Agilent 4 x 44 K whole rat genome oligo microarray. The principle component analysis and hierarchical clustering revealed that the gene expression of PCB47-treated male rats was most closely resembled that of PB-treated male rats, while PCB47-treated females showed a much stronger and differing effects than males. Enzyme activity assays confirmed the induction, but with the opposite gender difference and much higher fold inductions. Functional classification revealed that only PCB47 up-regulated cell adhesion-related genes and down-regulated PB induced CYP2B genes, CYP2B1 and CYP2B2 were much more induced in females. Other PCPs were used as the positive and negative controls. The results suggest that PCB47 exerts its toxicity through both CAR-dependent and CAR-independent mechanisms and female rats show much stronger and differing effects on expression level than males. (Supported by NIEHS P42 ES013661 and DAMD17-02-1-0241).

**2015 HUMAN ORGANIC ANION TRANSPORTING POLYMERPEPTIDE 1B1 AND 1B3-MEDIATED UPTAKE OF A PREDOMINANT POLYBROMINATED DIPHENYL CONGENER, BDE 47.**

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Polybrominated diphenyl ethers (PBDEs) are chemical additives serving as flame retardants found in a variety of manufactured goods. PBDEs are ubiquitously detected in the environment and are considered to be persistent and bioaccumulative. 2,2',4,4'-tetrabromodiphenyl ether (BDE47, MW 485.8) is the predominant congener detected in human and wildlife samples. Although associated health risks to humans are currently not clear, numerous studies report neurological, reproductive and developmental toxicities as well as increased oxidative stress in rodents exposed to BDE47. The goal of the present study was to determine the ability to mediate BDE47 uptake in rodent models. Following exposure, BDE47 highly accumulates in the liver. We have shown that BDE47 activates the hepatic xenobiotic nuclear receptors PXR and CAR to induce Phase I and II metabolizing enzymes. Mechanisms by which PBDEs enter the liver have yet to be elucidated. Human organic anion transporting polypeptides (OATPs) are polycpecific organic anion transporters mediating uptake of numerous drugs and chemicals. Given that their substrates are in general amphiphilic molecules with molecular weights of more than 350, the liver specific OATP1B1 and 1B3 are promising candidate transport systems for BDE47 uptake. Therefore, we tested the hypothesis that BDE47 is transported by OATP1B1 and 1B3. To test this hypothesis, we used Chinese Hamster Ovary (CHO) cell lines stably expressing OATP1B1 or 1B3 and determined uptake of radiolabeled BDE47. The results showed that BDE47 was transported by both, OATP1B1 and 1B3. Furthermore, we performed a detailed kinetic analysis and determined that OATP1B1 and 1B3 transported BDE47 with Km values of 251 and 389 nM respectively, and with Vmax values of 28.4 and 2.3 pmol/mg protein x min, respectively. The current study demonstrates that BDE47 is transported by OATP1B1 and 1B3. Based on the kinetic data we conclude that OATP1B1 has an approximately 20 fold higher capacity to mediate BDE47 uptake. Our results should help in better understanding the mechanism for the liver-specific accumulation of BDE47.

**2016 IN VITRO STUDY OF THE AIR DELIVERY OF PARTICLE-BOUND PBDES TO LUNG CELLS.**

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Polybrominated diphenyl ether (PBDE) exposure is thought to occur primarily via inhalation of particles and dust-bound PBDEs. However, few studies have examined the effects of particle-bound PBDEs on human health. We hypothesized that human lung cells exposed in vitro to airborne particle-bound PBDEs would produce intracellular reactive oxygen species (ROS) and undergo cytotoxicity. In order to determine the effects of particle-bound PBDEs, we cultured A549 human lung epithelial cells in the presence of an exposure chamber holding Transwell® inserts. To validate and characterize the exposure system, the size, morphology, and spatial variability of particles were determined by scanning electron microscopy and image analysis. Cytotoxicity and intracellular ROS in human epithelial lung carcinoma cells (A549 and NCI-H358) were used to evaluate the biological effects of particle-bound PBDEs. The silica particles and particles coated with PBDEs were discrete spheres of approximately 1 μm in diameter. The silica particles and particles coated with PBDEs were observed to be evenly distributed in the culture medium.

**2013 INDUCTION OF OXIDATIVE STRESS AND APOPTOSIS IN MOUSE CEREBELLAR GRANULE NEURONS BY VARIOUS PBDE CONGENERS.**

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Polybrominated diphenyl ether (PBDE) flame retardants have become widespread environmental contaminants. Exposure is believed to occur primarily through diet and dust. The ability of PBDEs to cause apoptotic cell death has been reported in various cell types including cerebellar granule cells, human astrocytoma cells, neuroblastoma cells and liver hematopoietic cells. Although the mechanisms involved in PBDE-induced apoptosis have not been fully understood, they are most likely related to oxidative stress and may involve the mitochondrial pathway. Aims of the present study was to determine whether PBDE congeners (the tetramobromodiphenyl ether 47, the pentabromodiphenyl ethers 99 and 100, the hexabromodiphenyl ether 135, and decabromodiphenyl ether 209) induce oxidative stress-mediated apoptosis in mouse cerebellar granule cells. All congeners except BDE-209 caused a concentration-dependent increase in cell death as assessed by MTT assay, which could be mostly ascribed to apoptosis (as assessed by Hoechst staining); they also increased formation of reactive oxygen species (as measured using dichlorofluorescein), and of lipid peroxidation (assessed measuring malondiadehyde levels). Comparison of tetra- and penta-brominated congeners indicated that BDE-100, which has the highest number of ortho bromines, was the most effective in causing apoptosis and oxidative stress, followed by BDE-47 and BDE-99 which appeared to have similar potency and efficacy. In contrast, BDE-209 was almost ineffective in inducing apoptosis and oxidative stress, probably due to its higher hydrophobicity which generally increases with the number of bromines. These findings suggest that PBDEs caused oxidative stress-mediated apoptotic cell death in mouse cerebellar granule neurons and that ortho-substituted congeners appear to be more toxic. (Supp. in part by P30ES07035).

**2012 PCB47: MORE THAN A CONSTITUTIVE ANDROSTANE RECEPTOR AGONIST.**

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PCBs are persistent organic pollutants that were once widely applied industrially until their production was banned in the 1970s. PCB47 (2,2',4,4'-tetrabromodiphenyl ether) is a substantial constituent of former commercial PCB mixtures and has recently been reported to be present in Chicago air. Because of its ortho-para-chlorination pattern, PCB47 is expected to exert toxicity through activation of the nuclear receptor CAR. However, few studies have been carried out to test this. The objective of this study was to compare the global gene expression in PCB47-treated rats with classical inducers-treated ones to explore the mechanisms of PCB47 toxicity. Sprague Dawley rats received short-term i.p. exposure to corn oil, phenobarbital (PB), 3-methylcholanthrene, β-naphthoflavone, dexamethasone, and PCB47. Liver RNA was extracted, pooled in each group and hybridized against a reference on Agilent 4 x 44 K whole rat genome oligo microarray. The principle component analysis and hierarchical clustering revealed that the gene expression of PCB47-treated male rats was most closely resembled that of PB-treated male rats, while PCB47-treated females showed a much stronger and differing effects than males. Enzyme activity assays confirmed the induction, but with the opposite gender difference and much higher fold inductions. Functional classification revealed that only PCB47 up-regulated cell adhesion-related genes and down-regulated PB induced CYP2B genes, CYP2B1 and CYP2B2 were much more induced in females. Other PCPs were used as the positive and negative controls. The results suggest that PCB47 exerts its toxicity through both CAR-dependent and CAR-independent mechanisms and female rats show much stronger and differing effects on expression level than males. (Supported by NIEHS P42 ES013661 and DAMD17-02-1-0241).
 posed over the Transwell. We found a strong positive correlation between particle loading mass and particle number (R²=0.97). Twenty four hours after airborne exposure to particles, no significant cytotoxicity was observed in any experiment. However, a statistically significant (p<0.05) increase in intracellular ROS was observed with 46 μg/cm² exposure to BDE 35 particles in both NCI-H358 and A549 cells and 29 μg/cm² exposure to BDE 35 and BDE 47 particles in A549 cells. These results demonstrate the reproducibility and sensitivity of this in vitro exposure system for application of particle-bound test compounds to cells and its potential for studying the toxic effects of inhaled particles. (Supported by NIH P42 ES013661 and P30 ES05605)

**2017 DOES DIETARY SELENIUM INFLUENCE THE EFFECTS OF PCB126 ON RAT PARAOXONASE 1?**

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Paraoxonase 1 (PON1), an antioxidant enzyme, is synthesized in the liver and secreted into the blood where it is associated with HDL and protects LDL against lipid peroxidation, a major risk factor in atherosclerosis. Our previous studies showed that short term exposure to various PCB congeners significantly (up to 54%) reduced PON1 activities in serum/plasma and liver microsomes. This study was to analyze the effects of PCB126 on PON1 activity and whether selenium (Se), an antioxidant, can mitigate such effects. Male Sprague-Dawley rats, 4-6 Se-deficient (0.02ppm), adequate (0.2ppm), or supplemented (2ppm) diet for 3 weeks, were injected ip with PCB126 (a single injection of 0.2, 1, or 5 μmol/kg) and euthanized 2 weeks later. Serum and liver microsomal PON1 activities were measured spectrophotometrically using two substrates, paraoxon and phenylacetate. Dietary Se levels had no effect on serum PON1 activities, but seemed inversely related to liver microsomal PON1 activities. PCB126 significantly increased in a dose-dependent manner serum and liver microsomal PON1 activities within each Se-diet group, with the exception of a slight reduction of liver microsomal PON1 activity with high dose (5 μmol/kg) compared to the medium dose (1 μmol/kg) in Se-adequate and Se-supplemented rats. Thus our preliminary findings suggest a compensation for low levels of one antioxidant (Se) by an increase of another one (PON1) and that a single exposure to a very low dose of PCB126 may increase PON1 activity, possibly as a protective mechanism. Time and extended dose studies as well as lipid peroxidation determinations are needed to evaluate whether this protective effect of increased PON1 activity is maintained and sufficient to mitigate the oxidative damage caused by PCB126, thereby reducing the risk of cardiovascular and other diseases by this and possibly other ubiquitous contaminants. (Supported by NIEHS P42 ES013661 and DOD DAMD17-02-1-0241)

**2018 “POLYCHLORINATED BIPHENYLS (PCBs) AND POLYBROMINATED DIPHENYL ETHERS (PBDEs) ARE INHIBITORS OF THE BREAST CANCER RESISTANCE PROTEIN (ABCG2) TRANSPORTER**

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PCBs and PBDEs are structurally similar persistent contaminants that elicit a variety of biological and toxicological responses. ABCG2 is a novel 72 kDa protein that functions as a multidrug transporter and is expressed in a variety of tissues throughout the body. It is involved in the efflux of many substances such as lipophilic drugs, xenobiotics, and trace metals. This study was aimed at investigating the ABCG2 transport activity of PCBs and PBDEs in the human breast cancer cell line, MCF7/MX100, which specifically over express ABCG2. Exposure to efflux a specific substrate of ABCG2, mitoxantrone. Flow cytometry was used to determine the effect of isolation/industrialization on PBDE body burden, the blood plasma from three First Nations (Cree Nation of Ouijé-Bougoumou, Quebec; Fort Albany First Nation, Ontario; and Weenusk First Nation [Paw气象], Ontario, Canada) were collected (n=54) and analyzed using a log-linear contingency model. Results and Conclusions: The mean values of PBDEs in wild meats and fish adjusted for standard consumption values and body weight, did not exceed the US EPA RfD. However, maximum values for certain species did exceed RfD values and potentially pose a threat to small children or those who would consume several portions of meat a day. Log linear modeling of the human PBDE body burden showed that PBDE body burden increases as access to goods increases. Thus, household dust from material goods containing PBDEs is likely responsible for the human exposure; the traditional diet appears to be a minor source of PBDEs.

**2019 DIETARY EXPOSURE TO PBDEs FROM A SUBSISTENCE DIET: FIRST NATIONS OF THE HUDSON BAY LOWLANDS, CANADA.**

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Introduction: Concerns regarding the persistence, bioaccumulation, long-range transport, and adverse health effects of polybrominated diphenyl ethers (PBDEs) have recently come to light. PBDEs may potentially be of concern to First Nations (indigenous) peoples of Canada who subsist on traditional foods, however there is a paucity of information on this topic. Methods: To investigate whether the traditional diet is a major source of PBDEs in sub-Arctic First Nations populations of the Hudson Bay Lowlands (James and Hudson Bay), Ontario, Canada, a variety of tissues from wild game and fish were analyzed for PBDE content (n=147) and dietary exposure assessed and compared to the US EPA RfDs. In addition, to examine the effect of isolation/industrialization on PBDE body burden, the blood plasma from three First Nations (Cree Nation of Oujé-Bougoumou, Quebec; Fort Albany First Nation, Ontario; and Weenusk First Nation [Paw气象], Ontario, Canada) were collected (n=54) and analyzed using a log-linear contingency model. Results and Conclusions: The mean values of PBDEs in wild meats and fish adjusted for standard consumption values and body weight, did not exceed the US EPA RfD. However, maximum values for certain species did exceed RfD values and potentially pose a threat to small children or those who would consume several portions of meat a day. Log linear modeling of the human PBDE body burden showed that PBDE body burden increases as access to goods increases. Thus, household dust from material goods containing PBDEs is likely responsible for the human exposure; the traditional diet appears to be a minor source of PBDEs.

**2020 FISH INTAKE AND POPC CONCENTRATIONS IN HUMAN MILK IN COASTAL CITIES IN CHINA.**

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In 2006-2007, we collected 60 and 48 donors of human milk in two coastal cities, Tianjin and Yantai, respectively, in China, in accordance with the WHO/UNEP global milk survey. We determined the concentrations of organochlorine pesticides by GC/MS/MS and dioxins by XDS-CALUX bioassay in the individual milk specimen collected. Geometric mean concentrations of PCBs, HCHs, dioxins and furans were measured. The human PBDE body burden was calculated and compared to the US EPA RfD. Maximum values for certain species did exceed RfD values and potentially pose a threat to small children or those who would consume several portions of meat a day. Log linear modeling of the human PBDE body burden showed that PBDE body burden increases as access to goods increases. Thus, household dust from material goods containing PBDEs is likely responsible for the human exposure; the traditional diet appears to be a minor source of PBDEs.

**2021 SCREENING-LEVEL ASSESSMENT OF RISK ASSOCIATED WITH EXPOSURE TO PBDEs IN VEHICLES.**

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Polybrominated diphenyl ethers (PBDEs) have received increased attention because of G2. This results in new insights into the health effects in combination with reports of adverse effects. Human exposure pathways are thought to be via dust ingestion primarily, followed by dietary intake and inhalation, for adults. Most recently, it has been suggested that exposure to PBDEs may be occurring in vehicles because of the use of PBDEs in automotive components such as textile, backings,
electronic insulation, polyurethane foams, and plastics. In this study, a screening-level risk assessment was conducted in an effort to provide preliminary estimates of theoretical risk associated with exposure to PBDEs in vehicles. Using standard approaches for calculating intake over a lifetime and for evaluating health risk, estimates of non-carcinogenic hazard and carcinogenic risk were assessed for a variety of scenarios involving adults and children exposed to PBDEs in car dust and air. Results indicated that non-carcinogenic hazard estimates for Σ tri-hexa BDEs and BDE 209 (evaluated separately) in dust were below one for both adult and child exposure scenarios. Though estimates of carcinogenic risk associated with BDE 209 in car dust were below acceptable risk levels (<10^-7) for all scenarios evaluated, estimates of hazard and cancer risk associated with air were <10^-3 and <10^-11, respectively. Though these preliminary evaluations are not representative of risk for all humans nor are they representative of risk from all types of cars worldwide, they provide an indication of human health risk associated with PBDE exposure. Further characterization of levels in cars, investigation of toxicity, as well as specific exposure variables (e.g., time in cars, absorption from dust and air), will decrease uncertainty in such quantitative assessments. This abstract does not reflect EPA policy.

2022 RESULTS OF A NEW SURVEY SHOW INCREASING LEVELS OF PBDES IN U.S. FOOD.

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We report for the first time PBDE levels in 50 matched food samples from Texas, California, and New York (90 total) collected in 2008. We note significant geographical differences in total PBDE levels. Levels in meat, dairy, eggs, oils, and bread are higher than previously reported, while levels in fish collected in 2008 are lower than in fish collected in 2003. In 2003, PBDE levels in fish were highest, while in 2008, levels in meat were highest. Median total PBDE levels in foods of animal origin (chicken breast, sausage, beef, baby food) from 2008 samples are 719 pg/g, 131 pg/g, 303 pg/g, and 17 pg/g, respectively. The maximum PBDE level in 2008 collected meat, 305 pg/g, exceeded the median PBDE levels in 2003 collected meat, 190 pg/g. The maximum PBDE level in 2008 collected meat, 3495 pg/g, exceeded the maximum PBDE level in 2003 collected meat, 1426 pg/g. PBDEs in dairy foods (butter, half and half, cheese, milk based infant formula) collected in 2008 exceeded those from 2003, with a median of 419 pg/g compared to a previous median PBDE level of 32.2 pg/g. The highest levels in dairy foods were observed in cheese and butter, with all six samples having PBDE levels above 1,000 pg/g. A 20 fold increase was noted in median PBDE levels in eggs; each of the samples collected in 2008 had PBDE levels over 1,300 pg/g (range 1350 – 1975 pg/g); previous median values were 85 pg/g. PBDEs in margarine also increased from 2003 levels, with a median value of 1485 pg/g compared to levels of 88 pg/g. Fruit and vegetable PBDE levels (median 11.2 pg/g, range 2.6 – 90 pg/g, n=14) were usually lower than meat, fish, and dairy, although highest levels in veg-

eatables overlap with lowest found in food of animal origin. Further research is needed to determine why levels in this larger 2008 survey were unexpectedly higher than previously measured in US food. This abstract has been reviewed by EPA but represents the personal views of the authors and is not Agency policy.

2023 EXPOSURE BASED WAIVING: THE APPLICATION OF THE TOXICOLOGICAL THRESHOLD OF CONCERN (TTC) TO INHALATION EXPOSURE FOR AEROSOL INGREDIENTS IN CONSUMER PRODUCTS.

P. Carthew, Unilever, Bedford, United Kingdom. Sponsor: P. Hepburn.

With the implementation of the 7th amendment to the Cosmetic Directive in Europe, repeat inhalation testing of toxic ingredients will longer possible after 2013, and alternative approaches to evaluating the toxicological safety of ingredients for use in aerosols needs to be developed. Exposure-based waiver refers to exemption from conducting toxicity studies when the justification for the waiver is based on the fact that there is minimal exposure of humans and the environment. Relevant exposure is interpreted as meaning that exposure remains within acceptable burden limits, and it can be assumed that the exposure is not associated with any hazard potential for human health and the environment. If a threshold of toxicologic concern (TTC) for inhalation of ingredients could be established, below which it could be confidently assumed that there was no hazard potential, then aerosols could be designed to minimize the consumer exposure to ingredients to the point where inhalation testing would not be considered necessary. Considering the publicly available inhalation toxicity study data for chemicals allows us to compile a database of toxicity NOAECs (no observable adverse effect concentrations) and NOAELs (no observable adverse effect levels) for both local and systemic adverse effects respectively, and determine an appropriate benchmark dose (5th percentile) to use in determining a safe level of exposure to an aerosol ingredient. In reviewing inhalation studies for respiratory tract and systemic effects certain types of chemicals, such as genotoxic carcinogens were excluded, as they were not considered representative of the ingredients that are, or could be used, in aerosols for consumer use. Application of an appropriate safety factor to this benchmark dose (BD) for local and systemic NOAECs and NOAELs gives an exposure threshold of toxicologic concern (TTC) for local and systemic toxic effects, below which there is no toxicology safety concern.

2024 EVALUATION OF IN VITRO MAMMALIAN GENOTOXICITY TESTS IN THE CURRENT CONTEXT.


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With the advent of new technologies, e.g., genomics, and new regulations, e.g., REACH, the field of regulatory genetic toxicology is in a state of flux. In the context of integrating new tests, we offer an analysis of current methods for evaluating mutagenic risk using the standard genotoxicity test battery. Some regulatory contexts consider genetic damage an important endpoint on its own, while others consider genotoxicity only in the context of carcinogenicity and in correlations with rodent cancer bioassays. Since there is no ideal assessment for carcinogenicity, we suggest that a focus on mechanisms may be more appropriate. We address issues related to the in vitro mammalian cell genotoxicity assays, including test results that are weak, associated only with toxicity or high concentrations, not accompanied by positive results in other tests, and/or not correlating well with rodent carcinogenicity tests. We summarize the results of international groups evaluating new tests and suggest options for integration of new technologies, including human monitoring. We conclude that improvements in data interpretation (e.g., FDA WoE document) and protocol updates provide justification for maintaining in vitro mammalian assays in the genotoxicity test battery. Disclaimer: The views and opinions expressed are solely those of the authors and are not official policy of the affiliated institutions.

2025 DEVELOPMENT OF A STRUCTURE ACTIVITY RELATIONSHIP PROCESS TO CHARACTERIZE ACUTE TOXICITY FOR GHS CLASSIFICATION.

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A critical health hazard endpoint in the Globally Harmonized System of Classification and Labeling of Chemicals (GHS) is acute toxicity. The GHS classification scheme allows for extrapolation of data from other non-oral toxicity tests, as well as the use of structure-activity relationships (SAR) to derive an Acute Toxicity Estimate (ATE). For mixtures lacking an ATE for any ingredient greater than 1%, the hazard communication (MSDS, label) under GHS must include a statement indicating the percentage of the mixture consisting of ingredient(s) of unknown toxicity. An internal process was developed to derive ATEs consistent with GHS guidance. For the chemical of interest, the process begins with collection of pertinent physical and chemical properties with applicability to toxicity, as well as collection of other non-oral toxicity endpoint data. This data is used in conjunction with commercially available and internally developed SAR software programs to provide estimates of predicted acute toxicity and the appropriate GHS acute toxicity category. A database was created to store the results of the estimation process, a description of the estimate rationale and the output from various software programs. The database also stores chemical structural information which allows for sub-structure searching and enhances consistency of estimates by allowing direct comparison of predicted values for similar compounds. Rule sets were developed to evaluate the likely reliability of the process based on chemical structure and properties, to facilitate consistency among estimates and to exclude estimates based on anticipated PBT properties. The process has been applied to over 100 chemical and/or toxicity endpoints. The availability of ATEs developed by this process increases the quality of the hazard determinations and classifications, eliminates the need for additional hazard communication statements concerning “unknown toxicity,” and reduces the need for animal testing.
2026 SURVEY METHOD FOR CHEMICAL SUPPLY CHAINS UNDER REACH.

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A new law concerning chemical control, "Registration, Evaluation, Authorization, and Restriction of Chemicals" (hereinafter called REACH), entered into effect in the European Union (EU) on June 1, 2007. REACH has a great impact on the activities of corporations, not only in EU industrialized nations, but also in Japan, U.S. and other non-EU industrialized nations because the law is a registration system vastly different from those adopted thus far by Japan, the USA, and Europe, in that the scope of advanced control by manufacturers and importers of chemical substances is expanded to existing chemical substances and chemical substances in articles that are already distributed around the world. A substance undergoes many processing operations by different manufacturers before it is imported in the EU in the form of an article or a product of any kind. As a result, one of the REACH mandates requires a "personalized" list re-quired to notify an importer of an article that includes the substances produced by the manufacturer of its registration information, may frequently require a huge amount of work to fill in complex missing links. However, the problem is not limited to the amount of work. If a specific company within a supply chain knows the complete picture of the network, it also knows the market and distribution channels of products for many of the other companies within the network to which it has business connections. This can pose a problem not only from the viewpoint of corporate ethics, but also of antimonopoly laws. For these reasons, manufacturers around the world are searching for effective solutions, with no fruitful results. In this research, we provide an outline of REACH and problems on a supply chain survey, propose revolutionary solutions of these problems, and discuss their effectiveness and usage.

2027 POLICY, SCIENCE, AND COMMUNICATION: PROTECTING CHILDREN FROM THE HEALTH EFFECTS OF LEAD.

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Increasingly sophisticated toxicological research on the health effects of lead and other chemical agents as well as direct experience provides ample knowledge that should drive policy changes that support an environment in which children can reach and maintain their full potential. Despite seemingly overwhelming scientific evidence it can be very difficult to institute meaningful policy directives. The efforts to change official policy on childhood blood lead action level provides an excellent case study of the science vs policy conflict. In 1991, CDC established the policy that recommended action if a child’s blood lead exceed 10 μg/dL. The national blood lead action level of 10 μg/dL is used as a standard by physicians and public health professionals to set state and local public health policy. Child health advocates in WA State worked to educate policy makers and public health professionals that there is no safe level of lead exposure and the blood lead action level should be lowered to 2 μg/dL based overwhelming data demonstrating the neurodevelopmental effects of low level lead exposure. These efforts had several positive outcomes: 1) lead was placed on the WA persistent bioaccumulative toxins list; 2) WA enacted a law with strict regulations on lead in child products (now being preempted by a less strict federal law); 3) WA Department of Ecology developed a lead chemical action plan that documents use of lead and makes recommendations on restricting the use of lead; 4) WA Department of Health worked to revise the state policy on childhood blood lead testing; and 5) submitted resolutions at the WA Public Health Association supporting a blood lead action level of 2 μg/dL. The WA lead cap provides an overview of the human health and environmental concerns about lead and recommendations for reductions in the uses and releases of lead. These efforts have encountered resistance and failures to adopt a precautionary approach to childhood lead exposure. We will examine why this resistance occurs despite scientific evidence the children are harmed a blood levels above 2 μg/dL.

2028 BACKGROUND RATES OF LYMPHOMAS/LEUKEMIAS AND LEYDIG CELL TUMORS IN SPRAIGE-DAWLEY RATS.

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The European Ramazzini Foundation (ERF) has conducted numerous bioassays with Sprague Dawley (SD) rats. When evaluating the results of animal bioassays, it is important to consider the overall background rates of disease in the animal model being used. However, in some ERF studies the background rates of certain cancers were higher than usual, making it difficult to evaluate the study results. Using published literature, we compared the rates of Leydig cell tumors and hematopoietic cancers in untreated SD rats in animal bioassays conducted by the ERF; to background rates reported by other laboratories. The endpoints were chosen due to their importance in evaluating MTBE carcinogenicity. For Leydig cell tumors, the mean ERF lab background rate was 5.8% vs 1.3% in the literature. In the total lymphomas/leukemias category, the percent of female rats in the ERF bioassays was, on average, more than triple the percentage identified in the other scientific literature (9.2% vs 2.6%). In contrast to other labs, the ERF lab reported background rates of lymphoid/monoblastic lymphoma in males and females, lymphoblastic lymphoma in females, and lymphoblastic leukemia in females. The rates of lymphocytic lymphomas and myeloid leukemia were similar for the ERF and other published literature. We recognize that changes in classification schemes over time and the limited data available make it difficult to evaluate the significance of findings for individual types of leukemia/lymphoma. Nonetheless, we conclude that the background rates for individual hematopoietic cancers in SD female rats as reported in the ERF studies are inconsistent with rates reported elsewhere and that overall, the ERF consistently reports higher percentages of female SD rats in the total leukemias/lymphomas category as compared to other published studies. If cancer rates in test animals are elevated, the rates in control animals should be considered when interpreting results, as elevations in cancer that are still within historical control rates could indicate a chance, rather than a significant, finding.

2029 TOXICITY CHARACTERISTICS OF CHEMICALS ON THE U.S. EPA DRAFT CONTAMINANT CANDIDATE LIST (CCL3).


The Safe Drinking Water Act Amendment of 1996 requires the United States Environmental Protection Agency (USEPA) to develop a Contaminant Candidate List (CCL) every 5 to 10 years to assist in priority-setting efforts. The Agency is required to make regulatory determinations for at least 5 contaminants 3 years after its publication. For CCL3, USEPA developed a process that considered a wide spectrum of potential drinking water contaminants. The process began with a Universe of about 6000 chemical contaminants. The screening criteria for potency and occurrence plus expert judgment, the Universe was reduced to a preliminary CCL (PCCL). The draft CCL3 was selected using computerized classification tools and expert judgment to prioritize the PCCL based on potential toxicity (potency and severity) and occurrence (prevalence and magnitude). The draft CCL3 developed using this process was published in the Federal Register on February 21, 2008. EPA examined the toxicity data from the draft CCL3 to characterize its distribution and its sources. Based on the 10 point scale used to select chemicals had potency scores at $5. Risk assessments conducted by the agency were the source of 63% of the toxicity data; other data sources included assessments by CalEPA, ATSDR, RAISHE and WHO (20%). The severity score was based on a 9 point scale with a score of 1 for no adverse effect to a score of 9 for death. More than 50% of chemicals had critical effects scored at six or higher. Approximately 33% were scored based on cancer as an outcome, 6% were scored based on reproductive and developmental effects, and scores for 13% were based on effects for other serious conditions or disorders assumed to be irreversible but not necessarily leading to death. [The views expressed in this abstract are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.]

2030 BEST PRACTICE FOR INCURRED SAMPLES REPRODUCIBILITY BIOANALYSIS IN PRECLINICAL STUDIES.

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During the 3rd AAPS/FDA (American Association of Pharmaceutical Scientists/Food and Drug Administration) Bioanalytical Workshop, it was suggested to test the reproducibility in the analysis of incurred samples for preclinical studies in addition to validation activities. FDA guidelines and published literature provide recommendations concerning the number and types of samples that should be reanalyzed and the way to evaluate these results. However follow-up activities and data reporting are still matter of discussion. Nevertheless, the application of these recommendations should improve the reliability of the bioanalytical results and ensure a better pharmacokinetic and safety assessment in preclinical settings. Usually, the validation of a bioanalytical method relies on data generated using spiked control biofluid samples. Actually incurred biological samples can vary in their composition when compared with the standards and quality control samples used to validate the method and analyze these samples. Laboratories where bioanalyt-ical activities are performed have now to be aware of these new FDA recommendations and there is an increasing need to apply them in order to achieve compliant Operating Procedures. In this paper, we focused on dealing, reporting, and using repeated results, need to be prepared referring to critical FDA recommendations as major guidelines. This Poster describes the results obtained from several set of incurred samples reanalysed to evaluate the reproducibility of bioanalytical methods supporting different GLP toxicokinetic studies.
The evaluation and promotion of alternatives for acute systemic toxicity testing is one of ICCVAM's four highest priorities because (1) acute toxicity testing is the most commonly required product safety test worldwide and (2) it causes significant pain and distress to test animals. We cosponsored a public workshop in February 2008 to review and consider standardized procedures to collect information pertinent to understand the mechanisms of lethality that should be included in future acute systemic toxicity studies to support further development of predictive mechanism-based in vitro test methods. This international workshop implemented one goal of the NICEATM–ICCVAM Five-Year Plan to identify approaches that would further reduce the potential pain and distress associated with acute toxicity testing by identifying more humane acute toxicity endpoints. The workshop reviewed public health significance and regulatory testing needs; human and animal assessments, biomarkers, and key pathways; humane endpoints; and the state of the science regarding in vitro methods that predict acute systemic toxicity. Breakout Groups identified knowledge gaps in understanding key toxicity pathways; recommended earlier humane endpoints for animal testing; suggested ways to obtain, from current in vivo testing models, mode of action and mechanistic information needed to develop and validate in vitro methods for assessing acute systemic toxicity; and explored avenues that would encourage industry to share information on in vitro and in vivo studies conducted in-house. This workshop recommended how mechanism-based in vitro test systems and earlier, more humane endpoints, could be developed to further reduce, refine, and eventually replace animal use for acute systemic toxicity testing while ensuring the protection of human and animal health. ILS supported by NIEHS contract N01-ES-35504. This presentation may

**2034** ESTIMATION OF A TETRACHLOROETHYLENE INHALATION CANCER RISK FACTOR: COMPARISON OF MASSDEP AND U.S. EPA DOSIMETRIC DECISIONS UNDER UNCERTAINTY.

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MassDEP derived a revised inhalation unit risk (IUR) for tetrachloroethylene (PCE) of 1 x 10⁻³ and 1 x 10⁻⁴ per µg/m³ in 2007, finalized in September 2008. Keeping with this, USEPA presented a range of IURs based on results from three human PBPK models. They then estimated an upper limit of human occupational exposure (UHE). The following derivation, USEPA released their draft 'Toxicological Review of PCE in June 2008 with IURs ranging from 2 x 10⁻⁶ to 2 x 10⁻⁵ per µg/m³. Both groups selected mononuclear cell leukemia in rats as the most sensitive endpoint from the key inhalation bioassay studies, NTP 1986 and JISA 1995; concluded that some form of action was uncertain; used default linear low-dose extrapolation in calculation of the IUR; and considered multiple approaches for adjusting animal bioassay dosimetry to lower levels of human exposure. The dosimetric adjustments need to take into account the differences in metabolism across species and dose-dependent differences in rates of metabolism in both animals and humans. Both groups selected total metabolized dose as the dose-metric because the active moiety is not known and multiple metabolites could be responsible for the carcinogenic effects. However, MassDEP used Michaelis-Menten steady-state kinetic equations to estimate animal total metabolized dose, while USEPA used a pharmacokinetic (PBPK) model. To estimate human population metabolism at relevant human environmental exposure levels MassDEP and USEPA were informed by different PBPK models and estimates of expected extent of human metabolism. Using the analysis by Chui and Bois (2006) to estimate the fraction of PCE metabolized by humans while accounting for human population variability in low dose metabolism: they estimated an upper limit of human total metabolized dose of PCE of 6 x 10⁻³ at 1 ppb. USEPA presented a range of IURs based on results from three human PBPK models yielding central estimates of human low dose metabolism ranging from 3–55%, as calculated by MassDEP. While different decisions were made in the dosimetric extrapolation, MassDEP's IUR falls within the range of USEPA values.

**2035** WEIGHT OF EVIDENCE PROTOCOLS FOR SKIN NOTATION ASSIGNMENT IN OCCUPATIONAL HAZARD ASSESSMENT.

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Skin contact is a significant route of exposure in occupational settings. However, many occupational health risk assessments rely on qualitative ‘skin’ notations to provide information on the potential for dermal exposures to contribute significantly to the systemic dose, and in particular, to inform the interpretation of quantitative risk assessments based on concurrent inhalation exposures. The underlying decision criterion for making such assignments is often not documented. Moreover, the traditional skin notation approach does not provide information on other effects of dermal exposure. We have reported previously on an enhanced notation strategy developed by the National Institute for Occupational Safety and Health
(NIOSH) that provides for the assignment of multiple skin notations that address systemic toxicity, direct skin effects, and dermal sensitization. The current work presents experience in applying the NIOSH strategy to over 70 chemicals. Weight of evidence decisions to address conflicting or limited data sets is highlighted. Examples of common situations include: 1) assigning a systemic effects notation where data or model predictions indicate absorption, but no or only limited dermal toxicity data are available, 2) differentiating among irritant severity levels when relying on qualitative studies that used different material dilutions and test systems, 3) developing notations for sensitization when limited human studies and standard animal assays provide conflicting results. The lessons learned in evaluating such problematic data sets provide the basis for refining weight of evidence evaluation procedures for hazard notations.

2036 ICCVAM PERFORMANCE STANDARDS FOR THE MURINE LOCAL LYMPH NODE ASSAY (LLNA).

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ICCVAM develops performance standards to facilitate the efficient validation of modified versions of adequately validated alternative test methods. ICCVAM recently developed performance standards based on the ICCVAM-recommended LLNA protocol (ICCVAM 1999). The protocol was revised recently to reduce the minimum number of mice per dose group from five to four, and to provide guidance on reducing the number of positive control animals and determining the appropriate highest test dose. The performance standards include essential test method components, a minimum list of reference substances, and standards for accuracy and reliability. Essential test method components are the structural, functional, and procedural elements of a validated test method that must be included in a modified method in order for it to be evaluated using the established performance standards. Essential components of the LLNA include topical application of the test substance to the ears of mice, measurement of lymphocyte proliferation in the lymph nodes draining the area of test substance application, and use of the maximum soluble dose that does not result in systemic toxicity or excessive local irritation. The minimum list of reference substances for these LLNA performance standards includes 13 sensitizers and 5 non-sensitizers. The accuracy and reliability standards to be achieved by a modified LLNA are based on the performance of the traditional LLNA. These LLNA performance standards will facilitate rapid and efficient validation of modified LLNA protocols, such as those using non-radioactive markers of lymphocyte proliferation. New versions of the LLNA that provide improved performance or other advantages are expected to result in broader use of the LLNA, which will further reduce and refine animal use for allergic contact dermatitis assessments while ensuring human safety. ILS staff supported this abstract by NIEHS contract N01-ES-35504.

2037 LLNA: CURRENT REGULATORY ISSUES.


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Legislation in the EU requires evaluation of the skin sensitization potential for more than 30,000 substances. For many of these the local lymph node assay (LLNA) is the required test, unless a scientific rationale can be provided for use of an alternative approach. Elements of the rationale may be that open application is unsuitable, or that the chemical is a member of a class for which there is evidence of common association with false positive or false negative results. Certain chemical classes, for example fatty alcohols, have been reported as potential false positives in the LLNA. It is important that the evidence for this is reviewed rigorously and documented, so that there is a general consensus about which substances truly represent false positives, rather than weak allergens for which hazard classification would rather be avoided. The OECD Test Guideline 429 for the LLNA provides for a minimum of 4 mice per dose group, or 5 mice per group if statistics are required. Recent discussions at the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) have suggested a change to LLNA requirements to mandate a minimum of 5 mice per group. This has prompted a retrospective analysis of published data from our own laboratories to determine whether use of 4 or 5 of mice has had any impact on performance of the LLNA. Of the datasets for 4 mice per group the 4 and 5 animals per group, 1 uncertain and 2 negative 40 results were identical in the 5 animal assay. One marginally positive result in the 4 animal assay was negative in the 5 animal assay. Where potency determinations were made, the outcomes were essentially identical in both forms of the LLNA. It is concluded there is no scientific justification for removing the option of a 4 mice/group LLNA. This will help to keep to a minimum the number of animals used for skin sensitization testing under REACH.

2038 NONCLINICAL RODENT STUDIES FOR A NOVEL PACLITAXEL-PEPTIDE CONJUGATE THAT TARGETS THE BRAIN.

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Sponsor: I. Daniels.

ANG1005 is a novel cytotoxic drug that combines a receptor-targeting peptide vector to 3 molecules of paclitaxel. The peptide vector can be applied to other molecular classes, including biologics, and is designed to enable transcytosis across the blood-brain barrier by targeting the low-density lipoprotein receptor-related protein. The toxicity profile of ANG1005 in Beagle dogs has been investigated in a series of intravenous (IV) infusion toxicity studies. ANG1005 was administered at single IV dose levels of 0, 100, 200, or 400 mg/m² with both 400 mg/m² animals and 10% of the animals (STDₜₒ) was estimated to be 75 mg/m². The ANG1005 rodent studies supported in part the initiation of 2 Phase 1 clinical trials in brain cancer patients.

2039 BEAGLE DOG TOXICITY STUDIES FOR A NOVEL PACLITAXEL-PEPTIDE CONJUGATE THAT TARGETS THE BRAIN.

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**2040 AERODYNAMIC AND SAFETY PROFILES OF NEBULIZED ANTI-C5 MABS IN A GLP INHALATION TOXICOLOGY STUDY.**

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Complement C5 plays a key role in the pathogenesis of asthma. A previous study demonstrated that a single nebulization delivery (10 min) of murine Anti-C5 was effective in ameliorating disease in rodents. To support the utility of aerosol delivery of anti-C5 mAb for intrapulmonary C5 inhibition in patients, a GLP toxicology study was performed with CD-1 mice to assess the safety of nebulized ALXN5100, a humanized anti-human C5 mAb and its surrogate, BB5.1, a mouse mAb specific to mouse C5 (Frei et al, 1987). Mice were exposed to low and high concentrations of BB5.1 (~0.12 mg/kg: 90 and 120 min) for 4 and 14 days and ALXN5100 (~0.32 mg/L: 30 and 120 min) for 4 days. Vehicle control was also included. Particle sizes for both BB5.1 and ALXN5100 were ~1.5-2.2 micon (GSD ~2.2) after nebulization with a Part L1C plus jet-air nebulizer.

Antibodies collected after nebulization showed intact mAb integrity and comparable activity as pre-nebulization controls. Average daily calculated inhalated/deposited (assumed 100%/ 10% pulmonary and tracheobronchial respectively) doses were ~3.4/3.4 mg/kg (Low); ~3/3.3 mg/kg (High) for BB5.1 and ~9/9.9 mg/kg (Low); ~37/3.7 mg/kg (High) for ALXN5100 after 4 and 14 days of exposure. Aerosol exposures to ALXN5100 were confirmed in serum and lung tissue of exposed mice. Exposures of BB5.1 caused no significant changes in serum C5 mediated hemolysis compared with vehicle control values. Exposures to both test articles were well tolerated. No mortality, morbidity, adverse clinical signs or treatment-related effects in clinical pathology, body weight, organ weight, or BALF parameters were observed. Histopathology revealed minimal to mild nasal and tracheal epithelial degeneration consistent with inhalation exposures in rodents in both vehicle control and treated groups. No other treatment-related histopathology was observed. These results indicate that ALXN5100 has a safety profile consistent for nebulization delivery in clinical studies.

**2041 NON-CLINICAL SAFETY ASSESSMENT OF A BI-FUNCTIONAL ANTIBODY FUSION INHIBITOR TARGETING CD4 AND HIV.**


Bi-functional fusion inhibitor (BFFI) is designed for treatment of HIV infection in treatment-experienced patients. BFFI is a fusion protein consisting of a humanized Immunoglobulin G1 (IgG1) isotype antibody that targets CD4 tagged with a fusion inhibitor peptide, T-651, which is derived from the H2R region of HIV-1 gp41. The antibody carries the L23A4 and L23A5 double mutation, which attenuates Fc-mediated antibody dependent cell-mediated cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). The peptide is linked to the antibody via a glycine-serine linker sequence. To assess the non-clinical safety of BFFI, an in vitro immunosafety screening strategy was employed, followed by an in vivo two-week range-finding toxicology study in cynomolgus monkeys. In vitro immunosafety was assessed in peripheral blood mononuclear cell assays that examined T cell activation end points, including cell proliferation, CD25/CD69 expression, and cytokine secretion. Induction of effector function (CDC and ADCC) and cytokine release in the blood assay were assessed. No perturbations in the in vitro assessments were observed. In addition, BFFI was well tolerated to up to 10 mg/kg in cynomolgus monkeys when administered via extended intravenous bolus every 3 days for 15 days. In vivo immunophenotyping was consistent with in vitro results, indicating no effect on T cell populations or markers of activation.

**2042 PRECLINICAL SAFETY EVALUATION OF AN ANTI-β7 INTEGRIN ANTIBODY TARGETING LYMPHOCYTE HOMING TO AND RETENTION IN THE GASTROINTESTINAL TRACT.**


rhuMAB Beta7 is a humanized anti-β7 integrin monoclonal antibody that is being investigated as a potential therapeutic for inflammatory bowel disease. It binds to and inhibits β7 integrins, which mediate lymphocyte homing to and retention in the intestinal mucosa. Studies in mice demonstrated that rhuMAB Beta7 selectively inhibits lymphocyte homing to mucosal tissues, such as the gastrointestinal (GI) tract, without affecting their trafficking to peripheral lymphoid tissues or the CNS. Repeat-dose toxicity studies were conducted in cynomolgus monkeys to evaluate the toxicity, toxicokinetics and pharmacodynamics (PD) of rhuMAB Beta7. It was well tolerated in cynomolgus monkeys following weekly intravenous (IV) or subcutaneous (SC) injection for 12 weeks at doses up to 50 mg/kg. Core safety pharmacology (cardiovascular, respiratory and neurological) endpoints were incorporated into the toxicokinetic study, and no drug-related effects were observed. Clinical signs including post-dose vomiting, hypoactivity and recumbency were observed in a subset of the animals given 5 mg/kg via IV injection. These clinical signs were manifest on the day of dosing following 3 or more doses, correlated with high-titer anti-drug antibodies, and were attributed to injection of a heterologous protein. Increases in peripheral blood gut homing memory T cells were observed, consistent with the anticipated PD effect of specific inhibition of lymphocyte homing to the GI tract. In contrast, no effect was observed on naive or peripheral-homing memory cells. The PD effect was reversible following drug clearance. Other than minimal to slight peripheral infiltration of lymphocytes and macrophages in the SC injection sites that was attributed to injection of heterologous protein, no drug-related microscopic changes were noted. In conclusion, the NOAEL following weekly IV or SC injection of rhuMAB Beta7 for 12 weeks is 50 mg/kg, the highest dose tested.

**2043 NONCLINICAL SAFETY EVALUATION OF XOMA 052, A MONOCLONAL ANTIBODY TARGETING IL-1β IN REPEAT DOSE TOXICITY STUDIES IN RATS AND CYNOMOLGUS MONKEYS.**

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XOMA 052 is a potent Human Engineered™ monoclonal antibody that specifically binds to IL-1β (IL-1β) and blocks activation of the IL-1 receptor. IL-1β is a proinflammatory cytokine involved in the development of diabetes, rheumatoid arthritis, gout and other diseases. Binding to IL-1β is expected to prevent the cellular signaling events that produce inflammation. XOMA 052 is currently being developed for acute, chronic and orphan indications, including its evaluation in two Phase 1 clinical studies in Type 2 diabetes designed to assess safety, pharmacokinetics and measures of systemic inflammation. The rat and cynomolgus monkey were selected for safety evaluation due to similar binding affinity and in vivo functional activity with XOMA 052 and human, rat and monkey IL-1β. XOMA 052 was administered intravenously weekly for 4 weeks at doses up to 100 mg/kg in rats, and 4 and 13 weeks at doses up to 90 mg/kg in monkeys, each study with a recovery period of 2 months. The nonclinical safety evaluation included daily clinical observations, food consumption, body weights, hematology, clinical chemistry, coagulation parameters, urinalysis, ophthalmologic examinations, electrocardiography (ECG), heart rate/blood pressure, T cell-dependent antibody response (TDAR), immunogenicity, as well as macroscopic and microscopic pathology. Pharmacokinetic analysis showed a half-life for XOMA 052 of 10-12 days in both species. The results showed no toxicologically significant findings, and the no-observed-adverse-effect level (NOAEL) was considered to be 100 mg/kg in rats and 90 mg/kg in monkeys. These studies supported the Phase 1 clinical evaluation of XOMA 052 in patients with Type 2 diabetes. Clinical studies in rheumatoid arthritis and acute gout will be initiated in 2008.

**2044 SAFETY ASSESSMENT OF A NON-DEPLETING ANTI-CD4 MONOCLONAL ANTIBODY (MAB) IN BAOBONS.**

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MTRX1011A is a non-depleting, humanized mAb being evaluated in rheumatoid arthritis patients. It binds to CD4 resulting in receptor downmodulation and inhibits the function of residual surface CD4 molecules by blocking interaction with the major histocompatibility complex (MHC) II. The nonclinical safety assessment designed to support Phase 1 clinical trials of up to 8 weekly IV/SC doses consisted of a toxicology study evaluating toxicity, toxicokinetics, and safety pharmacology in baboons at 0, 5, 15, or 50 mg/kg/wk, hemolytic potential and blood compatibility testing, and tissue cross-reactivity analyses. The baboon was chosen as a relevant species due to the crossreactivity of MTRX1011A to human and baboon CD4+ T cells. MTRX1011A was well tolerated in baboons; no adverse effects were observed during the dosing phase or the 10-week recovery phase. There was no impact of MTRX1011A on a core battery of intravascular and neurological endpoints assessed as part of the toxicology study. MTRX1011A demonstrated nonlinear kinetics driven by saturable CD4-mediated elimination. Serum concentrations correlated with the downmodulation and saturation of CD4 on peripheral-blood T cells. MTRX1011A bound to CD4 on T cells and resulted in rapid internalization of the toxin, toxicokinetics and pharmacodynamics (PD) of rhuMAB Beta7. It was well tolerated in cynomolgus monkeys following weekly intravenous (IV) or subcutaneous (SC) injection for 12 weeks at doses up to 50 mg/kg. Core safety pharmacology (cardiovascular, respiratory and neurological) endpoints were incorporated into the toxicokinetic study, and no drug-related effects were observed. Clinical signs including post-dose vomiting, hypoactivity and recumbency were observed in a subset of the animals given 5 mg/kg via IV injection. These clinical signs were manifest on the day of dosing following 3 or more doses, correlated with high-titer anti-drug antibodies, and were attributed to injection of a heterologous protein. Increases in peripheral blood gut homing memory T cells were observed, consistent with the anticipated PD effect of specific inhibition of lymphocyte homing to the GI tract. In contrast, no effect was observed on naive or peripheral-homing memory cells. The PD effect was reversible following drug clearance. Other than minimal to slight peripheral infiltration of lymphocytes and macrophages in the SC injection sites that was attributed to injection of heterologous protein, no drug-related microscopic changes were noted. In conclusion, the NOAEL following weekly IV or SC injection of rhuMAB Beta7 for 12 weeks is 50 mg/kg, the highest dose tested.
antibody-CD4 receptor complex. These constituted expected pharmacologic re- sponses. A subset of baboons developed anti-therapeutic antibodies but most main- tained expected drug exposure and pharmacodynamic effects throughout the dos- ing phase. Additionally, MTRX1011A did not cause hemolysis of baboon or human erythrocytes and was compatible with baboon and human serum and plasma. In the tissue cross-reactivity study, boitin-MTRX1011A binding was ob- served in a pattern consistent with the expected distribution of CD4 expression in both human and baboon tissues. The IND-enabling toxicology program enabled entry of MTRX1011A into Phase I human clinical trials with an adequate safety margin.

2045 THE ANTI-DIABETIC METFORMIN IS TOXIC TO LIVER MITOCHONDRIA OF ZUCKER DIABETIC FATTY (ZDF) RATS: A CELLULAR METABOLIC STUDY WITH 13C-LACTATE AND CARBON 13 NMR.

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Metformin, one of the most widely used antidiabetic drugs, has been previously shown to decrease hyperglycemia in diabetic subjects and in diabetic animal models. This effect results in part from the inhibition of hepatic gluconeogenesis. Since lactate is the main gluconeogenic precursor, the objective of this study was to investi- gate in vitro the effect of metformin on hepatic lactate gluconeogenesis by using a cellular metabolic approach. Precision-cut liver slices from adult male ZDF rats were incubated in oxygenated Krebs-Henseleit medium with variously 13C-labeled lactates (5 mM) or with 5 mM unlabeled lactate plus 25 mM 13C-bicarbonate. Substrate removal (lactate and glycerol) and product formation were measured by enzymatic and 13C-NMR methods. In slices from fasted lean ZDF rats, metformin dose-dependently inhibited 13C-glucose synthesis from [2-13C]-lactate (IC50 2.3 mM). The labeling patterns of the glucose synthesized indicated that lactate gluco- neogenesis involved not only the passage of carbons through pyruvate carboxylase and the reversible equilibration of oxaloacetate with fumarate but also pyruvate de- hydrogenase and the entire tricarboxylic acid cycle. In slices from fed diabetic ZDF rats, 2 mM metformin did not change glycogen degradation but inhibited lactate consumption by 97%, glucose production from glycogen plus lactate by 18% and the synthesis of all the 13C-labeled glucose carbons from all 13C-labeled substrates by 70, 70, 71 and 63%, respectively. By contrast, metformin increased lactic acid production by 96% and ketogenesis by 78%. Metformin also caused a reduction of cellular ATP levels and of 13C-O2 production from 13C-lactates but increased the beta-hydroxybutyrate/acetoacetate ratio which reflects the mitochondrial redox state. These results strongly suggest that a compound found to be toxic to liver mitochondria in vitro should not necessarily be withdrawn from drug development.

2046 TOXICITY AND TOXICOKINETICS OF METFORMIN IN RATS.


Metformin is the first-line drug for the treatment of type 2 diabetes mellitus (T2DM) and is often prescribed in combination with other drugs in order to control a patient’s blood glucose level and achieve HbA1c goal. New treatment options for T2DM will likely include fixed dose combinations with metformin, which, according to current FDA guidance, may require combination toxicology studies. Although metformin has been on the market for many years, there is little to no preclinical data to aid in design of these complex toxicology studies. Therefore, to understand toxicity of metformin alone, CrlCD(SD) (12/sex/group) were administered metformin at 6, 200, 600, 900, or 1200 mg/kg/day by oral gavage for 13 weeks. Three animals/sex were added at each dose for toxicokinetic evaluation. Administration of 5/900 mg/kg/day resulted in mori- boutony/mortalit and clinical signs of toxicity (reduced food intake and fre- quent nonformed feces). Other adverse findings included increased minimal necro- sis with inflammation of the parotid salivary gland for males given 1200 mg/kg/day, and body weight loss (20%) and clinical signs (associated with mori- boutony at the higher doses) in rats given ≥600 mg/kg/day. Metformin administra- tion was associated with evidence of minimal metabolic acidosis (increased serum lactate and beta-hydroxybutyric acid, and decreased serum bicarbonate and urine pH) at doses ≥600 mg/kg/day. There were no marked (≤2-fold) sex differences in mean AUC0-24 or Cmax, nor were there marked differences in mean AUC0-24 or Cmax following repeated dosing compared to a single dose. The mean AUC0-24 tended to increase in approximate proportion with the increase in dose. The mean Cmax tended to increase less than proportionally with the increase in dose. The no observable adverse effect level (NOAEL) was 200 mg/kg/day (mean AUC0-24 = 41089 ng/h/mL; mean Cmax = 10272 ng/mL based on gender average end of study values). These effects should be taken into consideration when assessing potential synergistic toxicities of metformin fixed dose combinations.
PS 2049 IMMUNOTOXICITY TESTING IN RATS FOLLOWING 28-DAY ORAL EXPOSURE TO A NOVEL P38 MAP KINASE INHIBITOR.

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Pamapimod is a small molecule pyridone which selectively inhibits p38(α) MAP kinase in efficacious doses. 0.1-0.4 μM (0.04-0.16 μg/mL) of pamapimod resulted in >50% inhibition of IL-1β and TNFα production. Its immunotoxic effects were evaluated in an oral 28-day study in Wistar rats (10 rats/group) with 50, 150 and 500 mg/kg/day of pamapimod (AUC0-24 98-585 μg*h/mL). No mortality was observed. Following pamapimod administration, clinical signs were mild (yellow material on body surface, salivation, ≥150 mg/kg/day) with lower body weights/food consumption at ≥50 mg/kg/day. At necropsy epididymal changes were noted in the 500 mg/kg/day group males. Higher white blood cell counts (lymphocytes and neutrophils) at the 150 and 500 mg/kg/day dose levels were noted but no alterations of the major organs in the immune system (including thymus and spleen weights). There were minor non-adverse differences in erythrocytic parameters (females ≥50 mg/kg/day and 500 mg/kg/day males). No effect of pamapimod was noted during the phenotypic analysis of splenic subpopulations (total B cells, T cells, natural killer cells, and macrophages). Cell-mediated immunity (anti-CD3-mediated proliferation) exhibited a slightly enhanced response ≥150 mg/kg/day. A dose-dependent decrease in the humoral immune response (T-cell dependent antibody-forming cell response) was observed. A lower innate immunity response (NK cell assay) was noted at the 500 mg/kg/day dose level. However, pamapimod changes were relatively minor compared to those of the positive control articles (cyclosporinamide and antiasialo-GM1 treatment). In summary, dose-related effects of pamapimod on the various components of the immune system were consistent with the pharmacological action as a p38 MAP kinase inhibitor and the associated cytokine inhibitory mode of action and as such, represent enhanced pharmacological effects.

PS 2050 REPRODUCTIVE TOXICITY ASSESSMENT OF PAMAPIMOD, A NOVEL P38 MAP KINASE INHIBITOR.

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Pamapimod is a small molecule pyridone which selectively inhibits p38(α) MAP kinase in efficacious doses. 0.1-0.4 μM (0.04-0.16 μg/mL) of pamapimod resulted in >50% inhibition of IL-1β and TNFα production. Its potential reproductive effects were assessed according to ICH recommendations. In a Segment I rat study (M: 30-1000 mg/kg/day for 28 days; F: 3-100 mg/kg/day for 15 days), when the males were given 1000 mg/kg/day (AUC0-24 656 μg*h/mL), there was a slight decrease in the pregnancy rate (70.8%) compared to controls (88%). That dosage decreased the pregnancy rate (70.8%) compared to controls (88%). Thus, hematotoxicity may be a primary event associated with AZT toxicity in HIV-infected patients. Future therapies of Hepatitis C will involve antiviral agents in a combination with today’s standard of care (PegInterferon and Ribavirin). A rat 28 day toxicity study, was performed to investigate the potential synergistic effects on toxicity profiles and mutual drug exposure of VCH-759 or VCH-916 either with or without Ribavirin co-treatment. Sprague-Dawley rats were treated by oral gavage with VCH 759 (200 mg/kg/day) or VCH 916 (100 mg/kg/day) twice daily with or without once daily Ribavirin (40 mg/kg/day) co-treatment for 28 days. The doses were selected to achieve therapeutic systemic exposure and the standard toxicology parameters were monitored. There was no significant toxicity observed for the rats treated with VCH-759 or VCH-916 alone. The main observations in the Ribavirin alone group were effects on the haematological parameters (slight reduction in Red Blood Cell, Haemoglobin, Hematocrit) and elevated bone marrow myeloid to erythroid ratios with erythrocyte hypoplasia. These effects were significant relative to the reported toxicity of Ribavirin in rats namely an anaemia associated with a bone marrow suppression. Co-administration of Ribavirin with either VCH-759 or VCH-916 resulted in minor, but reversible, reductions in weight gain or food intake. The hematotoxic changes attributed solely to Ribavirin were neither mitigated nor exacerbated by co-administration of VCH-759 or VCH-916. Furthermore there was no significant interaction in drug exposure to the co-treated groups. In conclusion, the co-administrations of VCH 759 or VCH-916 with Ribavirin did not exacerbate the changes observed with Ribavirin alone and this favourable drug combination safety study in rats further support the clinical evaluation of VCH-759 or VCH-916 in combination with Ribavirin.

PS 2051 ZIDOVUDINE (AZT) ALTERS HEMATOLOGIC PARAMETERS AND HEPATIC MITOCHONDRIAL FUNCTION IN B6C3F1 MICE.

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Sex-based differences in adverse effects of antiretroviral treatment have been observed in HIV-1 infected patients. The basis of the adverse effects is multi-facotorial, with mitochondrial toxicity being recognized as a key element underlying most side effects of antiretroviral drugs. To get better insight into the mechanism of sex-related differences in drug toxicity, we measured hematologic parameters and hepatic expression profiles of 542 mitochondria-related genes in male and female B6C3F1 mice administered antiretroviral drug, zidovudine (AZT). Beginning at 8 weeks of age, animals were treated orally by gavage with 0, 400 or 800 mg/kg/day of AZT for 14 days. Male body weight/day for 12 weeks and were humanely sacrificed 24-hr following the last dose. In both male and female mice, red blood cell (RBC) count was lowered in conjunction with significantly higher levels of mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) at all AZT doses tested. Sex-based differences were also noted in MCV and MCH levels at 400 mg/kg AZT, where female mice had higher levels compared to males. AZT also influenced expression levels of mitochondria-related genes, but only at the highest dose where several pro-apoptotic genes were up-regulated while genes involved in lipid metabolism were down-regulated. Sex-related difference in the transcriptional level of genes associated with oxidative phosphorylation was seen only in control mice. The results suggest that AZT-induced higher MCV and MCH levels may be in response to the lower RBC count that occurred even at the lowest AZT dose. Changes in mitochondrial-related gene expression in the liver were evident only at the highest dose. Thus, hematotoxicity may be a primary event associated with AZT toxicity in B6C3F1 mice [support: OWH/FDA and in part by IAG (224-07-007) between NCTR/FDA and NIEHS/NTTP].
14 compared with an 11% gain in controls. Asparagine aminotransferase (AST) and alanine aminotransferase (ALT) values were significantly elevated in male and female rats on Day 8. At Day 8 interim necropsy, minimal hepatocellular necrosis was seen in the 300 mg/kg dose group for both male and female rats. In the 14-day study there was an 11-20% increase in ALT and AST values for males and females administered 150 mg/kg on Day 15. These results indicate that the liver is the primary target organ of K777 in rats. All hepatic changes resolved in the recovery period. In conclusion, oral administration of K777 in male and female rats resulted in hepatotoxicity at doses of 150 mg/kg and above, but the insult observed adverse effect level (NOAEL) was approximately 50 mg/kg, and the hepatic effects are not expected to be dose limiting in clinical trials. Work supported by NIAID Contract N01-AL-60011.

2054 MECHANISTIC INVESTIGATION OF N, N-DIETHYL-4-(PHENYL-PIPERIDIN-4-YLIDENEMETHYL)-BENZAMIDE-INDUCED INSULIN DEPLETION IN THE RAT AND RINm5F CELLS.


These studies describe the effect of N,N-diethyl-4-(phenyl-piperidin-4-ylideneethyl)-benzamide (AR-M100390), a delta opioid agonist, on the pancreas and its mechanisms for pancreatic toxicity. Rats were treated with 5, 100, and 600 μmol/kg of AR-M100390 for 3 and/or 7 days; another group treated with 600 μmol/kg of compound were allowed to recover for 14 days. AR-M100390 (600 μmol/kg) caused vacuolation in the β-cell of the rat pancreas that was associated with depletion of insulin and hyperglycemia after 7 days of dosing. The loss of insulin by AR-M100390 was due to specific inhibition of rat insulin2 (rIns2) mRNA transcription in vivo. Insulin depletion and hyperglycemia were reversible. The effects of AR-M100390 in rats were reproduced in the rat pancreatic β-cell line RINm5F, where it inhibited intracellular insulin content and secretion without affecting cell survival. Loss of insulin in vitro was also a result of specific inhibition of insulin2 mRNA transcription and was reversible. Pre-treatment of cells with the δ-opioid antagonist naltrindole or pertussis toxin did not reverse loss of insulin in AR-M100390-treated cells suggesting that the effects were not mediated by the δ-opioid receptor. AR-M100390 inhibited K+-mediated calcium mobilization in RINm5F cells, suggesting that L-type calcium channels found in these cells and in pancreatic β-cells may partially play a role in the inhibition of insulin secretion by this compound. In summary, the in vitro and in vivo studies suggest that inhibition of insulin by AR-M100390 is due to a combination of inhibition of insulin synthesis and/or release.

2055 TREATED WITH KNS-760704 SHOW A FAVORABLE SAFETY AND TOXICOLOGY PROFILE WITH HIGH BRAIN:PLASMA LEVELS THAT SUPPORT CLINICAL DEVELOPMENT IN ALS.


KNS-760704 is an orally administered small molecule currently in Phase 2 clinical trials for the treatment of amyotrophic lateral sclerosis (ALS). Safety and toxicity were evaluated across several studies in the Sprague Dawley rat, the primary rodent toxicity species. KNS-760704 was generally well-tolerated in IND-enabling and subsequent toxicology studies when dosed by oral gavage up to 2000 mg/kg as a single dose and with daily doses up to 500 mg/kg for 2 weeks and 300 mg/kg for 6 months, including a 3-month interim assessment. The most prominent effect was mortality in males and females at single doses greater than 500 mg/kg/day. Significant decreases in body weight gain in males were observed after 2 weeks of dosing at 500 mg/kg/day and 6 months of dosing at 300 mg/kg/day. The NOAEL after 6 months of dosing was 100 mg/kg/day in males and a 300 mg/kg/day in females. In general, plasma levels of KNS-760704 followed first order kinetics and increased with dose with T1/2 ~4 hours and Tmax ~2 hours. Exposure (AUC 0-24) to KNS-760704 after 6 months of dosing at 300 mg/kg/day was 247,471 ng/hr/mL in males and 235,014 ng/hr/mL in females. In a separate study, oral bioavailability approximated 100% following single oral and IV doses. KNS-760704 was also evaluated in a CNS functional observational battery with no neurobehavioural effects seen after single doses up to 500 mg/kg. A pulmonary safety pharmacology study with single doses up to 500 mg/kg showed an increase in respiratory rate and minute volume and a decrease in tidal volume following single doses of 150 and 500 mg/kg. The NOAEL was 50 mg/kg. In a single-dose distribution study, brain:plasma levels of KNS-760704 ranged from 6:1 to 15:1 over 3 hours, which suggests that KNS-760704 may also concentrate in the human brain relative to plasma. These data demonstrate the preclinical safety of KNS-760704 and support its further clinical development.

2056 PRECLINICAL SAFETY EVALUATION OF OT-551 HCL, AN OPHTHALMIC CLINICAL CANDIDATE FOR THE TREATMENT OF DRY AGE-RELATED MACULAR DEGENERATION.


A preclinical safety evaluation of OT-551 HCl was completed in support of an IND Submission for an oculcar clinical evaluation in patients with early stages of dry age-related macular degeneration. An acute toxicity study in Sprague-Dawley rats was completed by infusion over 10 minutes with single i.v. OT-551 doses of 0, 10, 30, 100, and 200 mg/kg at 30 mL/kg. The no-observed-effect level (NOAEL) was 10 mg/kg; the maximum tolerated dose (MTD) was 30 mg/kg with immediate post-dose decreased motor activity and labored breathing/respiration. Ocular toxicity studies with OT-551 were conducted in rabbits and dogs. Both species received 0, 1, 2, or 3% ophthalmic solutions of OT-551 (10 μL/dose) 5 times daily at 3-hour intervals for 28 days. In rabbits, no drug-related effects were found on daily observation, or with monitoring of body weight or food consumption, except for slight conjunctiva redness in some animals at all dose levels, including vehicle. Several animals treated with the mid and high doses showed mild conjunctival congestion, reflective of minor local irritation. No other ophthalmologic changes were noted. In rabbits, slight conjunctival redness was noted in some animals at all dose levels and moderate redness in one high-dose animal. At necropsy, no clinically meaningful changes were observed following macroscopic or histopathology examinations. The no-observed-adverse-effect level (NOAEL) for OT-551 was 2% in dogs and 3% in rabbits. Plasma levels collected at 0.5 hr and later of OT-551 and TP-H (active metabolite) at all sampled time points on Study Days 1 and 27 were below quantifiable limits of the LC/MS/MS assay (50 and 20 ng/mL, respectively). The in vitro genetic toxicology studies conducted with OT-551 determined that the compound was not mutagenic or genotoxic in the Bacterial Reverse Mutation Assay. Mouse toxicity was observed in a 90-day repeat-dose study performed in CD-1 mice. Electron scanning microscopy and histological evaluation showed that the compound caused vacuolation in the retina, which was reversible. Work supported by the National Eye Institute and Research to Prevent Blindness.

2057 HEMOSTATIC TEXTILE WOUND DRESSING STASILON™ EXHIBITS REDUCED SKIN ADHESION AND LOW TOXICITY.

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A hemostatic textile wound dressing (brand named Stasilon™) was developed using a proprietary weave of 65% (wt/wt) vitreous continuous filament e-glass and 35% (wt/wt) bamboo rayon fiber. The diameter of the continuous filament e-glass fibers is 7 microns + or - 10% and can not be reduced by any degree of rough handling. The underlying reason for the absolute inability to produce smaller diameter fibers with wear and/or damage is that the vitreous (like glass) nature of the parent fiber will not permit longitudinal fracture. Therefore, potentially carcinogenic fiber diameters of 1.5-3 microns are not generated from this textile. Mechanical agitation of the parent 7 micron diameter e-glass fibers can shorten the original length of the wound dressing fibers, but can not reduce the original diameter. The chemical composition of the e-glass fibers is not intrinsically toxic, with tissue irritation for certain fiber size ranges being due to mechanical processes only. A battery of toxicity tests were conducted successfully, leading to 510(k) approval of the hemostatic textile for external application to breached skin for up to 30 days. The toxicity battery included evaluations of cytotoxicity in mammalian fibroblast cells; delayed contact sensitivity in guinea pigs; intratracheal injections and dermal irritation in rabbits; systemic injections in albino mice; 31 day implantation into the paravertebral muscles of rabbits; hemolysis; Ames mutagenicity in strains TA97a, TA98, TA100, TA102 and TA1535 with and without S9 activation; and bacterial endotoxins and pyrogens. Stasilon™ wound dressing outperforms gauze in both stopping bleeding and not adhering to the wound site while exhibiting a highly favorable toxicological safety profile.

2058 PRECLINICAL SAFETY ASSESSMENT OF INHALED XYLITOL: SUPPORTING STUDIES FOR CLINICAL TRIALS IN CYSTIC FIBROSIS.

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Xylitol is a 5-carbon sugar that lowers airway surface liquid salt concentration potentially enhancing innate immunity and reducing airway infections. In order to support repeat dose clinical studies, a preclinical toxicology program was performed. Three groups of rats and dogs were exposed to nebulized xylitol (in water)
by nose-only or face mask inhalation for 14 days. Control (0.0% saline) and recovery groups (14 days) were included for each species. Mean daily aerosol exposures were -3 and 3.5 mg/L for the rodent and dogs respectively. Particle sizes were in the inhalable range for both species (~2.5 micron MMAD, ~1.7 GSD). Mean calculated inhaled (100%/tracheal-bronchial + pulmonary: ~10% rat, 20% dog) doses for each exposure group were ~55, 5.6, 171, 18.3, 354, 37.9 and 27, 5, 53/11, 105/21 mg/kg for rats and dogs respectively. Both species tolerated exposures well. There was no morbidity or mortality associated with treatment and no treatment-related findings in clinical observations, body weight, clinical pathology or organ weights in either species. No treatment related histopathology lesions were observed in dogs. Some rats among exposure groups sacrificed prior to the 24h post-exposure sacrifice had mottled lungs. These findings resolved after 24h. Consistent with rodent inhalation toxicology, histopathology revealed nonspecific modeling and inflammatory responses of the upper airway. Findings were of similar incidence among treated and control groups. No other treatment-related histopathology was observed in rodents. These results suggest that inhaled silylol has a safety profile consistent for use in human clinical populations.

**PS 2059 14-DAY TOXICITY STUDY OF METASTIN 45-54 (NSC-D741805) IN FISCHER 344 RATS.**

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Metastin 45-54 plays an important role in reproductive function through a G protein coupled receptor, GPR54. This decapetide may be a potential candidate for clinical evaluation in patients with reproductive disorders. The objectives of this study were to determine target organ toxicity and its reversibility after administration of Metastin 45-54 (NSC-D741805) to Fischer 344 rats treated daily by the intravenous route of administration (tail vein) for 14 consecutive days followed by a 14-day recovery period. Thirteen male and ten female rats per group were administered vehicle (sterile normal saline) or Metastin 45-54. Dosages of 0, 30, 300, or 3000 μg/kg (0.18, 1.8, and 18 mg/m2) were administered at a dosing volume of 5 mL/kg. Administration of Metastin 45-54 did not elicit any clinical signs of toxicity, changes in body weight or food consumption compared to control. There were no drug-related hematology, serum chemistry, or coagulation effects. Increased Leutinizing Hormone levels in treated rats indicated that exposure to Metastin 45-54 occurred during the study. FOB evaluations did not result in persistent or sub-acute neutrotoxicological findings of any significance. There were no treatment-related findings in terminal body weight, organ weights, or gross findings at necropsy. There were no drug-related microscopic findings and, therefore, no target organs were identified. Based on these results, the no-observed-adverse-effect-level (NOAEL) was greater than 3000 mg/kg following 14-days of intravenous injection and 14-days of recovery. Furthermore, these data demonstrate a reasonable preclinical margin of safety of Metastin 45-54 following 14 day iv administrations in rodent species to support its clinical development. (This work was supported by NCI Contract No. N01-CM-42200 and by NICHD and NIDDK under NIH-RAID pilot program).

**PS 2060 ELECTRORETINOGRAPHIC ASSESSMENT OF ROD AND CONE FUNCTION IN CYNOMOGUS MONKEYS: EFFECTS OF DIGOXIN AND VORICONAZOLE.**


In non-clinical studies, the evaluation of electroretinogram (ERG) according to a standardized procedure in humans is considered necessary in order to collect clinically relevant data. In the present study, we investigated whether the effects of 1) digoxin and 2) voriconazole, which have been reported to cause visual disturbances and reversible attenuation of ERGs in humans, can be detected by ERGs in cynomolgus monkeys. According to the procedure that was established based on the recommendation of the International Society for Clinical Electroretinography of Vision (ISCEV), rod response (rod ERG), combined rod and cone response (standard combined ERG), cone responses (single-flash cone ERG and 30-Hz flicker ERGs) were evaluated. 1) After single dosing (0.1 mg/kg i.v. in 10% rat, 10% dog) doses for each exposure group were repeated dosing (0.01 mg/kg/day i.m. for 1 month, n=5) of digoxin, increased implicit time in the combined response and increased implicit time and decreased amplitude in the cone responses were detected as suppressive effects. The above changes showed reversibility. No significant change was noted in the rod response repeated dosing of digoxin. Thus, the effect of digoxin on ERGs was reversible and more prominent in the cone responses than in the rod response. 2) After single dosing (3, 6 mg/kg i.v. at 1-week intervals, n=6) of voriconazole, decreased amplitude and implicit time in the combined response and in the rod response and decreased amplitude in the cone responses were detected with a dose-dependent manner. The above changes showed reversibility. In addition, the effects of voriconazole on ERGs were more prominent in the rod response than in the cone responses. In conclusion, the effects of digoxin and voriconazole that have been reported in humans were detected by ERGs in cynomolgus monkeys. This method, by which drug-induced effects on the rod and cone function could be detected, is considered to be useful for the prediction of visual disturbances in humans.

**PS 2061 THRESHOLDS IN PHOTOSAFETY ASSESSMENT: A MODEL CASE SUPPORTING TIERED TESTING AND RISK ASSESSMENT.**


Photosensitization – sunlight-induced toxicity – is a common phenomenon medi- ated by many natural and synthetic compounds. There are well-established and routinely used test methods available to investigate phototoxicity (e.g. UV-vis light absorption spectrum, in vitro ST3 NRU Phototoxicity Test, UV Local Lymph Node Assay). Despite the fact that the individual test systems usually provide well-defined results, translation of positive findings into a meaningful risk assessment still appears as a challenge. Some of the relevant determinants (e.g. reactivity of the excited state, sufficient distribution into light-exposed tissues) are not easily mea- surable and thus often not available for risk assessment. Therefore, empirical thresh- olds for specific endpoints (e.g. for molar extinction, photo irradiation factor PIF or EC50 values under irradiation) are important in order to clearly indicate a remaining concern or to justify further testing. Based on accumulated in-house data (comprising Novartis development as well as reference compounds) we have ana- lyzed different approaches in order to better quantify a) UV-vis absorption spectra (generation of critical absorption profiles in different solvents) and b) the endpoints of the in vitro ST3 NRU Phototoxicity Test (EC50 value under irradiation, PIF value). The analysis of the UV-vis spectra database indicates that a standardized ap- proach appears feasible allowing the definition of generally accepted critical absorp- tion profiles (threshold spectra). The analysis of in vitro phototoxicity endpoints shows a clear correlation of PIF values with positive in vivo results (still without considering tissue concentrations). Although the current OECD Test Guideline 432 considers a PIF value of >5 as a positive result our dataset indicates that this threshold might be too conservative. It is evident that both approaches require much larger databases including well-defined datasets from different institutions in order to provide generally acceptable thresholds of toxicological concern.

**PS 2062 AN IN-VITRO MOTILITY ASSAY AS A PREDICTIVE MODEL OF GASTROINTESTINAL ADVERSE DRUG REACTIONS.**

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Gastrointestinal adverse drug reactions (GADRs), such as constipation and diarrhoea, are among the most frequently reported events in all phases of clinical drug development and a significant cause of compound attrition during pre-clinical development. Consequently, pharmaceutical discovery requires robust, simple and predictive models capable of detecting and quantifying potential GADRs during the early phases of drug development. Here we report upon the validation of an in vitro assay, developed to detect and quantify changes in intestinal motility as a sur-rogate marker for the presence of constipation/diarrhoea-type events in humans. In vitro recordings of intraluminal pressure were used to monitor the presence of colonic peristaltic motor complexes (CPCM) in segments of mouse colon mounted horizontally in an organ bath and perfused with Krebs solution (37°C). CPCM frequen- cy, amplitude and total activity generated were assessed. Twelve compounds, with or without identified motility-related GADRs were assayed, in a blind manner, for their effects on CPCMs. For all compounds, cumulative concentration-res-ponse curves were constructed at concentrations covering the maximal free therapeu- tic plasma concentration in humans. The model detected stimulatory and inhibitory responses, likely correlating to the occurrence of diarrhoea or constipa- tion, respectively. Dose-related effects could be identified and for several com- pounds, a potential mechanism of action (neurogenic vs myogenic) could be inferred. The model identified 7 true positives and 3 true negatives, whilst only 1 false positive and 1 false negative were detected. This resulted in a model sensitivity of 88%, specificity of 75% and a predictability of 83% (considered “good” by the European Centre for the Validation of Alternative Methods criteria). These results support the potential value of this model as a screening method in safety pharmaco-logy assessment of compounds in development.
In vitro assessment of drug effects on the protein encoded by the human ether-a-go-go gene (hERG) is frequently used to screen for proarrhythmic side effects of new chemical entities in early development. Although a good correlation exists, between prolongations in QTc (LQT) (measured in vivo or in vitro) and those of ventricular action potential duration (APD), the correlation between hERG current inhibition and QTc prolongation is not always accurate. It is common practice to work with hERG peak amplitude; however, changes in current amplitude may not provide enough information on how compounds affect the ventricular repolarisation process to fully account for their potential arhythmogenicity. In this study, using hERG-expressing cells, current kinetics and total amount of charge transferred (AUC) along with current amplitude were analysed to evaluate their relevance as LQT predictors. Methods: The whole-cell patch-clamp technique was used to evaluate the effects of two diuretics and one proprietary compound A on the hERG channel current expressed in HEK293 cell line. The effects of these same molecules on the ECG and APD were evaluated using the isolated heart perfusion system (Langendorff) and the high-impedance microelectrode technique. Results: Compound A (hERG amp IC50= 1.1 μM, hERG AUC IC50= 0.7 μM), prolonged QTc by 10% at IC50. Droperidol (hERG amp IC50 = 4.80 μM, hERG AUC IC50= 4.3 μM) prolonged QTc by 14% at IC50. Cisapride (hERG amp IC50=0.04 μM, hERG AUC IC50=0.04 μM), prolonged QTc by 10% at IC50. Conclusion: hERG current data successfully predicted the changes in QTc induced by Compound A, but failed to predict the changes in QTc for Droperidol and Cisapride which were 5.7% and 3.5% respectively. Changes in APD90 and APD30 were not more predictive than hERG results, whether it be amplitude or amount of charge transferred. More research is necessary to determine if the use of additional hERG current parameters could enhance the predictive power of the hERG inhibition assay.

ASSESSMENT OF HERTG PARAMETERS IN LQT PREDICTIONS.


IPS Therapeutics, Sherbrooke, QC, Canada.

In vitro assessment of drug effects on the protein encoded by the human ether-a-go-go gene (hERG) is frequently used to screen for proarrhythmic side effects of new chemical entities in early development. Although a good correlation exists, between prolongations in QTc (LQT) (measured in vivo or in vitro) and those of ventricular action potential duration (APD), the correlation between hERG current inhibition and QTc prolongation is not always accurate. It is common practice to work with hERG peak amplitude; however, changes in current amplitude may not provide enough information on how compounds affect the ventricular repolarisation process to fully account for their potential arhythmogenicity. In this study, using hERG-expressing cells, current kinetics and total amount of charge transferred (AUC) along with current amplitude were analysed to evaluate their relevance as LQT predictors. Methods: The whole-cell patch-clamp technique was used to evaluate the effects of two diuretics and one proprietary compound A on the hERG channel current expressed in HEK293 cell line. The effects of these same molecules on the ECG and APD were evaluated using the isolated heart perfusion system (Langendorff) and the high-impedance microelectrode technique. Results: Compound A (hERG amp IC50= 1.1 μM, hERG AUC IC50= 0.7 μM), prolonged QTc by 10% at IC50. Droperidol (hERG amp IC50 = 4.80 μM, hERG AUC IC50= 4.3 μM) prolonged QTc by 14% at IC50. Cisapride (hERG amp IC50=0.04 μM, hERG AUC IC50=0.04 μM), prolonged QTc by 10% at IC50. Conclusion: hERG current data successfully predicted the changes in QTc induced by Compound A, but failed to predict the changes in QTc for Droperidol and Cisapride which were 5.7% and 3.5% respectively. Changes in APD90 and APD30 were not more predictive than hERG results, whether it be amplitude or amount of charge transferred. More research is necessary to determine if the use of additional hERG current parameters could enhance the predictive power of the hERG inhibition assay.

REPEATED TRAINING FOR PRIMATE CHAIR RESTRAIN DOES NOT AFFECT CARDIOVASCULAR SAFETY PARAMETERS IN CYNOMOLGUS MONKEYS.

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It is generally accepted that training of nonhuman primates can significantly impact study conduct and outcome depending on the parameters studied. However, current studies used training and quantitative endpoints in the field of primate toxicity are rare. The present work evaluated the effects of repeated training for simulated infusion in a primate chair using cardiovascular endpoints in telemeterized monkeys. Four conscious male animals (M. fascicularis, 4-6 yrs, 5-8 kg), fitted with telemetry implant providing recordings of heart rate, and respiratory rate, blood pressure and body temperature, were used. Animals were studied during periods of free movements (ca. 60 min), handling/restrain (ca. 30 min), chair restraint infusion (ca. 60 min) and again during free movement (ca. 60 min). Data logging rate was 60 seconds yielding 210 individual data sets for each parameter and animal. This sequence was repeated four times with a 3-4 day interval between each repetition. During the first session, systolic and diastolic blood pressure as well as heart and respiratory rate increased, on average, by 39%, 38%, 79% and 35%, respectively, during handling/restrain compared to free movement. During the infusion period, parameters remained elevated to the same extent whereas within 30 min of return to free movement condition, all parameters returned to baseline ranges (119 mmHg, 87 mmHg, 148 bpm and 31 bpm). Surprisingly, the magnitude of increase in cardiac parameters and respiratory rates diminished on all subsequent three sessions without any evidence of acquisition to the experimental conditions. Body temperatures remained basically unaffected despite handling on all four occasions. In conclusion, training of male cynomolgus monkeys for repeated primate chair restrain – at least for four sessions - was not accompanied by measurable adaptations using cardiac parameters as endpoint. These observations strengthen the need for control animals in any cardiovascular safety studies and general toxicity studies.

NESTIN STAINING AS A MARKER OF NEUROMUSCULAR JUNCTION (MJ) INTEGRITY IN ROUTINE TOXICOLOGY STUDIES.

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Introduction: In toxicology studies where muscle is a potential target organ, it is important to monitor changes in NMJs. In complement to functional evaluation through electrophysiology, NMJ changes can be assessed in teased muscle preparation...
largest dose vol. that are considered good practice via iv, i.p. and po routes. Each mouse was then obsd. for toxic signs for up to 14 days after dosing. The automated data collection system (Provanis) was used to collect and file the screening results effectively. The biol/therapeutic effects evaluated by in vitro cultured NCI 60 cell lines showed tumor cell death by representative ionic liqs. in a dose-dependent manner. Our studies for the first time illustrate the possibilities in tailoring of clin.

2067  TEN HELA S3 CELLS CAN BE TUMORIGINIC IN SUPER IMMUNODEFICIENT NOG MICE.

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The test for tumorigenicity (TT) is an essential checkpoint for safety assessment of cellular materials used in cell therapy. To select suitable mice as a xenogenic host for TT, susceptibilities of NOG mice (NOD/Shi-il2rg-/-) mice to xenotransplanted HeLaS3 tumor cells were examined in comparison with those of NOD/Shi-il2rg-/- and nude mice. When ten thousand HeLaS3 cells were subcutaneously inoculated into the flank of female mice, incidence of tumor takes at day 22 was 5/5 (100%) in NOG, 1/5 (20%) in NOD/Shi-il2rg-/- and 0/5 (0%) in BALB/c-nu mice. The incidence at a cell dose of one hundred was 0/5 (0%) in NOD/Shi-il2rg-/- and 5/5 (60%) in NOG mice. Similar results were obtained by using male mice. NOG mice showed tumor formation even at inoculation with ten HeLaS3 cells (total of 5 from ten mice tested). We confirmed that the tumors formed were originated from inoculated HeLaS3 cells by histochemistry including staining with anti-HLA antibodies. Higher susceptibility of NOG mice to xenotransplanted tumor cells was recognized in experiments with another cell line, LM-2/Jc. These data suggest that NOG mice should be the most suitable host mice for TT.

2070  ROLE OF ACADEMIA IN A GLOBALLY COMPETITIVE DRUG DEVELOPMENT MARKET.

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Biotechnology companies are increasingly seeking sites within the US and foreign countries to outsource studies supporting drug development. This is driven by high costs for the up to date infrastructure needed to meet government regulations and testing requirements. New directions in drug development related to gene therapy and nanomedicine have created specific needs to move products forward. This support may include accredited vivarium capacity, specialized facilities and state of the art instrumentation. These are expensive and difficult to operate efficiently. Limited use in a company based research program makes it difficult to justify such expenses. Sophisticated systems with high acquisition costs are often found in academic institu-
tions to support large leading edge research endeavors. Academic institu-
tions leverage several users within their institutions to justify and operate such support systems. Frequently, these support systems are underutilized and require subsidies to operate within the academic environment. Such academic resources can be identified by drug development entities creating an opportunity for the aca-
demic community to leverage their assets. However, regulatory compliance rigor, administrative hurdles, timeliness and intellectual property issues associated with private industry generated research and development can lead to suboptimal research relationships with an academic institution. Preexisting relationships with an academic institution can provide a clear path to the utilization of the academic assets with mini-
mal delay. The leveraging of these expenses assets benefit both the academic institution and the drug industry to provide effective solutions to difficult problems with minimized costs.

2068 THE EXTRACTABLES AND LEACHABLES SAFETY INFORMATION EXCHANGE (ELSE) DEVELOPING AN EXTRACTABLES/LEACHABLES DATABASE.

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Assessing the safety of extractables and leachables (E&L) is an essential element of the research and development process for a great variety of pharmaceuticals, biolog-
ics and medical devices. Detailed assessments of these compounds must be included in applications submitted to health regulatory authorities. Although safety data on E&L is publicly available in peer-reviewed scientific journals and government re-
ports and databases, these data have never been brought together into a single compre-
hensive and readily accessible database, nor has there been any industry-wide effort to compile, organize, appraise and summarize this data. Currently, industry and regulators have no central source of safety data on which to base decisions regarding the need for additional safety studies. Therefore, there is a risk that such studies may be conducted unnecessarily. Additional the same or similar container closure materials are used in many different pharmaceuticals, biologics, and medical de-
vice. There is no repository of extractables information about these materials that could provide a basis for screening and selecting materials for use in product develop-
ment and expedite further product-specific extraction studies.

The Extractables and Leachables Safety Information Exchange is developing a com-
prehensive database that will provide a jointly-developed and credible source of (i) safety information on E&L and (ii) extraction profiles and study protocols for a range of materials. Such a database will advance principles of quality by design by enhancing the prospects for identifying potential safety issues at the initial stages of the development process, when container-closure materials are being screened and selected; support member companies’ regulatory filings; reduce duplication of ef-
fort and minimize testing; decrease the risk of substantial, unanticipated delays and associated costs; and serve as a forum for exchanging experience and perspectives among industry and government experts.

2069 LACTOSE POWDER PHARMACEUTICAL ACTIVE BLENDS; BEHAVIOR IN RODENT AND NON-RODENT INHALATION EXPOSURE SYSTEMS AND EFFECT OF INCREASING ANIMAL NUMBERS.

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Generation of aerosol from non-micronised lactose powder formulations results in a significant change in the proportions of pharmaceutical drug and vehicle com-
pared with those in the original bulk material. The ability to predict this ‘fold-
change’ is a tool for predicting future operating conditions and test substrate usage. ‘Fold-change’ data were collated from multiple snout/head rodent/rabbit and face-
mark non-rodent inhalation exposure systems used at HLS. The fold change was lower and less variable with increasing drug binding strengths: 5.6 down to 1.6 for ro-
dent system using 64% and 40% blends respectively, and 8.5 down to 1.8 for ro-
dent system using 0.4% to 40% blends respectively. Test drug specificity was evi-
dent for both types of system. A rodent system and 20% blend, resulted in a fold-
change difference from 1.8 at 0.6g/L to 2.1 at 251g/L for Compound 1, com-
pared with no different in fold value of 1.6 at 3.0g/L and 17.7g/L for Compound 2. Inclusion of an additive reduced the propensity for increasing the drug:total particulate fold-change in both systems; 5.5 down to 3 at 1 active and 10 down to 3.5 at 1% drug and 1.3 at 40%. Animal numbers influenced the sys-
tem exposure performance. Twenty-six rats in a 1 week study compared with 132 in a 2 year study reduced aerosol concentration from 22% to 30%; similarly 42 and 156 mice reduced concentration from 6% to 36%, and 6 and 22 rabbits reduced concentration from 1.5% to 20%. We are therefore able to predict with accuracy the drug requirements for a given study design, factoring in the effects of increasing an-
imal numbers. This is of particular importance when active drug is expensive and non-clinical programme time lines are critical, as well as achieving the required dose on day 1 of a study, vital in providing toxicokinetic data indicating achieved dose.
creased body weight gains and food consumption, elevated levels of hilirubin, protein, alanine aminotransferase (ALT), cholesterol, blood urea nitrogen and blood urea nitrogen/creatinine ratio, and decreased concentrations of inorganic phosphorus. Treatment-related anemia, increases in leukocyte, lymphocyte, monocyte, and platelet counts and in fibrinogen levels, decreases in prothrombin time, and increases in serum thyroxine and thyroid-stimulating hormone levels were also seen. Plasma drug levels were increased with increasing dose of PMCol. Relative liver, spleen and kidney weights were increased. Treatment-related histopathology findings in kidney and stomach were seen. Neurotoxicity of PMCol was revealed by FOB as an increase in supported rears. The no-observed adverse effect level (NOAEL) for PMCol in male rats was 100 mg/kg/day. In female rats, the NOAEL was not established due to meaningful increase in cholesterol levels seen in the 100 mg/kg/day dose group. The toxicity of PMCol was also assessed in male dogs following 4 week oral treatment by capsule administration at doses of 0, 50, 200 and 800 mg/kg/day. Toxicity was observed at 200 and 800 mg/kg/day. There were clinical signs of toxicity, decreases in body weights, body weight gain and food consumption, decreased levels of protein and calcium, increased levels of alkaline phosphatase and ALT, increased platelet counts, decreased triiodothyronine and thyroxin levels. A microvesicular vacuolization of hepatocytes and atrophy of the thymus were observed. The NOAEL in male dogs was 50 mg/kg/day. PMCol did not show any mutagenic activity in the standard genotoxicity battery testing (bacterial mutagenesis, in vitro chromosomal aberration, and in vivo mouse micronucleus).

**ROLE OF HUMAN CYP450 ENZYMES IN MDMA METABOLISM AND CYTOTOXICITY.**

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There is ongoing discussion if toxicity of 3,4-methylenedioxyamphetamine (MDMA) is caused by parent compound or metabolites. If metabolites play a role in MDMA toxicity, the role of specific P450 enzymes is still unclear. MDMA is metabolized via two routes: 1) O-demethylation leading to 3,4-dihydroxyamphetamine and 3,4-dihydroxyamphetamine metabolites and 2) N-dealkylation followed by oxidation to benzoic acid derivatives. P450s are responsible of MDMA oxidation and at least CYP2D6 is playing a role in this process, but involvement of other P450s can not be excluded. Knowledge about involvement of specific P450s is relevant because of large differences in human susceptibility exist. By using immortalized human liver epithelial cells (THLE) transfected with P450s (T5-CMV1A2, T5-CMV2D6, T5-CMV3A4) we studied cytotoxicity of MDMA metabolites. Cytotoxicity measurements (MTT) indicate a dose dependent reduction starting at concentrations itself (0.25-1 mM) also inhibited CYP1A2 activity. If this was caused by cytotoxicity or substrate inhibition is yet unknown. Similar experiments with CYP3A4 indicate a significant role of the parent compound in cytotoxicity at similar concentrations. Further studies with CYP3A4 inhibitors have to show the specific role of MDMA metabolism and this P450 enzyme. Additional experiments with CYP2D6 are ongoing. These results indicate that the THLE cells expressing high constitutive P450 activities are a good model to study bioactivation and detoxification mechanisms. Further experiments are ongoing in our laboratory to elucidate the role of human relevant P450s in MDMA toxicity.

**PROTECTION OF RED BLOOD CELL ACETYLCOLINESTERASE BY ORAL HUPERZINE A AGAINST EX VIVO SOMAN EXPOSURE: NEXT GENERATION PROPHYLAXIS AND SEQUESTERING OF ACHE OVER BChE.**

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In a Phase I clinical trial to determine the tolerability and safety of the specific acetylcholinesterase (ACHE) inhibitor huperzine A, twelve healthy elderly individuals received an escalating dose regimen of huperzine A (100, 200, 300, and 400 μg doses, twicely/day for a week at each dose), with three individuals receiving a placebo. Red blood cell ACHE and plasma BChE were measured in unprocessed whole blood samples following each dose, and then for up to 48 h following the final and highest (400 μg) dose to monitor the profile of inhibition and recovery of ACHE. Significant inhibition of ACHE was observed, ranging from 30 – 40% after 100 μg to >50% at 400 μg, peaking 1.5 h after the last dose. Gradual recovery of ACHE activity then occurs, and 48 h after the last dose, red blood cell ACHE had recovered to 95% of control (pre-dose) values. Huperzine A levels in plasma peaked 1.5 h after the final 400 μg dose. Plasma BChE was unaffected by huperzine A treatment (as expected). Aliquots of huperzine A-containing and placebo blood samples were exposed ex vivo to the irreversible nerve agent soman (GD) for 10 min, followed by removal of unbound huperzine A and soman from the blood by passing through a small C18 reverse phase spin column. Eluted blood was diluted in saline and assayed for total BChE activity. After 1.5 hours, the remaining huperzine A was then taken to achieve full return in free ACHE activity, and the proportion that is protected from soman exposure. Huperzine A-inhibited ACHE activity was restored almost to the level that was initially inhibited by the drug. The increased doses of huperzine A were well tolerated by these patients, with a higher proportion of red blood cell ACHE sequestered (protected) than has been previously demonstrated for pyridostigmine bromide (PB), indicating the potential improved prophylaxis against OP poisoning.
SOT 2009 ANNUAL MEETING

2077 EVALUATION OF CORTICOSTERONE AND METABOLITES IN A CHRONIC MILD STRESS RAT MODEL.

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Corticosterone is a steroid hormone secreted by the adrenal gland and is involved in the regulation of fuel metabolism, immune reactions, and stress responses. When clinical signs of stress and changes to immune organs are observed during drug development studies, differentiating between direct immunotoxicity and changes mediated by stress can be difficult. Recent research suggests that when corticosterone levels are elevated by either administration of exogenous corticosterone or by exposure to environmental stressors, changes in tissues known to be affected by stress result. The measurement of corticosterone during drug development studies may allow researchers to predict if changes in immune organs are the indirect result of stress or a direct drug related effect on the immune system. Previously, assessment of corticosterone levels required multiple blood samples collected over a period of time. Serial blood sampling is unavailable for small animals, and the use of urinary corticosterone is becoming the preferred method for corticosterone assessment. A correlation between blood and urinary corticosterone has been observed and studies are ongoing to assess the use of urinary corticosterone as a pre-clinical biomarker of stress in rats (J. Immunol Toxicol, 4:25, 36.: 2006). To aid in biomarker development, an assay to measure corticosterone and two metabolites in female rat urine has been developed. The assay utilizes HPLC coupled with mass spectrometric detection. The linear range is 0.05 to 20 ng/ml and assay precision is within 22%. The assay was used to measure corticosterone and two metabolites in the urine of rats exposed to chronic mild stress with and without treatment with Imiprime. Higher levels of corticosterone and 6-α hydroxycorticosterone were observed in the urine of rats exposed to chronic mild stress without Imiprime treatment when compared to the vehicle control rats. Corticosterone sulphate levels were below the LLOQ for most of study samples but future plans include concentrating or extracting the urine to enhance the measurement of the sulphate metabolite.

2080 EFFECT OF PARTICLE SIZE ON THE REGIONAL DEPOSITION OF TECHNITIUM-99M LABELED PARTICLES IN RODENTS.


There is extensive information detailing the deposition patterns of various particles in the human respiratory tracts and several manuscripts detailing deposition in preclinical species. However, the majority of the data in rodents was generated prior to significant advancements in rodent imaging capabilities. In order to address this need and determine the effect of particle size has on the deposition patterns in rodent species these experiments were conducted. Rats and mice were exposed, via nose only inhalation, to particles ranging from 0.5 to 5 microns mass median aerodynamic diameter (MMAD). The aerosols were composed of technetium-99m radio-labeled sulfur colloid particles. Aerosols were generated with a series of compressed air jet nebulizers to achieve each desired particle size (0.5, 1.0, 3.0 and 5.0 micron). Aerosol samples were collected to characterize the activity aerosol concentration and the particle size distribution. Impactor analysis detailed that mass and activity median aerodynamic diameters (AMAD) correlated with each other. For example for the target particle size of 0.5 micron the MMAD was 0.62 micron and the AMAD was 0.57 micron. These data indicate that the aerosols of Tech-99m and the sulfur colloid particles were homogeneous. This trend of MMAD and AMAD agreement was consistent from 0.5 to 5 microns. In order to determine deposition animals were sacrificed immediately following inhalation exposures and were imaged using a small-animal SPECT-CT camera. Data analysis indicated that 11.2 ± 1.1% of material in lungs of the mouse and 60 μCi in the lungs of the rat after exposure to the 0.5 micron aerosol. Increasing particle size resulted in an increase in deposition in the nasal region and resulted in an increase in stomach uptake.

2079 AMIODARONE INDUCES PRO-FIBROTIC AGENTS IN RAT PLEURAL MESOTHELIAL CELLS.

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Clinical studies have demonstrated an association between amiodarone therapy and a variety of pulmonary complications ranging from sub-acute necrotizing pneumonitis to pulmonary fibrosis. Fibrosis is characterized by increased expression of extracellular matrix proteins and such expression may involve pro-fibrotic agents such as Angiotensin II (Ang II), Transforming Growth Factor β1 (TGF β1) and Connective Tissue Growth Factor (CTGF). This study investigated the effect of amiodarone on the levels of the pro-fibrotic proteins TGF β1, CTGF and Ang II and the extracellular matrix protein elastin in rat pleural mesothelial cells (RPMCs). RPMCs were maintained in Ham’s F-12 medium at 37°C in a humidified atmosphere of 5% CO2 / balanced air. Confluent cultures of RPMCs were treated with 5μg/ml of amiodarone for 24hrs, followed by protein extraction and western blot analyzes for TGF and CTGF. To investigate the effect of amiodarone on elastin, confluent cultures were treated with 5μg/ml of amiodarone for 24hrs, after which the original medium was replaced with medium containing 100μg/ml of β-aminopropionitrile to prevent elastin cross-linking. Effects of amiodarone on Ang II levels were studied by treating RPMCs with 5μg/ml of amiodarone for 24hrs. After 5 days of additional culture, levels of soluble elastin and Ang II present in the medium were analyzed using Enzyme Linked Immunosorbent Assays. Amiodarone treated cultures had increased levels of TGF β1 (1.32±0.5, P<0.05) and CTGF (202.2±97.7, P<0.05) in comparison to controls. Perturbation of RPMCs by amiodarone increased the accumulation of soluble elastin (197.8±17.2, P<0.001) and Ang II when compared to controls. Such findings suggest that amiodarone induces the expression of pro-fibrotic proteins that in turn increase levels of extracellular matrix proteins creating a fibrotic-like response in RPMCs.

2081 ADHESION MOLECULE EXPRESSION AND CYTOTOXICITY IN DIACETYL EXPOSED RAT LUNGS.


Diacetyl (2,3-butanedione), a volatile component of butter and butter flavoring, gives the flavor and aroma of butter, and has been associated with food-related airways obstruction in the microwave-poppcorn and flavoring industries. Inhaled diacetyl vapors cause epithelial necrosis in the rat and mouse upper respiratory tract. Many cytotoxic agents cause both apoptotic and necrotic cell death but the role of apoptosis in diacetyl-induced cell death is unknown. Dicarbonyl/xylulose reductase (DCXR) metabolizes diacetyl to acetoin and appears to play a role in mucosal absorption of diacetyl. In human skin, DCXR localizes to the cytoplasm near the adhesion molecules, e-cadherin and β-catenin. Therefore, we hypothesized that diacetyl causes apoptosis and changes in epithelial adhesion molecules. Lungs were collected from Sprague Dawley rats one day after a 6 h exposure to inhaled air (n=6) or 317 ppm diacetyl vapor (n=6). Apoptosis was assessed by the TUNEL assay and immunofluorescence for activated caspase-3. DCXR, e-cadherin, and β-catenin were visualized by immunofluorescence and confocal microscopy. Diacetyl...
increased the number of TUNEL and activated caspase 3 positive cells in mainstem bronchus epithelium. However, most cells in necrotic foci did not exhibit these apoptotic markers. DCXR staining was most intense in terminal bronchiolar cells morphologically consistent with Clara cells. In some necrotic foci in the mainstem bronchus epithelium, the normal thin linear staining of β-catenin at intercellular junctions was replaced by more focal globular expression. Detaching necrotic cells often also lost β-catenin and e-cadherin expression. Such alterations may play a role in, or possibly result from, necrosis, apoptosis, and/or epithelial detachment. These findings indicate that both apoptosis and necrosis contribute to diacetyl epithelial injury and suggest that diacetyl may alter intercellular adhesion complexes of respiratory epithelium.

2082 REPEATED EXPOSURE OF HUMAN BRONCHIAL EPITHELIAL CELLS TO OZONE SUPPRESSES THEIR RESPONSE TO STAPHYLOCOCCUS AUREUS.

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Ozone (O3) is a common air pollutant that causes a variety of adverse health effects including altered host vulnerability to pathogens. Pulmonary clearance of Staphylococcus aureus is decreased after O3 exposure, but the mechanisms that underlie altered host susceptibility are poorly understood. Our goals were to determine whether UNC3T cells, a human bronchial epithelial cell line grown at air-liquid interface, secrete pro-inflammatory cytokines in response to repeated exposure to environmentally relevant levels of O3, and if repeated O3 exposures alter responsiveness of these cells to a S. aureus filtrate challenge. We determined that this cell line did not release cytokines following exposure to O3. We found that repeated exposures to a low dose (0.2 ppb) and high dose (0.8 ppb) of O3 followed by a S. aureus filtrate challenge attenuated the release of MIP-5α, Gro-α, IL-6, ENA-78, and G-CSF, as compared to air control cells. Furthermore, O3 pre-exposure significantly decreased phosphorylation of ERK1/2 20 minutes after the S. aureus filtrate challenge. These results indicate that repeated exposures to low level O3 attenuates airway epithelial pro-inflammatory responses to S. aureus filtrate. This response could increase host susceptibility to this prevalent pathogen.

2083 OZONE-INDUCED EXACERBATION OF ACUTE LIVER INJURY IN MICE.

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Ozone (O3), an oxidant air pollutant in photochemical smog, principally targets epithelial cells lining the respiratory tract. However, changes in global gene expression have also been reported in livers of O3-exposed mice. Overexpression of aceta-
morphin (APAP) is the most common cause of drug-induced liver injury in developed countries. In the present study, we examined the effects of a single, ambient O3 exposure on livers of mice treated with a hepatotoxic dose of APAP. C57BL/6j male mice were fasted overnight and then given APAP (300 mg/kg ip) or saline vehicle (0 mg/kg APAP). Two hours later, mice were exposed to 0 or 0.5 ppm O3 for 6 hours. They were sacrificed 1 or 24 hours after O3 exposure. Animals killed at the 24-hour timepoint were given bromodeoxyuridine (BrdU) two hours before sacrifice to identify hepatocytes undergoing reparative DNA synthesis. Liver tissue samples were processed for light microscopic examination and morphometric analyses. Blood samples were analyzed for plasma alanine aminotransferase activity (ALT). Saline-treated mice exposed to either air or ozone had no liver injury. All APAP-treated mice developed marked hepatic centrilobular necrosis that increased in severity with time after APAP exposure. O3 exposure increased the severity of APAP-induced liver injury as indicated by a 60% and 33% increase in necrotic hepatic tissue and plasma ALT, respectively. APAP also induced a 10-fold increase in BrdU-labeled hepatocytes that was 80% attenuated by O3. Gene expression analy-
sis at 1 hour after O3 revealed that APAP and O3 coexposure resulted in greater expression of p21 and Socs3 mRNA and less IL-6 mRNA expression compared to APAP alone. These results suggest that acute exposure to oxidant air pollution exacerbates drug-induced liver injury and delays hepatic repair.

2084 INHALED GLUCOCORTICOID CAUSES RAPID VASOCONSTRITION IN THE ISOLATED AND PERFUSED RAT LUNG.


Rationale: Recently it was shown in the clinic that inhaled corticosteroids cause rapid bronchial vasconstriction. The aim of the present study was to investigate whether vasconstrictive effects on pulmonary circulation could be detected after short inhalation of a glucocorticoid in a preclinical model: the isolated and perfused rat lung (IPL). Methods: Using the recently developed DustGun Technology, we exposed the IPL by inhalation to a dry powder aerosol of budesonide (BUD). The pulmonary perfuse flow rate was assessed during 100 min post dose. A reduction in perfusion flow was interpreted as vasconstriction. Main Results: The onset of vasocstriction was significant within 10-40 min after inhalation exposures to 10 and 50 μg BUD compared to vehicle (lactose) (p<0.012 and 0.049, respectively). Throughout the whole perfusion period (100 min), 2 μg BUD had no effect while 10 μg had more pronounced effect than 50 μg BUD. Co-administration of a selective α1-adrenoceptor antagonist (prazosin 50 nM added to the perfuse) with 10 μg BUD reduced vasocstriction by approximately 50% during 100 min of perfu-
sion (p<0.003).

Conclusions: The results suggest that corticosteroid-induced airway vasconstric-
tion is at least partly driven by an α1-adrenoceptor mediated mechanism. The rapid onset of vasocstriction after inhalation of budesonide suggests that this is a nongenomic effect. This ex-vivo study supports clinical observations of vasoc-
striction after inhalation of corticosteroids. This model could serve as a comple-
ment to clinical models to uncover effects and mechanisms of pulmonary/bronchial circulatory changes.

2085 IMMUNE RESPONSES IN NEONATAL AND ADULT RATS EXPOSED TO SOOT.


Exposure to particulate air pollution (PM) has been correlated with increased mor-

bidity and mortality in adults and undernourished children. PM contains combustion products including organic compounds bound to particulates and in the gas phase. We exposed neonatal (7d old) and adult male Sprague Dawley rats to inhaled soot generated by diffusion flame for 6 hrs. Control rats were exposed to filtered air. This soot was low in organic carbon; simulating the particulate fraction of PM. Airway epithelial cell injury was assessed 2 hrs post exposure using differential permeability to fluorescent dyes, high resolution histopathology and by measuring LDH release into bronchoalveolar lavage fluid (BALF). BALF and peripheral blood cell differentials as well as cytokine profiles in serum and BALF were measured at 6, 24 and 48 hours post PM. We found that exposure to soot stimulated an increase in circulating neutrophils in adult rats and an increase in lavage neutrophils in neonates. LDH release was significantly increased in the BALF of neonates only at the 2 hr timepoint but cellular injury was not apparent at either age. Cytokines measured by a multiplex assay in both BALF and the peripheral blood were altered by exposure to PM compared to age matched filtered air controls. There were tem-
poral changes in cytokines by age. BALF IL-1β was significantly increased at 2 hrs following PM in adult rats. TNF-α (P<0.0092) and IL-6 (P<0.0211) cytokine levels in serum were decreased in neonates 48 hrs post PM. We conclude that low PAH soot does not cause acute cytotoxicity in airways of neonatal or adult rats but that exposure to soot causes neutrophil abundance and changes in key cytokines in both the lavage and peripheral blood. There are temporal and spatial differences in neonatal responses compared to adult responses. Funded by EPA RD82541401 and NIH ES/HL67000. Although funded in part by the USEPA this research has not been subject to USEPA required peer and policy review and therefore does not reflect the views of the Agency and no official endorsement should be inferred.
FA groups were also pre-injected (ip) with either saline or 200 μM n-acetyl-cysteine (NAC), a known thiol-containing scavenger of NO. Following 1 or 2 h of exposure, plasma NOx, a measure of total nitrates and nitrites, were determined. Predictably, pure NO exposures lead to a doubling of plasma NOx from 4 μM in the FA group to 10 μM in the DE group. Whole DE, which contained ~3.5 ppm NO, for 1 h also lead to a doubling of plasma NOx; contrary to expectations, the presence of NAC increased the levels of plasma NOx in the DE exposure group. After 2 h of exposure, the effect of NAC was lost, potentially because the T1/2 of NAC is ~45 min in the circulation. In a separate series of experiments, human subjects were exposed to DE or FA on separate occasions for two hours and plasma was taken immediately following exposure and 20 h post-exposure. Plasma NOx was increased immediately following exposure compared to FA and this increase was reversed 24 h post-exposure. The role of NO-generated biochemical species on cardiovascular disease is at present unknown, but the lack of change in carboxyhemoglobin in both rat and human studies, plasma NOx appears to be a useful biomarker of acute exposure to complex emissions. Based on the findings with NAC, we predict that individual nutritional and physiological status may prove essential to determining the biochemical transformation of NO systemically, and thereby influence toxic outcomes. (This abstract does not necessarily reflect EPA policy).

2087 HYPOXIA INDUCIBLE FACTOR 1 ALPHA (HIF-1α) MEDIATES OZONE-INDUCED LUNG INJURY IN MICE. Y. Sani,1, 2, D. L. Abo,2, 3, K. Y. Kim,2, 3, D. B. Hamrick2, 3, and D. L. Laperle3. Genetics, Michigan State University, East Lansing, MI; Center for Integrative Toxicology, Michigan State University, East Lansing, MI, 2Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI, 3Department of Pathobiology and Diagnostic Investigation, Michigan State University, East Lansing, MI, 4The National Food Safety and Toxicology Center, Michigan State University, East Lansing, MI.

Ozone, a potent oxidant gas in photochemical smog, poses a threat to the respiratory tract. Its increased ambient levels have been linked to lung injury, inflammation and airway hyper-responsiveness. In children, ozone is known to worsen the asthmatic symptoms even at concentrations below the U.S. Environmental Protection Agency standard. Hypoxia Inducible Factor 1 alpha (HIF1α) is a hypoxia-regulated transcription factor that is critical to the development of the lung. The role of HIF1α in ozone-induced lung injury has not been determined. To characterize HIF1α’s role in this process, an inducible, lung-specific HIF1α knock-out mouse model was generated. In this study, wild type (WT) and HIF1α long-deﬁcient female mice were exposed for ﬁve nights (8 hours per night) to filtered air alone (0 ppm ozone) or 0.5 ppm ozone. Interestingly, we observed that HIF1α-deﬁcient mice were more resistant to ozone-induced toxicity when compared to WT controls. Bronchoalveolar lavage ﬂuid analysis revealed increased numbers of neutrophils and total inﬂammatory cells following ozone exposure in WT mice as compared to HIF1α deﬁcient mice. Finally, inﬂammatory cytokine proﬁling in the lung revealed perturbations in the ozone-induced release of cytokines such as KC, IL-4, and osteonectin. Moreover, a signiﬁcant increase in IL-6 was only observed in the HIF1α deﬁcient mice following 5 days of ozone exposure. These results suggest that HIF1α mediates the ozone-induced pulmonary inﬂammation in response to airway injury following inhaled toxicant challenge.


Hypoxic insult to the lung initiates a cascade of events that ultimately results in tissue damage. These include the production of reactive oxygen species and oxidative stress, inflammation, and alterations in mitochondrial function and apoptosis. In the present studies we analyzed the effects of hypoxia on the responses of macrophages, which are highly sensitive to oxidative stress. For these studies RAW 264.7 macrophages were exposed to hypoxia for 3 h. This was accomplished by culturing cells in DMEM/1% FBS at 37°C in a modular incubator chamber adjusted to 0.5-5% O2 and 5% CO2 balanced with nitrogen. Exposure of macrophages to hypoxia resulted in a time-dependent increase of mRNA expression for the oxidant enzyme NADPH oxidase (NOX2). The NOX2-derived molecular oxygen is reduct by vascular endothelial growth factor (VEGF), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and the adenosine A2A receptor (A2AR), a marker of cell injury. These effects were most pronounced after 16 h exposure to hypoxia. In contrast, hypoxia had no effect on superoxide dismutase or tumor necrosis factor-alpha expression. To investigate potential mechanisms regulating hypoxia-induced alterations in gene expression, cells were transfected with MAPK1 siRNA for 6 h using HiPerFect transfection reagent, 48 h prior to hypoxia. This siRNA transfection suppressed MAPK1 expression by about 70%. Knockdown of MAPK1 in macrophages resulted in significant decreases in hypoxia induced expression of iNOS, COX-2, VEGF and A2AR. These data demonstrate that macrophages are highly responsive to hypoxia and that MAK1 is involved in their activity. This may be important in the pathophysiology of lung injury associated with oxidative stress. Supported by AR055073, ES004738, ES005022 and APERG.

2089 NQO1 MODULATES LUNG RESPONSES TO OZONE IN ADULT AND NEONATAL MICE. E. N. Potts1, N. Mason2, R. L. Auren2 and W. Foster1. 1Pulmonary Division, Duke University Medical Center, Durham, NC and 2Neonatology, Pediatrics, Duke University Medical Center, Durham, NC.

Rationale: Mice exposed to ozone during postnatal development fail to gain weight normally and also develop airway hyperresponsiveness (AHR, asthma phenotype). NAD(P)H quinone oxidoreductase (NQO1) has been identified in epidemiologic studies as a host factor for susceptibility to ozone. We have reported that humans homozygous for a single nucleotide polymorphism (SNP) on NQO1-1 (c.1019C>A) or expressing a non-functional/NQO1 variant enzyme are protected from acute ozone-induced airflow obstruction, and that adult NQO1 null (-/-) mice have less lung inflammation and reduced AHR after ozone versus WT mice. We hypothesized that during early postnatal life the absence of NQO1 may also prevent the adverse effects of ozone impaired weight gain and ozone-induced AHR. Methods: C57Bl/6 (WT) and NQO1-/- pups were exposed beginning at birth to repetitive exposure to FA or 1 ppm ozone (3 h/day, 3d/wk x 4 wk). At day 28, pulmonary mechanics were measured by forced oscillometry in response to increasing doses of MCh aerosol. Results: for equivalent litter sizes, both WT and NQO1-/- pups had decreased weight gain compared with unexposed pups, with more severe effects in NQO1-/- pups (12% reduction for WT, vs. 22% reduction in the NQO1-/-). Compared to FA, both WT and NQO1-/- mice exhibited ozone-induced AHR; despite the greater weight loss, ozone-exposed NQO1-/- pups were consistently less sensitive to aerosol MCh challenge at each dose escalation as compared to airway responsiveness of ozone-exposed WT pups. Conclusions: Ozone-impaired postnatal weight gain was dissociated from ozone-induced AHR to MCh challenge: absence of NQO1 in neonatal mice reduces (or delays) ozone-induced lung injury and induction of AHR. Supported by: EPA-RD83329301 and NIH ES11961 & ES16547.


Epidemiological studies report a positive correlation between traffic-related air pollution and increased rates of cardiovascular morbidity and mortality; however, the underlying cellular mechanisms have not yet been elucidated. We have previously reported that vascular oxidative stress levels are significantly elevated in apolipoprotein KO (ApoE/-) mice exposed to vehicular emissions, which appears to mediate increases in endothelin-1 and matrix metalloproteinases, expression of which are associated with progression of atherosclerosis. Since ROS production promotes formation of oxidized LDL (oxLDL), we investigated whether the effects of oxLDL on the vasculature are mediated via the lectin-like-ox-LDL receptor (LOX-1), the major receptor for oxLDL in endothelial cells. To test this hypothesis, 10 wk old male ApoE/- mice on a high fat diet received either mouse IgG control (16 μg protein/ml, 0.1 ml/mouse, i.p.) or the neutralizing antibodies to LOX-1 (anti-mouse LOX-1/IR-E1 antibody: 16 μg protein/ml, 0.1 ml/mouse, i.p.) every other day. Mice were randomly assigned to inhalational exposure of either filtered-air (FA: n=12 anti-LOX-1, n=12 IgG) or a mixed exposure of 250μg PM/m3 diesel exhaust +50μg PM/m3 gasoline engine exhaust (n=12 anti-LOX-1, n=12 IgG) for 6 hr/day for 7 days. Upon sacrifice, aortas were split and snap frozen. TBA5R analysis of the aorta show significant elevations in oxidative stress from mice exposed to vehicular exhaust (exposed, 25.7 ± 1.6 nmol/ml/mg) compared to FA (9.6 ± 1.4 nmol/ml/mg). Furthermore, anti-LOX-1 treatment ameliorated the increase in vascular lipid peroxidation in vehicular exhaust-exposed animals (6.7 ± 1.1 nmol/ml/mg). No significant difference was noted in between IgG and anti-LOX-1 treatment in FA-exposed mice. Such findings indicate that effects of ROS (and
subsequent oxLDL) generated from vehicular exhaust exposure in the vasculature are likely mediated through the LOX-1 receptor. Funded by NIH K99ES016586 (AKL) and NIH 1R01ES014639 (MJC).

Based on this concept, the cytotoxicity of PM2.5 on A549 human bronchoalveolar carcinoma cells was studied, utilizing a Promega Cell Titer 96 Aqueous assay. Cell viability curves were generated in triplicate for three different batches of PM 2.5 suspensions. Batch 1 was utilized at concentrations of 600μg/ml, 300μg/ml, 200μg/ml, 100μg/ml, and 50μg/ml; batch 2 was utilized at concentrations of 250μg/ml, 200μg/ml, 100μg/ml, 50μg/ml, and 10μg/ml; batch 3 was utilized at concentrations of 181.8μg/ml, 145.4μg/ml, 109.0μg/ml, 72.7μg/ml, 36.3μg/ml, and 18.1μg/ml. Batch 1 showed an IC50 of 238μg/ml; batch 2 showed an IC50 of 59μg/ml; and batch III showed an IC50 of 130μg/ml. The variation in IC50 is likely due to the fact that batches were collected at different locations. The presence of transition metals in PM2.5 particulates is believed to be the major cause of toxicity. As a result, cytotoxicity was also measured in the presence of deferoxamine mesylate, a well known transition metal chelating agent, at deferoxamine concentrations of 0.75mM, 0.50mM, and 0.0375mM. Tests showed a remarkable reduction in PM2.5 cytotoxicity. The reduction in cytotoxicity suggests that deferoxamine mesylate provides protection to A549 cells, and, that transition metals are indeed involved in mediating PM2.5 toxicity. Utilizing the highest PM2.5 concentration (600μg/ml), mechanisms of cell death were investigated utilizing fluorescence microscopy. At this concentration, the three PM 2.5 batches showed a 70 ± 20 %, 62 ± 25 %, 60 ± 19 % cellular necrosis (Acridine Orange/Etidium Bromide).

**2092 CHOLESTEROL SECOALDEHYDE ACTIVATES PHOSPHOLIPASE A2 AND CAUSES BARRIER DYSFUNCTION IN VASCULAR ENDOThelial CELLS: IMPLICATIONS IN VASCULAR DISEASES.**

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Oxidative modification of cholesterol by ozone leads to the formation of several products, including a major product, 3-hydroxy-5-oxo-6,6-secocholest-4-enal (cholesterol secoaldehyde or ChSeco). ChSeco has been shown to induce lung lavage of the oxygen-exposed animals, arterial plaque, and in the brain of Alzheimer’s patients. In vitro, ChSeco has been shown to initiate the foam cell formation, promote misfolding of proteins, and is cytotoxic to several mammalian cells. As the vascular endothelium is in constant contact with the circulating oxysterols, here, we investigated the mechanism(s) of ChSeco-induced cytotoxicity, alterations in morphology, activation of PL2A, and barrier dysfunction in cultured bovine pulmonary artery ECs (BPAECs) and pulmonary microvascular ECs (BLMVECs). Cells were treated with different concentrations of ChSeco (10 to 100 μM) for 1 and 2 h, following which the cytotoxicity (LDH release and cell morphology), release of [3H] arachidonic acid (as an index of PL2A activation), and barrier function (electrical resistance in EC monolayers grown on gold electrodes) were determined. It was observed that ChSeco induced cytotoxicity, alterations in cell morphology, release of [3H] arachidonic acid, and decline in the electrical resistance in the EC monolayers with increase in dose of ChSeco and time of treatment of cells with ChSeco, as compared to that in the control untreated cells. Using specific inhibitors (AAOCOF, methyl arachidonate fluorophosphates, and PN-18), our study also revealed that ChSeco specifically activated both the Ca2+-dependent and Ca2+-independent PL2A. The results of the current study provides mechanistic insights into the oxysterol-mediated vascular dysfunctions observed during the onset and subsequent progression of the cardiovascular and cerebrovascular diseases.
viro hERG evaluation of all pharmaceutical compounds targeted for human use is fundamental. The Human ether-a-go-go (hERG) related gene potassium channel (IKr) plays a major role in the repolarizing phase of cardiomyocyte action potential. It is the blockade of this channel and the consequent possibility of QT prolongation and increased susceptibility of torsade de pointes that is of primary concern. It has been suggested that there is a potential difference in endogenous voltage-activated potassium currents between the Chinese Hamster Ovary (CHO) and Human Embryonic Kidney (HEK) cell lines expressing the hERG channel, therefore, both were tested. Using whole cell patch clamp technique, reference compounds were tested for hERG inhibition while cells were being held at constant physiological temperature for testing. Data were captured using the 21 CFR Part 11 compliant PatchMaster Pro (HEKA). Electrophysiological characteristics of hERG behaviour were studied in those three stimulus-response relationship, voltage range and gating kinetics. The hERG tail current was pharmacologically verified using both negative controls (0.3% DMSO, Aspirin – 100 μM) and a positive control (Cisapride – 1 to 100 nM). Results obtained were comparable to published data. The conditions for testing the whole cell patch clamp were within a range that was acceptable in our facility for use in determining effects on cardiovascular potassium channels.

**2006 ENDOTHELIAL CELL-SPECIFIC ARYL HYDROCARBON RECEPTOR NULL MICE DISPLAY LOW SYSTEMIC BLOOD PRESSURE AT MODEST ALTITUDE.**

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Aryl hydrocarbon receptor (AHR) null mice are hypertensive at modest altitude, but hypotensive at sea level. We tested the hypothesis that endothelial cell-specific deletion of AHR would result in a similar blood pressure phenotype as ubiquitous deletion. Mice harboring AHR with exon 2 flanked by loxP sites (AHRfl/fl) were crossed to mice expressing cre-recombinase driven by the Tek endothelial cell-specific promoter to produce Cre+/AHRfl/fl (ecAHR KO) as conditional knockouts and Cre-/AHRfl/fl (ecAHR WT) as littermate controls. To confirm AHR excision, ecAHR KO and WT mice were treated with 450 ng/kg 2,3,7,8-tetrachlorodibenzop-dioxin (TCDD) and cardiac cytochrome P4501A1 (CYP1A1) mRNA measured. Mean arterial blood pressure (MAP) was measured in ecAHR KO and WT male mice with the endothelial nitric oxide synthase inhibitor, L-NNA, (250 mg/kg) iv. Finally, to assess nitric oxide (NO) production we measured urinary excretion of total nitrates/nitrites (NOx). We found that TCDD significantly induced CYP1A1 mRNA in endothelial cells in ecAHR WT mice, but failed to induce it in ecAHR KO mice. MAP of ecAHR KO mice was significantly lower as measured by tail cuff and confirmed by radiotelemetry (MAP: ecAHR KO: 120±2 mm Hg, ecAHR WT: 120±2 mm Hg), but no significant difference was detected in urine NOx. Interestingly, however, while L-NNA increased MAP in ecAHR null mice, the response was even greater in ecAHR WT mice (MAP: ecAHR null, +8.12 mm Hg; ecAHR WT, +18.20 mm Hg). These data suggest that hypertension at modest altitude observed in mice with complete deletion of AHR is not a result of loss of AHR from endothelial cells. Further, our data suggest that loss of AHR solely in endothelial cells may reduce NO-dependent control of blood pressure, although this cannot account for the apparent hypertensive phenotype. Supported by HL07843.

**2009 THE IMPORTANCE OF ASSESSING THE PHARMACOLOGICAL EFFECTS OF NOVEL CHEMICAL ENTITIES ON LEFT VENTRICULAR PRESSURE AND CONTRACTILITY.**

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The ICH S7A guideline requires effects of test substances on the cardiovascular system be assessed with respect to BP, HR and ECG intervals. The guidelines then states that where adverse effects may be suspected additional supplemental safety pharmacology studies, including ventricular contractility should be conducted as deemed appropriate. However the definition of an adverse effect is ill defined and as such traditional approach to assessing effects is primarily limited to the core battery requirements. Failure to conduct supplementary studies is also in part due to limitations of study designs, where LVP and contractility assessment were limited to acute studies due to limitations of surgical and monitoring techniques. However with advances in telemetry it is now feasible to develop chronic models for assessing LVP and contractility in conjunction with BP, HR and ECG. The objective of this study was develop a model for continuous evaluation of LVP and contractility and highlight potential adverse effects not detected by traditional assessments. Dogs were surgically implanted with Data Sciences International (DSI) telemetry transmitters. LVP, contractility, ECG and BP were assessed over a 4 week baseline period and again for up to 24 hours following administration of Atenol and Pemobendan. Atenol caused a rapid decrease in HR ~30%, which was also followed by a rapid and sustained decreased in maximum left ventricular contractility of a similar magnitude. No effects were noted on blood pressures. Pemobendan caused a rapid increase in maximum left ventricular contractility (~180% mmHg/sec). Contractility increased by ~100% and remained elevated for up to 4 hours. Over the same period no significant changes in heart rate or systemic blood pressure were noted. In conclusion this model demonstrates that assessment of heart rate and blood pressure alone, may result in missing potential adverse pharmacological effects and that it is essential to assess contractility as part of full cardiovascular safety assessment program.

**2099 TRANSPLACENTAL EXPOSURE TO ARSENIC INDUCES HEPATIC CHANGES IN APOE-/- MICE.**

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Exposure to arsenic via drinking water is a serious health concern in the US, and also a worldwide problem. Epidemiological studies have shown that chronic consumption of arsenic contaminated drinking water is linked to higher rates of cardiovascular disease. It has recently been shown that transplacental exposure to 85 mg/L sodium arsenic (NaAsO2; 49 ppm As) in drinking water accelerates atherosclerosis in ApoE-/- mice without a high lipid diet. However, the mechanism by which arsenic induces atherosclerosis is not elucidated. We hypothesized that transplacental exposure to arsenic induces hepatic changes in the ApoE-/- mice which predisposes them to accelerated atherogenesis. To test this hypothesis, the livers of 10 week old mice transplacently exposed to arsenic (49 ppm) were collected for protein expression, global DNA methylation, and histology studies. Transplacental exposure to arsenic increased the expression of Hsp70, total Hsp70 and Hsp 70 + Hsc 70), and Sreb1p as determined by Western blot analysis of liver homogenates. Global DNA methylation was assessed by methyl acceptance assay. There was a significantly higher incorporation of methyl groups (hypomethylation) per microgram of DNA in control mice compared to arsenic exposed mice (hypermethylation). Liver sections fixed in formalin were processed for Hematoxylin/Eosin (H/E) staining to look for necroinflammation and liver morphology, but there was no difference in the liver histology between arsenic exposed and control mice. However, analysis of plasma samples for indices of liver damage showed that arsenic exposure significantly increased plasma levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) suggesting liver injury. Taken together, these results suggest that transplacental exposure to arsenic induces hepatic changes, which might be associated with inflammatory responses that are the driving force behind the accelerated atherogenesis. Supported in part by USPHS grants ES015812 and ES014445 and U of L Competitive Enhancement Grant.
2100 DIOXIN INHIBITS HEART REGENERATION IN ADULT ZEBRAFISH.

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Humans do not have the capacity to regenerate damaged heart tissue and instead form a scar that is non-contractile resulting in reduced cardiac function. It therefore becomes necessary to use other vertebrate models to understand the molecular mechanisms underlying the tissue regeneration process. One such model is the zebrafish, as it can replace damaged tissues and organs such as the heart, fins, spinal cord and the retina without forming scar tissue. We have found that the environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) inhibits regeneration of the zebrafish ventricle after surgical wounding. Adult male zebrafish were exposed to 70 ng/g TCDD or vehicle i.p. and 24 h later were anesthetized and 20% of the ventricle was surgically resected. The hearts were analyzed at 4, 7, 14 and 21 days post amputation and assessed for signs of recovery. TCDD exposed fish displayed a continued presence of a blood clot at the site of injury that was not replaced by new muscle compared to the vehicle control group. TCDD exposed fish also exhibited reduced cell proliferation as seen by BrU incorporation compared to vehicle control using confocal microscopy. Amputated TCDD exposed fish displayed signs of pericarditis while this was not observed in the control group. TCDD also markedly altered expression of the epicardial cell layer transcript, raldh2, as compared to controls. These findings indicate that TCDD has an effect on the maintenance of a normal adult heart, but interacts with a process that is critical in the healing of wounded myocardium. Elucidating the molecular mechanisms by which these occurs are currently being investigated. This work is supported by NIH Grant ES012716 and UW Sea Grant.

2101 CHEMOTHERAPEUTIC AGENT DOXORUBICIN INDUCES CARDIAC FAILURE IN MALES BUT NOT FEMALES EXPOSED AS NEONATES.

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Pre-pubertal girls exposed to doxorubicin for cancer therapy are more likely to develop cardiomyopathy later in life. Additionally, estrogen is cardioprotective against doxorubicin toxicity in adult rodents. To model this problem we randomly treated two litters of Sprague Dawley rat pups (n=3/group). Males or females pups were administered either saline or 1 mg/kg doxorubicin every other week intraperitoneally in a volume of 25-50 μl in one, two or three doses starting at day 14. Rats were evaluated monthly by trans-thoracic echocardiography. RNA was isolated from hearts of doxorubicin and saline treated rats and subjected to analysis using the Agilent 2100 Bioanalyzer and microarray analysis. Results: At 4-5 months of age, doxorubicin treated males showed a significant decline in left ventricular systolic function (FS%, fractional shortening) with a 15% mean ± SD of (51% ± 9%) compared to (69% ± 2%) in female doxorubicin treated rats (ANOVA p < 0.001). FS% was (70% ± 2%) for female controls and (70% ± 2%) for male controls. When FS% declined to 40%, (around month 5) male rats were euthanized. The FS% in female rats at 12 months was 65±3 demonstrating protection from doxorubicin cardiotoxicity. To understand the mechanism of protection, a second group of eight litters were treated similarly and euthanized 2 months after the last doxorubicin injection before a decline in heart function was observed by echocardiography. RNA was isolated from hearts of doxorubicin and saline treated males and females and screened by illumina microarray (n=5/group). The doxorubicin treated females have a preservation of pathways involved in ribosome biogenesis and translation, heart morphogenesis and steroid receptors compared to the doxorubicin treated males. Pathways important in apoptosis were not activated in the doxorubicin treated females compared to males. Conclusion: Susceptibility in males to doxorubicin cardiac toxicity may be partly due to a reduction of cardiac protein translation and an activation of apoptosis.

2102 PULMONARY COMPLICATIONS RESULTING FROM GENETIC CARDIOVASCULAR DISEASE IN TWO RAT MODELS.

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Underlying cardiovascular disease (CVD) has been considered a risk factor for exacerbation of air pollution health effects. Therefore, rodent models of CVD are increasingly used to examine mechanisms of variation in susceptibility. Pulmonary complications and altered iron homeostasis secondary to CVD may influence outcomes of air pollution exposure. In this study we characterized baseline pulmonary endpoints of iron homeostasis, oxidative stress, and inflammation in rat models of CVD. Healthy normotensive Wistar Kyoto (WKY), spontaneously hypertensive (SH) and the spontaneously hypertensive heart failure (SHHF) rats were evaluated at 11-14 wk old. Blood pressure and heart rate measurements taken by tail cuff were increased in SH and SHHF rats relative to WKY rats (SHR>SHHF>WKY). Breathing parameters measured using whole body plethysmography demonstrated increases in breathing frequency and tidal volume resulting in increased minute volume in SHHF and SH relative to WKY rats (SHR>SHHF>WKY). Real-time RT-PCR indicated differences in markers of inflammation and oxidative stress in lungs among strains. Pulmonary MIP-2, IL-1α, and HO-1 expressions were greater in SHHF and SHR relative to WKY. Affymetrix gene array data demonstrated alterations of several genes related to innate metabolism in lungs of SHHF but not SH rats when compared to WKY. BALF non-heme iron was also increased in SHHF relative to WKY and was associated with increases in transferin and ferritin. This study demonstrates that SHHF have greater pulmonary complications of oxidative stress, inflammation and altered iron homeostasis that WKY or SH rats which may play a role in their increased susceptibility to air pollution. Funded by EPA/UNC CR833237 (This abstract does not reflect US EPA policy)

2103 EXOGENOUS NITRIC OXIDE GAS MIMICS DIESEL EMISIONS-INDUCED IMPAIRMENTS OF VASCULAR ENDOTHELIAL NITRIC OXIDE SYNTHASE FUNCTION.

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Detrimental effects of vehicular emissions on the vascular system have been increasingly recognized as an important potential contributor to the overall cardiovascular disease risk. Our data show that diesel engine emissions (DE) can induce alterations in blood pressure and electrocardiographic indices that may represent inadequate coronary perfusion. Previously, we have shown that coronary arteries exhibited enhanced constriction to the peptide endothelin-1 (ET-1) after DE exposures. In this study, we have looked at the effects of DE and one of the principal gaseous components thereof, nitric oxide, on the level and function of a crucial vascular regulatory enzyme, endothelial nitric oxide synthase (eNOS). Healthy, male Sprague-Dawley rats were exposed whole body to DE (350 μg PM/m3) or nitric oxide (10 ppm) for a single 5 hour period. Aortic eNOS protein was significantly elevated (3 fold) following DE and NO exposures compared to that found in filtered air-exposed control rats. Further investigation revealed that while total NOS activity, measured by a citrulline formation assay, was elevated in the aortas of exposed rats, the activity to protein concentration ratio of NOS was reduced, suggesting dysfunction of this protein with a compensatory synthesis of new protein. Furthermore, coronary artery constriction to ET-1 was enhanced following NO exposure in a manner similar to that seen in DE experiments. Thus, it appears that exposure to DE not only has the capacity to disrupt normal eNOS function, leading to compensatory molecular responses in endothelial cells. These studies 1) characterize a potentially crucial pathway underlying air pollution-induced endothelial dysfunction and 2) identify monoxide gases as potential environmental drivers of this effect. This project was funded by NIH R01ES014639 and EPA STAR Award RD-83186001.

2104 DIFFERENTIAL OXIDATIVE PROPERTIES OF DIESEL EXHAUST Particles.


Diesel exhaust particles (DEP) accounts for a significant percentage of particulate matter (PM) released into the atmosphere and are associated with adverse pulmonary effects. Due to their extremely small size and high surface area, DE can adsorb toxic substances, thus potentially influencing its effects. DEP compositions vary and are often characterized by their ratio of elemental to organic carbon (EC/OC). We hypothesized that lung epithelial cells exposed to DEP rich in adsorbed OC compounds would result in greater antioxidant responses, enhanced inflammation and/or chemokine release, or possibly cell injury. Three samples of varying EC/OC ratios were evaluated: OC deficient ultrafine carbon black (CB), OC poor inflammatory chemokine release, or possibly cell injury. Three samples of varying EC/OC ratios were evaluated: OC deficient ultrafine carbon black (CB), OC poor inflammatory chemokine release, or possibly cell injury. Three samples of varying EC/OC ratios were evaluated: OC deficient ultrafine carbon black (CB), OC poor inflammatory chemokine release, or possibly cell injury. Three samples of varying EC/OC ratios were evaluated: OC deficient ultrafine carbon black (CB), OC poor inflammatory chemokine release, or possibly cell injury.
were determined using HPLC and real-time RT-PCR, respectively. Inflammatory chemokine release and expression of RANTES and MIP-2 were evaluated by ELISA and real-time RT-PCR. Cell injury was inferred by the release of LDH. Exposure to DEP resulted in significant increases in GSH, GSG and HO-1 expression, but only modest increases in cell number. Particle exposures had no effect on MIP-2 gene expression or release. While RANTES expression and release was not altered by CB, SRM 2975 reduced its release (most likely due to particle adsorption) without alterations in its expression, and DEP, not only reduced its release (again due to adsorption) but also decreased its gene expression. In conclusion, DEP can selectively bind mediators, thus interfering with their quantification which must be considered when interpreting their release. In addition, it appeared that epithelial exposure to simple elemental carbon particles was largely innocuous, while particles with increasing OC content (and related adsorbed matter) seemed associated with cellular antioxidant responses. Funded by the EPA CTR82512010 (Abstract does not represent USEPA policy).

**2105 METABOLIC ACTIVATION AND PULMONARY TOXICITY OF DAURINE IN CD-1 MICE.**

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Dauricine (DBC), a bisbenzylisoquinoline alkaloid, is the major bioactive component isolated from the root of Dauricine D.C., which has been commonly used in traditional Chinese medicine as a remedy for the treatment of throat swelling, amygdalitis and chronic bronchitis. The objective of the studies was to evaluate toxicity of DRC. We have found to produce selective pulmonary toxicity in mice. Animals were treated i.p. with DRC, dose-dependent increases in LDH activities were observed in lung lavage fluids recovered from DRC-treated mice (150 mg/kg) to levels that were as much as 3.5 times controls. Euthyroid-based staining studies showed that remarkable cell loss membrane integrity in the lungs obtained from the animals treated with DRC at the dose of 150 mg/kg. We examined the histopathologic changes by H&E stain, and significant alveolar edema along with the swelling of alveolar epithelia and some hemorrhage was observed in the mice given the highest dose of DRC. We also observed cytotoxicity of DRC in three human lung cell lines. DRC (40 μM) caused up to 60% cell death in all three cell lines and is much more cytotoxic than 4-ipomeanol and monocrotaline, known pulmonary toxins. In animal studies, we found that ketocanozone, an inhibitor of CYP3A, showed protective effect on the pulmonary injury induced by DRC. This indicates the pulmonary toxicity of DRC requires P450-mediated activation. We successfully identified a quinone methide metabolite of DRC after exposure to mouse lung microsomes. In addition, the presence of ketocanozone in the microsomal incubation inhibited the formation of the quinone methide metabolite. In conclusion, bisbenzylisoquinoline alkaloid DRC produced selective pulmonary injury. The pulmonary toxicity appears to depend on the metabolic activation of DRC, mediated by CYP3A. The electroquinone and quinone methide metabolite is speculated to be responsible for the pulmonary toxicity induced by DRC.

**2106 INFLAMMATORY EFFECTS OF THE SULFUR MUSTARD 2-CHLOROETHYL ETHYL SULFIDE IN MOUSE LUNG.**

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Sulfur mustard gas is a potent vesicant known to damage lung tissue. Toxicity is due to alkylation of critical proteins that maintain the integrity of the lung, resulting in inflammation and oxidative stress. To elucidate inflammatory mechanisms mediating sulfur mustard gas induced respiratory damage, we used 2-chloroethyl ethyl sulfide (CEES), a lung toxicant and model sulfur mustard analog. Male mice (B612FF, 8-10 weeks old) were intratracheally instilled with CEES (3 or 6 mg/kg) or control. Animals were sacrificed 3, 7 and 14 d later and bronchoalveolar lavage (BAL) fluid and lung tissue collected. Treatment of mice with CEES resulted in a time-dependent increase in cell number and total protein in bronchoalveolar lavage (BAL) fluid indicating lung injury to the lower lung. Increases in NOX levels were also detected in BAL fluid from CEES treated mice 7 and 14 d post exposure. This was associated with increased inducible nitric oxide synthase gene expression which was most prominent at the 6 mg/kg dose of CEES and was evident at all post exposure days. CEES instillation also resulted in increased expression of TNFR1 (p55 receptor for TNFα), amphiregulin (ligand for epidermal growth factor receptor) and but decreased expression of cycllooxygenase-2 (enzyme that mediates the production of prostanooids) and MIP-2 (macrophage chemotactic factor). The effects of CEES on inflammatory mediator gene expression were most pronounced 3 day after exposure. Taken together, these data suggest that CEES modulates redox status in the lung and the production of inflammatory mediators. These changes may be important in the cytotoxic pulmonary response to sulfur mustard. Supported by NIH grants AR055673, ES004758, and ES005022.

**2107 EXTRAPULMONARY EFFECTS OF INHALED OZONE IN A RODENT MODEL.**

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Ozone is a respiratory irritant known to cause lung damage. This is associated with macrophage accumulation and the release of cytokine and proinflammatory mediators, which have been implicated in toxicity. Evidence suggests that the pathogenic response to inhaled ozone is exacerbated in the patients with chronic inflammatory conditions such as COPD. Inflammatory mediators generated in the lung are known to cause extrapulmonary effects, targeting sensitive organs such as the liver. We speculated that exposure to ozone in the presence of chronic lung inflammation modifies the hepatic response to this oxidant. To test this possibility, we compared the effects of inhaled ozone on wild type mice and transgenic mice lacking surfactant protein-D (SPD), which exhibit hallmarks of COPD. Although no major structural differences were noted in histologic sections of livers of WT and SPD-/- mice, trichrome staining indicated greater fibrosis in sinusoidal endothelium and in areas surrounding the portal triad in the SPD-/- mice. Constitutive expression of cyclooxygenase-2 (COX-2), a key enzyme in inflammatory eicosanoid production, was also greater in liver macrophages from SPD-/- mice relative to WT mice, whereas hepatic expression of high mobility group box 1 (HMGBl), a marker of oxidative stress, was similar in the two mouse strains. Inhalation of ozone (0.8 ppm, 3 hr) was associated with a time-dependent increase in the number of macrophages in lungs of wild type mice, which was most pronounced 72 hr post exposure. In contrast, in SPD-/- mice, lung macrophage numbers decreased 48-72 hr after ozone, reducing pulmonary inflammation. In WT mice ozone inhalation caused an increase in liver HMGB1 and COX-2 expression while in SPD-/- mice, decreased expression of HMGB1 was observed with no effects on COX-2. These data suggest that chronic pulmonary inflammation leads to oxidative stress in the liver, which can be mitigated by exposure to inhaled irritants that exacerbate pulmonary inflammation (NIH ES004758, ES005022, CA132624, AR055673, HL074115).

**2108 COMPARISONS OF DIFFERENT LUNG MORPHOMETRY METHODS OF MEAN LINEAR INTERCEPT AND MEAN CHORD LENGTH IN QUANTIFYING MILD MOUSE EMPHYSEMA.**

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ABSTRACT BODY: Mouse emphysema models following chronic cigarette smoke exposures are widely used in efficacy testing of COPD drug candidates and potentially reduced exposure cigarette products. There are however currently no consensus in the morphometric evaluation procedures that are critical in quantifying phenotypic changes (i.e., airspace enlargement). Procedural information is scanty in literature and appears to vary as does methods and reporting of data. The objectives of the project were to define and compare two of most widely used morphometry procedures, the mean linear intercept (Lm) and the mean chord length (MCL) in detecting subtle emphysema changes in mouse lung following chronic smoke exposures, Potential variables and factors that may affect the outcomes were also identified and discussed with the intention of recommending standardization of each procedure. Female A KR mice (~12 wks at start) were exposed to mainstream smoke from 2R4F reference cigarettes (filter removed), 2.4 cig/day, 6 d/wk for 24 wks. Up to 10 mice/group were subjected to Wk 24 necropsy and histopathologic examination. Microscope slides of lung tissues were analyzed by image analysis (Image Pro Plus® Version 6.2.1. Media Cybernetics, Bethesda, MD) to determine Lm and MCL. The morphometry results were method dependent as well as sample acquisition dependent. The outcomes ranged from statistically insignificant to mild degree of emphysema (significant increases in Lm and MCL: -10-13%). Key variables include: 1) filed selection and the exclusion criteria – random or selective (e.g., fields containing large airways and vessels excluded), 2) the use of parallel horizontal lines only for MCL or both the parallel horizontal and vertical lines, 3) a Quality Control review or verification by histopathologist, and 4) automation and consistency in method application among groups. (Funded by Battelle Biology and Health Science Initiative)
Intratracheal (IT) bleomycin (BS), one of the most commonly used animal models for pulmonary fibrosis (PF), causes patchy fibrosis in the lung. While these models do not elucidate the human condition, they are representative of many of the clinical and subclinical PF characteristics. This model allows 7-10 days for the pneumonia-like symptoms to resolve and then retains the animals through 14-28 days for the onset of PF. In this study, we compared the dose dependency and time-course of toxic response and cellular mediators (collagen, nsMxA, CTGF, TIMP1, TGFβ1, and fibronectin) associated with the manifestation of PF via IT and inhalation in F344 rats. The novel delivery of BS in rats should enhance the inflammation and other results (including time-course) associated with PF. Animals were examined for in-life clinical signs, gross and microscopic pathology, and changes in cellular mediators. Rats received 3.6 mg/kg, IT or 0.12 mg/kg, inhalation. 3 mg/kg IT induced minor cytokine and microscopic changes through 21 days. As a follow-on study, the dose was increased to 6 mg/kg, however the onset of pneumonia and PF caused mortality by day 11. 3.5 mg/kg was evaluated through 28 days and caused reversible airway hyperresponsiveness (AHR), with persistently high airway resistance on the 7th day after challenge as was seen 2h after the last exposure (P<0.05). To determine if an irritant response on the 7th day after challenge as was seen 2h after the last exposure (P<0.05). After 14 days, AHR was normal. To determine if an irritant response was present, nasal lavage was performed on the 7th day after challenge. In contrast, BS caused an increase in nsMxA only with slight inflammation and changes in pathology. The decreased response is likely due to the dose dependency of the model. A limitation during the initial development of the inhaled model was the solubility of BS in saline. Follow-on studies examined the use of water which increases the maximum feasible dose in the inhalation system. Higher inhaled doses generate increased toxicity and fibrosis. In conclusion, we have shown the administration method combined with the dose play an important role in the onset of PF symptoms and resulted in pathology in murine models.

Brevetoxins (PBtxs) are potent polyether neurotoxic compounds produced by the dinoflagellate Karenia brevis. Surf and wind can aerosolize these PBtxs resulting in inhalation exposure at or near beaches that can produce both immediate and chronic asthma-like symptoms in humans, especially those with pre-existing airway diseases. To understand the more chronic effects of inhaled PBtxs, we challenged 4 “asthmatic” sheep with 20 breaths of a 300 ppb solution containing equal amounts of PBtx-2 and PBtx-3, on 4 consecutive days. This combination of PBtxs represents the most prevalent PBtxs aerosolized during a red-tide event, and the dosing approximates exposure during light exercise. We measured airway responsiveness to inhaled carbachol, and performed bronchoalveolar lavage (BAL) before and serially after the last PBtx challenge. Compared to pre-challenge, the sheep developed airway hypersensitivity (a hallmark of asthma) that was as severe on the 7th day after challenge as was seen 2h after the last exposure (P<0.05). After 14 days, responsiveness was normal. To determine if a transient component remained in the airways that contributed to this prolonged airway hyperresponsiveness, we assessed the BAL fluid for bronchoconstrictor activity by aerosolizing cell free supernatants back into 5 PbTx-naive sheep and measuring airway resistance. BAL fluid obtained on the last exposure day and 1 day later, caused a mean 60% increase in airway resistance (P<0.05). A measurable constrictor effect in BAL fluid obtained 7 days after challenge, but not at 14 days, was seen. The BAL fluid-constrictor activity was partially blocked by pre-treatment with the PbTx antagonist, β-naphthyl-PbTx. We conclude that low level PbTx exposures can induce airway hypersensitivity that lasts for 1 week. The airway responsiveness may be triggered in part by residual PbTx in combination with soluble mediators that are present in the airways during this period. Supported by NIH-P01 ES 10594.

Pulmonary function analysis has become an important tool in the evaluation of different murine respiratory disease models. It is still unclear however, which test is optimal for a particular disease as the most commonly used pulmonary function variables of humans are not routinely applied in mice. With this study we wanted to delineate the potential and restrictions of several existing pulmonary function techniques and validate some common variables between humans and mice. We therefore evaluated one noninvasive (whole body plethysmography) and two invasive pulmonary function setups (forced maneuvers system from Busco and forced oscillation technique-Flexivent from Scireq) in different mouse models. Two models of asthma (occupational and allergic), a model of pulmonary embolism and a model of pulmonary fibrosis were compared with healthy controls. We found that noninvasive tests were not useful for our purpose. However, invasive forced expiratory flow-volume curves adequately measured hyper-responsiveness to methacholine, airflow limitation and airway obstruction, which best correlated with the invasive measurement of respiratory resistance. Both invasive techniques were efficacious for the quantification of restriction or hyperinflation via changes in total lung capacity or vital capacity, and compliance of the respiratory system was proved to be useful and specific in this setting. Forced oscillation variables may have less clinical value but were shown to be additional for the further description of the underlying lung disease. We conclude that invasive pulmonary functions are very useful in the description of respiratory disease models and may bring murine models more close to human pathology.
adjusment of height and weight. This finding suggest that exposure of POPs could be of importance for development of obesity in humans, but further analyses of the associations is needed before conclusions about causality can be made. Most importantly, the associations has to be confirmed also in women.

2114 DIESEL-EXHAUST PARTICULATE (DEP AND NANOPIRLE (NP) EXPOSURES: CAN DEP TELL U.S. ABOUT POTENTIAL HEALTH RISKS OF NP?


Potential health risks of engineered NPs are increasingly studied in vitro and in laboratory animal experiments, but there is a lack of data on human exposure to NP. However, there is an abundance of human data for another source of NP exposure, notably for NP found in DEP. In laboratory settings, human-volunteer exposure studies report DEP number concentrations (i.e., >1010 particles/cm3) that exceed the highest measured values reported to date in manufacturing and handling of engineered NPs. Recent human DEP exposure studies, using sensitive physiological instrumentation and well-characterized exposure concentrations and durations, suggest that elevated DEP exposures (from older diesel-engine technologies, and at levels 1 to 2 orders of magnitude higher than environmental or occupational concentrations) may trigger short-term changes in, for example, lung and systemic inflammation, thrombocytopenia, and brain activity. In these reports, considerable uncertainty remains as to (1) which DE constituents underlie the observed responses (i.e., NPs, DEP mass, DE gases), (2) the relevance of these findings to newer diesel-engine technologies, and (3) the implications of the observed changes for the development of disease. However, if we consider the elevated DE exposures that have existed in some occupational settings (and thus, high NP concentrations), and if we assume that DE NP are the most biologically active component of DEP, accumulated data from healthy volunteers do not appear to support the idea that NPs in DEP lead to enhanced toxicity compared to other inhaled particles. In summary, the elevated levels of NPs in DEP combined with available human data on DEP health effects, do not support the idea that high levels of NP exposure per se (at least in the DEP context) must be highly toxic by virtue of the NP content alone.

2115 INTERSPECIES EXTRAPOLATION OF TOXICITY DATA IN ECOCLOGICAL RISK ASSESSMENT: ISSUES AND SOLUTIONS.

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Extrapolation of toxicity data across species is a primary source of uncertainty in ecological risk assessment. In the 1990s, USEPA and other agencies recommended use of allometric equations for such extrapolations. Since metabolic rate is directly proportional to body weight, these equations could be used to modify results of toxicity tests in laboratory animals for wildlife species merely through comparison of body weights. The approach most generally used the ratio of the target to test species body weights raised to the 0.75, resulting in higher toxicity reference values (TRVs) for larger species than for smaller species. For example, using this approach, lead TRVs for beaver and muskrat are 58 times and 172 times smaller than for a 38 g grey squirrel. Similarly, the ratio of the TRVs for a 65 g house mouse and a 1 kg pen-tailed giant mouse is 9.5. The ratio of the TRVs for a 1.9 kg house cat and a 10 kg golden retriever is 0.18. However, the current use of allometric equations for interspecies extrapolation of toxicity data, citing field data that do not support their use. However, the current practice of assuming equivalent toxicity ignores the differences in body sizes and metabolic rates across species. From a regulatory standpoint, the chosen approach is a key component in differentiating between sites that require additional work and those that do not. For metabolically activated compounds, allometric equations should provide more accurate predictions of potential toxicity than the assumption of equivalent toxicity. For chemicals that do not require metabolic activation, another metric should be used that does not assume equivalent toxicity. Therefore, the current USEPA approach is not recommended as it ignores all physiological, anatomical, and toxicological knowledge of different species. New guidance, supported by both field data and toxicological literature, should be developed to reduce uncertainty in interspecies toxicity data extrapolation.

2116 A MECHANISTIC STUDY ON THE AMIDARONE-INDUCED PULMONARY TOXICITY IN RATS.

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Amiodarone (AM) is gaining support as a first-line antidysrhythmic drug despite its potentially fatal pulmonary toxicity. The goal of this study was to investigate some possible mechanisms associated with AM-induced pulmonary toxicity (AIPIT) in rats. To achieve this goal, Sprague-Dawley rats were given AM in a dose level of 80 mg/kg/day for one, two, three and four weeks. Rats were weighed at day 7, 14, 21 and 28 following AM injection. Bronchoalveolar lavage (BAL) was obtained from each group and used for determination of total leukocytic count. Lung samples obtained from each group were weighed. Homogenization was done finely and the homogenate was used for determination of biochemical parameters such as ATP content, lipid peroxides, (GSH, catalase, SOD, GPx, and GR activities), NO and hydroxyproline levels. Histopathology of lung fragments was also investigated. Results revealed that administration of AM for one week show a significant increase in body weight together with an increase in both lung weight and lung/body weight coefficient. GSH depletion was observed. Considerable increases in lung hydroxyproline level with some histopathological alterations were apparent. Treatment for two weeks produces significant increase in BAL fluid, total leukocytic count and elevation of both GR activity and NO level in lung homogenate. Loss of cellular ATP and inhibition of most antioxidative protective enzymatic system appeared. Alteration in SOD activity was appeared following daily treatment for three weeks. In rats treated with AM for four weeks, more severe toxicity was apparent together with significant reduction in GR activity. Histopathological diagnosis was mostly granulomatous inflammation and interstitial pneumonitis in rats treated for three and four weeks respectively. No fibrosis was observed in either one of the aforementioned groups. Lastly, it is evident that AIPIT might occur in rats in a sequence of edema, inflammatory reactions and severe imbalance between ROS production and antioxidant defenses ending ultimately in sever lung toxicity.

2117 INCREASE OF DOPAMINE LEVELS AFTER FIPRONIL TREATMENT.

A. Analon, M. A. Martinez, R. Pirig, M. Martinez, V. Caballero, I. Ares, E. Ramos and M. R. Martinez-Larrañaga. Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Universidad Complutense, Madrid, Spain. Fipronil, a phenylpyrazole insecticide is a potent blocker of GABA receptors, which are believed to be its primary target site. Because of its high insecticidal activity against many insects and other arthropod pests, fipronil has found many uses in agriculture, veterinary medicine and urban pest control. In relation to the oral LD50 values of fipronil in insects and rats, the sensitivity of insects to fipronil is 700- to 1300-fold higher than that in rats. Limited information is available on the in vivo CNS effects of fipronil. Because of a variety of putative biochemical and physiological target sites may contribute to fipronil toxicity, the objective of the present study was to investigate neurochemical effects following the administration of fipronil (5, 10 and 15 mg/kg/day, orally for 5 days) in male Wistar rats (n =6/group). Animals were sacrificed 24 hours following fipronil administration and their brains were rapidly removed. The hypothalamus, midbrain and striatum were dissected and analyzed for content of dopamine (DA) and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) using a HPLC method with electrochemical detection. Fipronil causes a dose-dependent increase of DA levels. Fipronil, at the highest dose, increased DA levels in hypothalamus (66%, P<0.001), midbrain (51%, P<0.001) and striatum (40%, P<0.001). In contrast, fipronil, at the highest dose, decreased DOPAC levels in hypothalamus (39%, P<0.001), midbrain (48%, P<0.001) and striatum (19%, P<0.001) respect to corn oil controls. Similarly, Fipronil decreased the turnover [(DOPAC+HVA)/DA] in hypothalamus (20%, P<0.01), midbrain (64%, P<0.001) and striatum (35%, P<0.001) respect to corn oil controls. These results demonstrate that fipronil toxicity may be mediated by dopamine receptors. This work has been supported by projects No. CCG07-UCM/AGR-2618 & Consolider-Ingenio 2010 No. CSD2007-063 (MEC), Spain.

2118 MULTIMODAL BIOSSENSOR FOR ANALYSIS OF BLOOD ESTERASES.

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The aim of this work was to develop a multimodal biosensor for simultaneous analysis of blood esterases including acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and carboxylesterase (CaE). The task can be solved when sensors and sensor arrays are used that are formed by layer-by-layer (LBL) technology. The sensor array application enables the measurement of multiparameter analytical responses that can be analyzed using modern mathematical approaches, thus providing simultaneous quantitative assay of mixtures of esterases. Based on LBL technology, a bielectrode measuring system was created for the simultaneous analysis of choline and phenol. The measuring process of the bielectrode system was optimized for the analysis of individual activities of AChE, BChE and CaE, as well as...
2119 KINETIC DIFFERENCES IN THE INTERACTIONS OF CHLORPYRIFOS OXON WITH BUTYRYLCHOLINESTERASE AND ACETYLCHOLINESTERASE.

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The organophosphorus insecticides are commonly utilized throughout the world, and account for about half of all insecticide use in the United States. The acute toxicity observed following exposure to toxic levels of these chemicals is the accumulation of excess acetylcholine resulting from inhibition of acetylcholinesterase (EC 3.1.1.7) and probably butyrylcholinesterase (EC 3.1.1.8). Previous studies from this laboratory have shown that the interactions of some organophosphates with acetylcholinesterase are far more complex than originally thought, with the inhibitory rate constant k2 changing as a function of inhibitor concentration (concentration-dependent inhibition kinetics). In the present study, chlorpyrifos oxon (O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphate) displayed concentration-dependent inhibition kinetics towards human recombinant acetylcholinesterase. For example, 2 mM chlorpyrifos oxon yielded a k2 of 9.32 nM h-1, whereas 10 mM inhibitor gave a k2 of 1.36 nM h-1. In sharp contrast, no evidence of concentration-dependent inhibition kinetics was observed with the inhibition of butyrylcholinesterase with chlorpyrifos oxon. A range of chlorpyrifos oxon concentrations from 0.3-10 nM, estimates of the k2 ranged from 30 nM h-1 to 70 nM h-1, with no pattern of concentration-dependent inhibition. Stopped-flow studies determined the Kd for chlorpyrifos oxon binding to butyrylcholinesterase to be 2.52 mM. These data suggest that active site gorge differences between these two enzymes have important mechanistic implications for concentration-dependent inhibition kinetics. (Supported by Grant ES012648 from NIEHS).

2120 DOSE-RESPONSE EVALUATION OF C57BL/6 MICE FOR MOTOR ABNORMALITIES AND PARKINSON-PATTERNED NEUROPATHOLOGY AFTER PARAQUAT AND MANEB EXPOSURE.

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The combination of parquat and maneb (PQ/MB) has been proposed to be a risk factor for Parkinson’s Disease. C57Bl/6 mice were dosed daily from PN 5-19 (0, 31.0 mg/kg PQ/MB) and/or twice weekly from week 28-31 of age (10/30 mg/kg PQ/MB), the levels reported in the literature to cause loss of substantia nigra pars compacta. This high dose was reduced to 5/15 mg/kg PQ/MB following lethality in naive males after the fourth of seven injections as adults. Additionally, two other doses were derived from human PQ exposure models and were administered as a middle dose (0.060/0.18 mg/kg PQ/MB juvenile, 0.61/1.8 mg/kg PQ/MB adult) and a low dose (0.0007/0.002 mg/kg PQ/MB juvenile and adult) to represent exposure levels more relevant to humans. Animals dosed as juveniles only, as adults only, or both, were tested for motor activity and arena behavior at 52 weeks, and then perfusion fixed for evaluation of neuropathology at 35 weeks. One additional group dosed as juveniles and adults was tested again at 62 weeks and sacrificed at 70 weeks of age. At 32 weeks of age, there were no effects of treatment on behavioral endpoints. At 62 weeks of age, treated animals were similar to controls in the arena and activity cage, except in high dose females where rearing activity was increased. Staining with anti-GFAP and deOsmon’s amino cupric silver method did not detect any degenerative response related to treatment across the entire brain at either 33 or 70 weeks of age in either gender. Stereological analysis and quantification of dopaminergic neurons in the substantia nigra is ongoing.

2121 PRENATAL EXPOSURE TO PERMETHRIN CAUSES VASCULAR MALFORMATIONS IN THE FETAL BRAIN AND DECREASES MOTILITY OF ADULT MICE.


Recently, permethrin, a pyrethroid insecticide, was shown to cause abnormalities in the central nervous system (CNS). Previously, we demonstrated that permethrin decreased both endothelial differentiation from mouse embryonic stem cells and angiogenesis of human brain microvascular endothelial cells in vitro. Abnormalities in the CNS may result from defects in formation of the brain vasculature. In the present study, we investigated the effect of prenatal exposure to permethrin on brain vascular formation in the mouse fetuses and on the behavior of mice after maturation. Mice at embryonic day (Ed) 10 were administered permethrin at the dose of 0, 2, 10, 50 or 75 mg/kg dissolved in corn oil. Fetal brains were obtained at ED17. The length of the anterior-posterior axis was shorter in the groups treated with permethrin at the dose of 10, 50 or 75 mg/kg than those treated with corn oil alone. Vascular malformation were observed in all of the permethrin treated groups, and included shortened lengths of vessels, an increased number of small branches and, in some cases, insufficient fusion of the anterior communicating arteries. For the behavioral test, mice at ED10 were administered permethrin at the dose of 0, 2, or 50 mg/kg dissolved in corn oil. Motility was examined at 8 weeks and 12 weeks using the Modified-SHIRPA test. At 8 weeks, although no change was observed in spontaneous behavior, motility of male mice in an open field was significantly decreased in both permethrin treated groups. At 12 weeks, no change was detected in female mice, while a significant decrease of spontaneous behavior and open field motility was detected in male mice. However, no significant change was detected in brain weight, body weight or brain morphology. Our results suggest that morphologic abnormalities in the fetal brain caused by prenatal exposure to permethrin recover in the postnatal period, but functional abnormalities in the brain were worsened.

2122 DELAYED EFFECTS OF ACUTE EXPOSURE TO CHLORPYRIFOS IN AN ANIMAL MODEL (TG2576) OF ALZHEIMER’S DISEASE.

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In mammals, exposure to organophosphates (OP) has been related to long lasting cognitive effects. Although the OP insecticide chlorpyrifos (CPF) is widely used in agricultural activities and household, it acts as a cholinesterase (ChE) inhibitor. In the present study, we evaluated the delayed effects produced by administration of a single s.c. dose of CPF (0.50 mg/kg) to male adult transgenic mice carrying the Swedish mutation for the familiar Alzheimer disease (Tg2576) and their corresponding wild-type mice. Body weight changes were recorded during the experiment. ChE activity was measured in brain 72 hr after CPF treatment in order to determine the inhibition percent achieved. A functional observation battery (FOB) was applied at 72 hr and at 8 weeks after CPF exposure. Effects on spatial learning and memory were evaluated 17 weeks after treatment in a Morris water maze test. Motor coordination and balance were tested using a rotarod 19 weeks after CPF administration. A 52.7% ChE inhibition, and a decrease in body weight 72 hr after treatment were observed. No differences between Tg2576 and wild-type were noted in spatial task acquisition. However, CPF-treated transgenic mice showed improvement in the retention task. On the other hand, motor learning and coordination were impaired in treated transgenic mice. The current results suggest that a moderately low CPF exposure improves long term memory in Tg2576 mice in the classic version of the water maze task, but it impairs motor function.

2123 COMPARATIVE SENSITIVITY OF EEG AND THE PHOTIC AFTERDISCHARGE (PHAD) TO BRAIN CHOLINESTERASE (CHE) INHIBITION BY CARBARYL.

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We have reported that inhibition of brain ChE by treatment with carbaryl decreased the duration of the flash-evoked PhAD (Mowzan et al, 2008) and may increase wild-type activity in the EEG (Graff et al, 2007). We determined if changes in the non-stimulus evoked EEG were more sensitive to brain ChE inhibition than the PhAD. Long Evans rats were implanted with epidural screw electrodes. After recovery, restrained animals were tested for 2 days using similar conditions (no flash stimulus) as PhAD studies for acclimation. On day 3, the rats’ pupils were dilated, light adapted, and tested for the PhAD and EEG in the absence of light. PhAD responses were elicited by a flash presented 5 min after the lights were turned off. The PhAD was measured in the EEG for 15 s. The EEG was recorded for 90 s before the flash, and for 30 s after the flash, to measure any change in the EEG activity. All data were collected and analyzed according to SOPs. The EEG results showed that carbaryl decreased the duration of the non-stimulus evoked EEG, but the PhAD was not altered.
and 10 min later they were dosed (po) with 0, 1, 10, 30, or 50 mg/kg carbasil (corn oil vehicle). EEG recording (50 segments of 2s) occurred 5, 15, and 30 min later. The EEG was transformed using a FFT and the spectra averaged. Immediately after testing rats were sacrificed, the brains removed, flash frozen, and stored at -80°C until determination of brain ChE activity by radiometric assay. Because the electro-physiological and ChE data were dependent measures, orthogonal linear regression was used to examine the relationship between %control physiological endpoints and %control brain ChE activity. Measurement error variances were estimated from independent studies of the physiological variables and brain ChE activity. Changes in Theta EEG activity of about 30% were significant, but the PhAD du-ration required about 50% change for significance. Considering animals with >10% brain ChE inhibition, regression indicated EEG changes associated with 96- 52%, and decreased PhAD with about 58%, control brain ChE activity. The simi-licity was due to the asymptotic levels of brain ChE inhibition produced over this dosage range of carbasil. Orthogonal regression improved the fit to the data, espe-cially for the PhAD. The results indicate that the non-stimulus driven EEG and the flash-evoked PhAD have approximately similar sensitivities to inhibition of brain ChE. Supported by a NRC Fellowship. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

2124 NEONATAL PARATHION EFFECTS ON COGNITIVE FUNCTION IN AGING RATS.

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Developmental exposure of rats to the organophosphate (OP) pesticides chlorpyri-fos, diazinon and parathion has been found in our studies and others to cause per-sistent neurobehavioral effects in juvenile and young adults. The current study was conducted to determine the persisting effects of early postnatal exposure on cogni-tive function in older adults and aged rats. On postnatal days 1-4, we administered parathion to male and female Sprague-Dawley rat pups at doses near the threshold for overt signs of systemic toxicity and spanning the threshold for barely-detectable cholinesterase inhibition (0, 0.1 or 0.2 mg/kg/day). The rats began training on the 16-arm radial maze starting when they were one year of age. The controls showed the normal sex difference in this spatial learning and memory task with the males having significantly fewer working memory errors than females. This normal sex ef-fect was not evident in either of the parathion-exposed groups. The males in the 0.1 mg/kg/day parathion exposure group were significantly worse than the control males. The parathion exposed females show slight though nonsignificant reduc-tions in errors. This study demonstrates the selective long-term behavioral alter-ations caused by exposure to the OP parathion at a low dose briefly during postna-tal development, which does not produce clinical signs of toxicity. As we have seen previously with chlorpyrifos exposure during the same postnatal period, normal sex differences in cognitive function were eliminated by parathion. Males are particu-larly at risk for OP-induced parathion-induced cognitive impairments. This effect was seen more than a year after the brief period of low-level postnatal exposure. (Supported by NIH ES10356)

2125 CRITICAL WINDOWS OF EXPOSURE FOR DEVELOPMENTAL CHLORPYRIFOS EFFECTS ON BEHAVIORAL FUNCTION IN ZEBRAFISH.

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Developmental exposure of rats to the pesticide chlorpyrifos (CPF) has been found to cause persistent neurobehavioral impairment. In a parallel series of studies with zebrafish, we also are finding persisting behavioral dysfunction after developmental CPF exposure. While we have developed a battery of measures of zebrafish behavior, which are reliable and sensitive to toxicant-induced induced damage. This study determined the critical window of developmental CPF exposure for causing persisting neurobehavioral effects. Tests of learning (3-chamber tank spatial discrimina-tion), stress response (novel tank diving test) and sensorimotor response (tap startle response and habituation) were conducted with adult zebrafish after early develop-mental CPF exposure. The CPF exposure level was 100 mg/L with durations of 0- 1, 0-2, 0-3, 0-4 and 0-5 days after fertilization. In the 3-chamber learning test, the 0-5 day CPF exposure group had significantly lower learning rate. Both the 0-4 and 0-5 groups were significantly hyperactive in the learning test. In the novel tank div-ing task the zebrafish exposed to CPF 0-5 days had a significantly reduced amount of time in the bottom of the tank. This group also was significantly hyperactive. In the tap startle response test the 0-5 day group had significantly less habituation to startle than controls. This study demonstrates the selective long-term behavioral alter-ations caused by exposure to CPF in zebrafish. We are in the process of evaluat-ing the effects of starting the exposure at a later time to attempt to define the pre-cise developmental window necessary for causing the behavioral effects. The characterization of critical windows of developmental CPF exposure will greatly facilitate the determination of the molecular mechanisms critical for long- term neurobehavioral impairment after developmental toxicant exposure. (Supported by NIH ES10356)

2126 RELATIVE POTENCIES OF TYPE I AND TYPE II PYRETHROIDS FOR INHIBITION OF SPONTANEOUS FIRING IN NEURONAL NETWORKS.

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Pyrethroids insecticides commonly used in pest control disrupt the normal func-tion of voltage-sensitive sodium channels. We have previously demonstrated that permethrin (a Type I pyrethroid) and deltamethrin (a Type II pyrethroid) inhibit sodium channel-dependent spontaneous network activity of rat cortical neurons in vitro; both compounds had similar effects. The current study further compared ef-fects of Type I and II pyrethroids by characterizing the concentration-response of inhibition of spontaneous activity by β-cyfluthrin, cypermethrin and λ-cyhalothrin (Type I), as well as bifenthrin, resmethrin. S-bioallethrin and resmethrin (Type I), and fenprothrin (mixed type). Primary cultures of rat cortical neurons from post-natal day 1-2 pups were grown on multiple electrode arrays. After 10-30 days in vitro, spontaneous network activity consisting of individual spikes and bursts devel-oped and stabilized. Concentration-dependent effects of pyrethroids (1nM to 50μM) on spontaneous network activity were determined in the presence of 10μM bicuculline and 20μM SCH50911 (to inhibit gabapentin input). All 11 compounds caused a concentration-dependent decrease in mean network spike rates. A. Cyhalothrin was the most potent compound, with an IC50 of 25 nM, while S-bioal-lethrin, fenprothrin and resmethrin were the least potent, with IC50 values of >1.5 μM. Using deltamethrin as the index chemical, relative potencies ranged from 0.12 to 7.0. In general, Type II compounds were more potent than the Type I com-pounds or fenprothrin. A mixture of 5 compounds (permethrin, cypermethrin cyfluthrin deltamethrin and esfenvalerate) similarly decreased spontaneous spike rate in a manner that was not effect additive. These data demonstrate that demon-strate that Type I and Type II pyrethroids inhibit network activity in a similar man-ner. Whether pyrethroid effects are dose-additive is currently being examined. (This is an abstract of a proposed presentation and does not necessarily reflect Agency policy)

2127 TYPE I AND TYPE II PYRETHROID ALTERATIONS IN SPONTANEOUS BURSTING PARAMETERS IN RAT CORTICAL NETWORKS MEASURED USING MULTIELECTRODE ARRAY RECORDINGS.

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Pyrethroids are widely used in agricultural, industrial and residential settings to control insect pests. Pyrethroids prolong sodium channel inactivation, although their complete mode of action is not fully understood. We previously reported that permethrin (a Type I pyrethroid) and deltamethrin (a Type II pyrethroid) disrupt bursting characteristics of rat hippocampal neurons in vitro by increasing the inter-spark interval (ISI) and decreasing the burst duration (BD). Here, we utilize corti-cal neurons grown on multielectrode arrays to analyze effects of 5 pyrethroid com-pounds or fenprothrin. A mixture of 5 compounds (permethrin, cypermethrin cyfluthrin deltamethrin and esfenvalerate) similarly decreased spontaneous spike rate in a manner that was not effect additive. These data demonstrate that demon-strate that Type I and Type II pyrethroids inhibit network activity in a similar man-ner. Whether pyrethroid effects are dose-additive is currently being examined. (This is an abstract of a proposed presentation and does not necessarily reflect Agency policy)

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Pyrethroid insecticides bind to voltage-gated sodium channels and modify their gating kinetics, thereby disrupting neuronal function. Using murine neocortical neurons in primary culture, we have compared the activity of 11 structurally diverse pyrethroid insecticides to evoke Na⁺ and Ca²⁺ influx. Pyrethroid insecticides including tefluthrin, deltamethrin, λ-cyhalothrin, β-cyfluthrin, esfenvalerate, S-bioallethrin, fenpropathrin, cyperthrin and biofenthrin produced concentration-dependent elevations of intracellular Na⁺ and Ca²⁺. Two pyrethroids, resmethrin and permethrin, did not produce Na⁺ or Ca²⁺ influx. The EC₅₀ values for active pyrethroids on Na⁺ influx ranged from 1.44 μM (tefluthrin) to 5.86 μM (fenpropathrin). The corresponding potencies on Ca²⁺ influx varied from 1.93 μM (esfenvalerate) to 11.9 μM (tefluthrin). Tefluthrin was the most efficacious pyrethroid insecticide as demonstrated by an increment of [Na⁺], greater than 20 mM. Biofenthrin was a low efficacy pyrethroid producing an increase of [Na⁺], less than 3 mM. The rank order of efficacy of pyrethroids in the Na⁺ influx assay was tefluthrin> deltamethrin> λ-cyhalothrin> β-cyfluthrin> esfenvalerate> S-bioallethrin> fenpropathrin> cyperthrin> biofenthrin. Similarly, pyrethroids also displayed distinct efficacies in the Ca²⁺ influx assay. The rank order of efficacy on Ca²⁺ influx was deltamethrin> tefluthrin> λ-cyhalothrin> β-cyfluthrin> esfenvalerate> fenpropathrin> cyperthrin> biofenthrin. These data suggest that the most salient differences between pyrethroids on Na⁺ and Ca²⁺ influx were in their respective efficacies rather than potencies. Assessment of the influence of pyrethroid mixtures on Na⁺ influx revealed predictable additive effects. These data indicate that comparison of pyrethroids on neuronal [Na⁺], using the fluorescent dye SBFI is a useful assay for comparison of pyrethroid efficacies. (This abstract does not necessarily reflect Agency Policy)

Pyrethroids are widely used insecticides in both agricultural and vector control programs. Given the widespread use of these compounds for the control of insect vectors of devastating human and animal diseases (particularly in urban environments) and additional exposure via dietary uptake, human consumption is likely. The aim of this study was to determine the effects of various pyrethroids on the current characteristics of the human T-type voltage-sensitive calcium channel (Cav3.2). Cav3.2 cDNA was transcribed into cRNA using the mMessage mMachine in vitro transcription kit and injected into defoliated Xenopus oocytes. Cav3.2 currents were electrophysiologically characterized using the two-electrode voltage clamp technique with BaCl₂ as a charge carrier. The toxic enantiomer of cypermethrin (IR) inhibited peak current at the highest concentration examined (10⁻⁷M) by approximately 20%, while the non-toxic enantiomer of cypermethrin (IR) had no effect on Cav3.2 peak current compared to DMSO control. Dose response curves were generated for bioallethrin, cypermethrin, deltamethrin, fenpropathrin, and permethrin. For cypermethrin and deltamethrin, decreased peak current in a concentration-dependent manner while fenpropathrin and permethrin had no effect on the peak current compared to DMSO control. Furthermore, deltamethrin was the most efficacious of the pyrethroids examined which resulted in a 40% reduction of Cav3.2 peak current at a saturating concentration (10⁻⁷M). These results indicate that pyrethroids are potent and stereospecific inhibitors of human Cav3.2. Supported by RI-INBRE Grant # P20RR16457 from NCRR, NIH

In this study we use a previously described model of OPIDN in rats (Jortner et al., Tox. Pathol. 33:378, 2005), to assess the ultrastructural effects of this process on the cell bodies of somatosensory neurons of dorsal root ganglia, a population of cells giving rise to affected fibers. Young adult male Long-Evans rats were administered two organophosphates over a 63-day period, with sacrifice on days 28, 63 and 90. The test compounds were tri-ortho-tolyl phosphate (TOTP) given at a dosage of 300 mg/kg on days 14-28 and 49-63 day periods (14 total doses) and/or chlorpyrifos in two 60 mg/kg subcutaneous exposures (on days 7 and 42). There was TOTP-related OPIDN, including the presence of induction of brain neurotoxic esterase and bilateral distal axonopathy progressing to myelination fiber degeneration. The lesions were maximal on day 90, following the full course of treatment and a subsequent four-week period without exposure to organophosphates. Previous light microscopic study of the dorsal root ganglia (DRG) revealed chromatolysis, and vacuolation of neurons at the 90-day interval (Jortner et al. Toxicologist 47:468, 2008). When examined by electron microscopy, the perikaryal vacuoles were present in occasional rats exposed to TOTP with or without chlorpyrifos, and consisted of one or more large membrane-bound vacuoles containing a sparsely granular material lacking organelles. Chromatolysis was defined by peripheral displacement of both the nucleus and Nissl substance, and was noted in all TOTP-exposed rats. Both of these effects have previously been associated with axonal degeneration or aging (Groves and Scaravilli in Dyck et al. Peripheral Neurology, 4th ed.). Their presence in OPIDN is likely associated with the toxicant-induced axonal injury. Supported by USAMRMC DAMD17-99-1-9498.

2129 STRUCTURE ACTIVITY RELATIONSHIP OF PYRETHROIDS ON THE HUMAN T-TYPE VOLTAGE-SENSITIVE CALCIUM CHANNEL

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Pyrethroids are used in insecticides for both humans and animals because combined exposures in environmental settings occur. We previously noted that OP compounds such as malathion and chlorpyrifos could disrupt BBB integrity using in-vitro models (Neurotoxicol 26, 277; SOT 2007 abstract 2277). This study examined the neurotoxicants alone and in combination for ability to disrupt BBB integrity by decreasing tight junction proteins. Rat brain endothelial (RBE4) cells were cultured in petri dishes and induced to form tight junctions with astrocyte-conditioned media. Cells were then exposed to malathion 10uM, malaoxon 1uM, or lead acetate 1uM and 10uM alone and in combination with an OP compound for a 24 hr period. OP compounds were applied first, lead was added 10 hr later, and cultures incubated for an additional 14 hr. Then, western blots were performed to determine total protein expression. Positive controls for tight junction proteins occludin, claudin 1, and zona occludens 1 (ZO1) were included. The results were analyzed utilizing the Odyssey infrared imaging system. Occludin was reduced by up to 75% and 80% by 1uM and 10uM lead acetate respectively, about 60% by malathion 10uM, and about 80% by malathion 1uM. Combinations of neurotoxicants decreased occludin 70% to 90%. ZO1 was decreased by lead acetate about 60%; together they decreased ZO1 about 60%. Claudin 1 was reduced about 30% by neurotoxicant mixtures and by lead alone about 40%, but not by OP compounds. Results suggest a relationship between induction of damage to BBB integrity and a decrease of proteins that form the tight junction in rat brain endothelial cells. In RBE4 cells, therefore, at least one of the mechanisms that OP compounds and lead utilize to disrupt BBB integrity is by decreasing occludin, claudin 1, and ZO1 tight junction proteins in the barrier.

2130 ULTRASTRUCTURAL STUDY OF CHROMATOLYTIC AND VACUOLAR CHANGES IN SENSORY NEURONS IN ORGANOPHOSPHATE-INDUCED DELAYED NEUROTOXICITY (OPIDN).

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OPIDN results in distal axonopathy progressing to myelinated nerve fiber degeneration. In this study we use a previously described model of OPIDN in rats (Jortner et al., Tox. Pathol. 33:378, 2005), to assess the ultrastructural effects of Dieldrin, a banned organochlorine pesticide, is considered one of the twelve most persistent, bioaccumulative, and toxic chemicals by the US EPA and is ranked seventeenth on the 2005 CERCLA Priority List of Hazardous Substances. A number of epidemiological studies have shown a correlation between Parkinson’s disease (PD) and pesticide exposure, particularly dieldrin. PD is a progressive disease that leads to the degeneration of dopaminergic neurons in the substantia nigra pars compacta. Increased concentrations of dieldrin have been found in the brains of PD patients. This compound has also been shown to adversely affect a number of cellular processes yielding toxic intermediates and oxidative stress, effects that may increase the propensity to develop PD. These processes include disruption of dopamine (DA) catalysis and trafficking, potentially leading to an increase in the
endogenous neurotransmitter 3,4-dihydroxyphenylacetaldehyde (DOPAL). The goal of this study is to determine which structural features of dieldrin are responsible for its ability to disrupt PD-relevant cellular processes. In order to achieve this aim, various analogs of dieldrin were used, including endrin, aldrin, and isodrin. Endrin is an isomer of dieldrin, aldrin lacks the epoxide found in dieldrin, and isodrin is an isomer of aldrin. The relative effects of these compounds on DA neurotoxicity were analyzed using nerve growth factor-differentiated PC6-3 cells. The extracellular concentrations of DA and its metabolites were monitored over a 4-hour time course and quantified by HPLC. It was found that dieldrin and endrin showed a time-dependent increase in extracellular 3,4-dihydroxyphenylacetic acid (DOPAC) whereas isodrin and aldrin showed a decrease in DOPAC formation, compared to controls. When analyzing DOPAL formation, endrin and dieldrin showed time-dependent increases, as compared to controls, and endrin and isodrin showed no effect. These results indicate there is a structure-specific effect on DA neurotoxicity by dieldrin and its analogs.

2133 DELTAMETHRIN EXPOSURE CAUSES CASPASE-3 MEDIATED APOPTOSIS IN SK-N-AS NEURONOBLASTOMA CELLS.

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Deltamethrin is a commonly used type II pyrethroid pesticide that has been demonstrated to affect dopaminergic neurons both in vitro and in vivo. Recently, we have demonstrated that exposure of SK-N-MC neuroblastoma cells stably expressing the dopamine transporter under neurotoxic conditions following exposure to deltamethrin (Elwan et al., 2006). However, the pathway by which deltamethrin induces apoptosis is not well understood. In the present study, we utilized a dopaminergic-like neuroblastoma cell line (SK-N-AS) to determine the molecular cascade and assess the involvement of deltamethrin-induced apoptosis. Exposure of SK-N-AS cells to deltamethrin (100 nM-50 μM) resulted in time and dose dependent cell death as measured by lactate dehydrogenase (LDH) leakage. After 48 hr of exposure, cell death (35-45%) was only observed at concentrations >10 μM. However, significant apoptosis (21-121%) increase, as measured by DNA fragmentation was observed at lower concentrations (100 nM-5 μM). Co-treatment with the specific caspase-3 inhibitor Z-DEVD-FMK (50 μM) completely blocked deltamethrin-induced apoptosis. These results suggest that caspase-3 may be the protease that mediates deltamethrin-induced apoptotic cell death in SK-N-AS neuroblastoma cells. Supported in part by NIHES grant R01ES015991.

2134 SAFETY OF PRALIDOXIME ADMINISTERED IN A RAT MODEL OF METHYLTOXICITY.

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Objective: The treatment of organophosphate (OP) insecticide poisonings includes the administration of antidotes including atropine and pralidoxime. A recent clinical trial showed large doses of pralidoxime significantly reduced morbidity and mortality in OP poisoning. However, OP-carbamates induce the same signs and symptoms while pralidoxime is efficient in OP and potentially harmful in carbamate poisoning. This study was designed to assess whether a therapeutic dose of pralidoxime would result in further respiratory toxicity in rats poisoned with carbamate poisoning. Results: Poisoned rats were treated with pralidoxime (base: 50 mg.Kg-1) administered IM at the time of maximal respiratory effects, 5 min post injection of M. Ventilation was measured using infra-red telemetry. Results are expressed as mean +/- SEM. Statistical analysis used parametric tests with p < 0.05. Results: M induced a significant decrease in core temperature, a decrease in respiratory rate resulting from an increase in respiratory time. M increased the tidal volume. Respiratory toxicity occurred 5 min after injection and lasted 20 min. In comparison with the M group, the administration of pralidoxime did not induce any significant differences regarding the core temperature (35.3 ± 0.6 vs 35.0 ± 0.2 °C), the respiratory rate (85 ± 2 vs 80 ± 4 breath/min), the respiratory time (0.55 ± 0.05 vs 0.56 ± 0.04 s), and the tidal volume (3560 ± 440 vs 3340 ± 850 μl). Discussion: In a previous study we showed a 50 mg/kg of pralidoxime (base) administered IM completely but transiently reversed paraaxon-induced respiratory toxicity. The use of pralidoxime for patients with carbamate intoxication is controversial as pralidoxime may increase the toxicity of monomethylcarbamate. In the present study, the administration of a single therapeutic dose of pralidoxime did not further increase methoxymethyl-induced respiratory toxicity.

2135 LONG TERM EFFECTS OF SARIN EXPOSURE ON COGNITION.

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Organophosphorus (OP) chemical warfare agents like sarin inhibit cholinesterases by phosphorylation at the active site serine. We have shown earlier by a series of biochemical, cellular, molecular and global gene expression approaches that sarin exposure at lethal (1 X LD50) and sub-lethal dose (0.5 X LD50) affects central nervous system for a very long time after the initial acute exposure. In this study, we investigated the effect of a single acute exposure of (1 X LD50) sarin on the cognition, six months post-exposure in Sprague-Dawley rats. Firstly, Water Maze test was used which consisted of training to swim to a platform location that was held constant throughout training to assess the reference memory. Rats received 3 trials a day for 9 days (4 acquisition / 1 probe test, 2 New location tests / 1 re-acquisition / 1 probe test). Behavior was tracked by a video camera and analyzed by video track software. Data obtained strongly suggest significant impairment of the reference memory. Secondly, prepulse inhibition test of the acoustic startle response was used to assess sensori motor reactivity which showed small difference between sarin treated and control mice. Thirdly, 12-Arm Radial Maze test was used to assess errors in reference and working memory. The data indicated that sarin treated mice showed significantly different pattern for both a) choices to criterion and b) latency to complete a choice. Sarin treated mice made more wrong choices and acted more quiedy, suggesting significant impairment in cognitive functions. Thus, this study adds further proof to our hypothesis that initial events mediated by disrupted cholinergic and non-cholinergic pathways decide the long term pathological outcome at several levels.

2136 EXPOSURE OF C. ELEGANS TO THE GLYPHOSATE-CONTAINING PESTICIDE ROUNDUP LEADS TO DOPAMINERGIC NEURONAL DEGENERATION AND MITOCHONDRIAL INHIBITION.

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The incidence of Parkinson’s disease (PD), a neurodegenerative disorder affecting dopaminergic (DA) neurons, has been positively correlated with areas that have high rates of pesticide usage. Additionally, data suggest neurodegeneration may result from increased ROS production and mitochondrial dysfunction. In the present study, three strains of the nematode C. elegans, N2 (wild type), NW1229 (neurons labeled with green fluorescent protein (GFP)) and BZ535 (GFP-labeled DA neurons), were exposed to the glyphosate-containing pesticide, RoundUp (RU), to examine potential mechanisms of toxicity. Acute treatments, in which worms were incubated with RU for 30 min, were conducted in synchronous L2 worms. For N2, the LC50 ≤ 7.28% (95% CI: 5.56 – 9.52; r2 = 0.6886). NW1229 nematodes were also treated with varying glyphosate concentrations (0, 1, 5, 10 and 15%). Compared to control NW1229, ~ 75.9% worms treated with 5% glyphosate died (p < 0.01). Photomicrographs indicate this dose corresponded to nerve ring and dorsal nerve cord neurodegeneration. In order to ascertain whether DA neurons were specifically sensitive, BZ535 were exposed to varying concentrations of glyphosate (0, 1, 5, 10 and 15%). Similar to data from NW1229, at 5%, ~ 56.2% of the worms were dead compared to controls (p < 0.01). Photomicrographs indicate loss of neuronal projections from the CEP neurons into the nose region. Mitochondrial dysfunction was determined by incubating worms with tetramethylrhodamine ethyl ester (TMRE), a fluorescent dye which is only taken up by mitochondria with an active membrane potential (ΔΨm). N2 and NW1229 worms were exposed, post-treatment (RU), to 50 μM TMRE for 30 min. Analysis of fluorescence indicates a negative correlation between TMRE uptake and increasing RU concentration. This is particularly pronounced in the nerve ring. Taken together, these data suggest that RU is capable of inducing neurodegeneration in C. elegans, and that mitochondrial dysfunction is a likely mechanism of action.

2137 TREATMENT OF CAENORHABDITIS ELEGANS WITH ROUNDUP AND MANZATE SUGGESTS LETHALITY MEDIATED BY MITOCHONDRIAL INHIBITION.

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Previous studies demonstrated a positive correlation between high levels of pesticide usage and Parkinson’s Disease (PD). PD is also known to involve mitochondrial inhibition. In order to better characterize the potential relationship between two common pesticides and neurodegeneration, we chronically and acutely exposed
wild type (N22) Caenorhabditis elegans to varying concentrations of RoundUp (RU; glyphosate) or Manzate (MZ; manganese/zinc ethylene-bis-dithiocarbamate) for 24 hours or 30 min, respectively. To further model environmental exposure, a additional population of worms was exposed first to RU for 30 min, followed by a 24-hour incubation with MZ. The LC50 was determined by counting the number of live worms remaining 24 hours post-exposure. The LC50 for acute RU was ~ 7.5% (95% CI: 5.6-9.5%; r2 = 0.6886), chronic RU, ~ 5.7% (95% CI: 5.1-6.3%; r2 = 0.9455) and for chronic MZ, ~0.80% (95% CI: 0.66-0.86%; r2 = 0.9719). Quite surprisingly, acute treatment with MZ, even at the highest concentrations (30%) only resulted in ~50% lethality (r2 = 0.6184). For the combination treatment (RU + MZ), the LC50 was ~12.5% (95% CI: 12.3-13.3%; r2 = 0.6670). Additional experiments were completed using tetrathymethyldihydride ethyl ester (TMRE), a fluorescent dye selectively taken up by mitochondria with intact mem-
brane potentials (Δψ). Following acute exposure to 10% RU, the LC40, worms were incubated with 50 μM TMRE for 30 min. Data analysis suggests a decreased uptake of TMRE, particularly in the worm’s nerve ring. Interestingly, the LC50 val-
ues for the individual treatments were well within concentrations available for pur-
chase at most major retail stores, and were similar to the recommended usage for each agrochemical (2% for RU and 0.658% MZ). Taken together, these data demonstrate that acute or chronic exposure to these common pesticides results in significant lethality at concentrations near the recommended application, and the mechanism of action may involve mitochondrial inhibition.

**Design/Method:** For the in vitro study, effect of diquat on cell viability (SH-SY5Y cells human neuroblastoma) was evaluated. We also studied the in vitro effect of di-
quat on complex-I activity and Reactive Oxygen Species (ROS) generation. For the in vivo study, C57BL6 mice were divided into two groups and intraperitoneally administered with vehicle or toxins twice a week for six weeks. Behavioral parame-
ters such as, akinesia, catalepsy, swim test, tremor, straub tail and rotord were ob-
served after the last treatment. Striatum was dissected and analyzed for the monoamineergic neurotransmitters employing HPLC-EC. Statistical data analysis was performed using Sigma stat 2.03 software.

**Results:** Diquat dose-dependently inhibited the cell viability of SH-SY5Y cells. Diquat increased the generation of ROS significantly. In the mice, diquat significantly reduced the locomotor activity but had no effect on the monoaminergic neu-
rotransmitter in the striatum.

**Conclusions:** Diquat inhibited the neuronal cell growth, increased the generation of reactive oxygen species and also induced behavioral changes. Thus, diquat may significantly increase the risk for nigral neurodegeneration.

**Acknowledgement:** This study was supported by Department of Pharmacal Sciences, Auburn University, Auburn, AL.

**References:**
2. University of Michigan, Ann Arbor, MI, 3. The Dow Chemical Company, Midland, MI and 4. Battelle Pacific Northwest Division, Richland, WA.

We evaluated the quantitative relation between measured red blood cell acetyl-
cholinesterase (RBC AChE) and plasma butyrylcholinesterase (BuChE) activities with exposure to chlorpyrifos (CPF) as assessed by measurement of urinary 3,5,6-
trichloro-2-pyridinol (TCPy) in a study group of workers occupationally exposed in the manufacture of chlorpyrifos and a referent group of chemical manufacturing workers. Measures of plasma BuChE and RBC AChE activity and urinary TCPy concentration collected over a year-long study in CPF-exposed workers (n=53) and referents (n=60) were analyzed using linear mixed models to characterize exposure-
response relationships. Median and 95th percentile urinary TCPy concentrations in CPF workers were 10-fold and >30-fold higher, respectively, than in the referents. RBC AChE activity was unrelated to exposure, up to the maximum of 1,000 μg TCPy/g Cr, while plasma BuChE activity was negatively related to uri-

nary TCPy concentrations above 110 μg TCPy/g Cr. No-effect levels for inhibition of plasma BuChE and RBC AChE corresponded to absorbed doses of CPF of approxi-

mately 5 and >50 μg/kg.d, respectively. PON1 phenotype was not significantly related to cholinesterase (ChE) inhibition at the observed exposure levels.

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mately 5 and >50 μg/kg.d, respectively. PON1 phenotype was not significantly related to cholinesterase (ChE) inhibition at the observed exposure levels.
These findings are consistent with previous no-effect level determinations for ChE inhibition in humans and suggest that general population CPF exposure levels are substantially below the identified no-effect levels. The dose-response relationships observed in this study are consistent with predictions from the previously published PBPK/PD model for CPF, supporting its use in other risk assessment efforts. 17% of unexposed refiners experienced one or more plasma BuChE measures more than 20% below baseline over a year of repeated, periodic measurements, suggesting that ongoing monitoring programs may have a substantial rate of false positive results.

We exploit the protein reactive and axonopathic properties of γ-diketone-like 1,2-diathylbenzene (1,2-DAB) or 2,5-hexanedione (2,5-HD) to elucidate protein modifications associated with nerve fiber (axon) degeneration. We used two differential in-gel electrophoresis (2D-DIGE), matrix assisted laser desorption ionization time-of-flight tandem mass spectrometry (MALDI-TOF/MS-M) and western blot to analyze and compare the lumbo-sacral proteome of male Sprague Dawley rats treated with 20 mg/kg/day 1,2-DAB, or equivalent doses of 2,5-HD i.e. 500 mg/kg/day, or equimolar doses of their respective non-protein-reactive and non-axonopathic isomers 1,3-DAB or 2,5-HD (negative controls), or equivalent volume of vehicle, 5 days a week for up to 3 weeks. Common protein targets of 1,2-DAB and 2,5-HD included unexposed triplet protein L, gelolin, protein disulfide isomerase (PDI), glutathione S-transferase, NADH dehydrogenase 1 tetramer, pyruvate kinase, and fatty acid synthase. In general, the expression of proteins involved in membrane-bound PDI was down-regulated and S-nitrosylated proteins were detected. Markers of oxidative stress (e.g. HO-1) including nitric oxide synthases (NOS isoforms) retained normal mRNA/protein expression. The similarity of the neuroproteomic patterns of 2,5-HD and 1,2-DAB axonopathy suggests common biomarkers and/or mechanisms of neurotoxicity associated with their parent solvents n-hexane and 1,2-diathylbenzene, respectively. It is possible that changes in PDI expression and/or activity mediate the pathogenesis of γ-diketone axonopathy via protein misfolding and/or S-nitrosylation. Supported by NIH grants P42ES01358, U19ES11384, K01NS02183 and the Oregon Worker Benefit Fund.

To study the effect of 2,5-hexanone, a metabolite of n-hexane, on the permeability of blood-nerve barrier, male adult Wistar rats were administered by gavage with 400 mg/kg.1-1 2,5-hexanone for 4 weeks as treatment group, and 0.99% sodium chloride with the same dosage as control group. Event Blue, a fluorescent dye was injected through left femoral vein to the treatment and control rats, after recycling, fixation, embedding and slicing, the distribution of Event Blue was observed under fluorescence microscopy. Integral absorbance was assessed by Image-Pro Plus. The difference between the 2,5-hexanone treated and the control groups was compared. In the transversal sections of sciatic-tibial nerves in the 2,5-hexanone treated group, the integral absorbance of fluorescence in the proximal section was stronger than those of the intermediate and the distal section (p<0.01), and the integral absorbance of fluorescence in the intermediate section was stronger than that of the distal section (p<0.01). In the control group, the weak fluorescence was widely distributed, and the integral absorbance in the distal section was stronger than that of the proximal section (p<0.05). In the treated rats, the integral absorbance of fluorescence in the proximal, intermediate and distal sections was stronger than those of the controls (p<0.01). For the vertical sections of sciatic-tibial nerves in rats the 2,5-hexanone treated rats, fluorescence was observed in both the proximal and distal sections. The weak fluorescence in the distal section of the sciatic-tibial nerves of the control was observed, and almost no fluorescence was showed in the proximal section. The fluorescence in the proximal and distal sections of the treated rats was stronger than the controls. The permeability of blood-nerve barrier could be increased by 2,5-hexanone.
maneb (MB) mouse model, a significant increase in oxidative stress has been observed and overexpression of superoxide dismutase (SOD) and glutathione peroxidase (Gpx) protect against this. The increases in oxidative stress are greater in males than females, with the females being spared from dopaminergic cell death. Thus, this study sought to determine whether there were differences in oxidative stress response and antioxidant defense mechanisms between the genders. Male and female C57BL6 mice were treated 2X a week for 6 weeks with saline or 7 mg/kg PQ + 30 mg/kg MB and were sacrificed 2 or 7 days after the last treatment. Changes in oxidative stress and enzyme activity were determined in the striatum, frontal cortex and olfactory bulb. A significant increase in lipid peroxidation in the striatum was observed 7 days after the last exposure in both males and females, with a larger increase seen in the males. A significant increase in SOD activity was observed in the striatum of females but not males, suggesting a possible neuroprotective response in the females. No significant changes in oxidative stress or enzyme activity were observed in the olfactory bulb or frontal cortex in either gender. This indicates that the effects of paraquat and maneb are relatively selective to the nigrostriatal system, as previously demonstrated. Further analysis of other antioxidant enzymes including GPx and glutaredoxin, changes in enzyme protein levels as well as changes in the ventral midbrain are underway. The results from these studies will further our understanding of the oxidative stress response in the PQ-MB mouse model and determine the gender-related differences.

2147 MOUSE CEREBELLAR ASTROCYTES PROTECT CEREBELLAR GRANULE NEURONS AGAINST TOXICITY OF THE POLYBROMINATED DIPHENYL ETHER (PBDE) MIXTURE DE-71.

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A large body of evidence indicates that polybrominated diphenyl ether (PBDE) flame retardants have become widespread environmental pollutants. Body burden is particularly high in infants and toddlers, due to exposure through maternal milk and house dust. Animal studies suggest that PBDEs may exert developmental neurotoxicity, via mechanisms that are still elusive. PBDEs have been reported to cause oxidative stress and apoptotic cell death in neurons in vitro, when tested in monolayer cultures. Here we report the results of experiments in which mouse cerebellar granule neurons (CGNs) are co-cultured with cerebellar astrocytes. Astrocytes were found to protect neurons against the toxicity of the PBDE mixture DE-71. Astrocytes from Gclm (−/−) mice, which lack the modifier subunit of glutamate cysteine ligase and, as a consequence, have very low GSH levels, were much less effective at protecting CGNs from DE-71 toxicity. In vivo, both these neurons and astrocytes would be either Gclm (+/+)/Gclm (−/−), the toxicity of DE-71 to CGNs is predicted to vary 16.8-fold, depending on genotype. Hence, in addition to being intrinsically more susceptible to DE-71 toxicity because of their low GSH content, CGNs in Gclm (−/−) mice would also suffer from the lack of a full protective effect provided by astrocytes. Since several polymorphisms, including some in the Gclm gene, cause very low levels of GSH, it may be speculated that such individuals might display a higher susceptibility to the neurotoxic effects of PBDEs. (Supp. in part by P30ES07053).

2148 CYTOTOXICITY OF DINITROBENZENE ISOMERS ON C6 GLIOMA CELLS.

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Dinitrobenzene (DNB) refers to a mixture of three isomers: 1,2-, 1,3- and 1,4-DNB. These DNBs are used as common intermediates in the dye and plastics industries. 1,2- and 1,4-DNB cause methemoglobinemia, in contrast, 1,3-DNB is a purported neurotoxicant in rats. To our knowledge, no studies have been conducted to determine if the cytotoxic effects of 1,2- and 1,4-DNB parallel those of 1,3-DNB in neuronal or glial cell-culture models. The purpose of this in vitro study was to compare the relative ability of different cytotoxicity assays to detect early changes in cell viability. C6 Gliona cells were exposed to 10 – 250 μM of each DNB for up to 6 hours. Cytotoxicity was then determined using MTT reduction for mitochondrial integrity, neutral red (NR) uptake for lysosomal integrity, and LDH release as a marker for cell death. C6 Gliona cells exhibited a concentration- and time-dependent decrease in NR uptake following a four hour exposure to 1,2- and 1,4-DNB with effects most evident between 50-250 μM; however NR uptake was the most apparent with 1,3-DNB exposure. In contrast to results obtained with NR, no reduction of MTT by C6 gliona cells was observed at any time or concentration used in these studies. Statistically significant increases in media LDH vs. total LDH after four hour exposure were observed only for 1,2-DNB and 1,3-DNB at 250 μM. Being the more efficient redox-cycler, 1,4-DNB caused LDH leakage beginning at 100 μM, thus revealing its greater potency in this model. The redox-cycler for potency in the LDH assay was 1.4-1.2-1.3-DNB. These results also indicate that differences do exist in the abilities of the 3 cytotoxicity assays to detect early changes in cell viability. However, each assay remains crucial in the overall study of cell viability because each assay is based on different cellular parameters and can give insight into cellular localization as well as mechanism of toxicity of toxicants. This project was supported in part by 5G12-RRO8124 from NCR to the BBRC at UTEP and by NIEHS/NHI grant ES011982 to RTM. The views stated in this abstract are not necessarily the view of NIH/NIEHS.

2149 AGING AND LIFE-STAGE SUSCEPTIBILITY: TOXICITY EFFECTS ON PROTEIN CARBONYL CONTENT IN FRONTAL CORTEX AND CEREBELLUM OF BROWN NORWAY RATS.

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The influence of aging on susceptibility to environmental contaminants is poorly understood, largely due to a lack of data on exposures in older adults and adequate animal models. We examined the acute effects of the volatile organic compounds, toluene, in a study investigating multiple toxicological endpoints. The objective of this study was to test whether oxidative stress plays a role in the adverse effects caused by toluene exposure, and if so, if effects are age-dependent. We selected protein carbonyl as an indicator of oxidative stress because carbonyl content of cells is a useful indicator of oxidative protein damage and has been linked to the adverse effects associated with chemical exposures. Brown Norway rats (4, 12, and 24 months) were dosed orally with toluene (0, 0.65 or 1.35) in corn oil. Four hours later, brains were removed and placed on ice. Frontal cortex and cerebellum were dissected, quick frozen on dry ice, and stored at 80°C until analysis. Protein carbonyls were assayed using commercial kits. One-way ANOVA indicated that hydroxyl peroxide, a positive control increased protein carbonyls in cortical tissue in vitro in a concentration-dependent manner. Two-way ANOVA indicated a significant effect of age and toluene on protein carbonyls in both frontal cortex and cerebellum. In control rats, there was an age-dependent increase in the protein carbonyls indicating increased oxidative stress in 24 month old rats compared to 4 or 12 month old rats. Although toluene increased protein carbonyls in both brain regions and in all age groups, step-down analyses indicated toluene effects were statistically significant only in 12 month old rats. These results indicate that frontal cortex and cerebellum of 12 month old rats are more susceptible to oxidative damage caused by toluene when compared to 4 month and 24 month old rats. (This abstract does not necessarily reflect USEPA policy).

2150 POSTNATAL EXPOSURE TO METHYLmercury ENHANCES DEVELOPMENT OF PARALYTIC PHENOTYPE IN SOD1:G93A FEMALE MICE.

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The effect of methylmercury (MeHg) on time course of development of a paralytic amyotrophic lateral sclerosis (ALS) phenotype (ALS like) was investigated using the transgenic mouse model SOD1:G93A (G93A). We postulated that MeHg exposure would hasten the onset of ALS phenotype and increase further changes in intracellular Ca2+ ([Ca2+ ]i) seen in brainstem motor neurons in this mouse model. Female mice expressing the mutated form of human superoxide dismutase 1 gene (G93A) or wildtype control (wt) were exposed chronically to 0, 1 or 3 ppm MeHg in drinking water from PND29. Motor function was tested using a variable speed rotarod. Hypoglossal motor neurons (NXII) in brainstem slices were loaded with a Ca2+ selective fluorophore and imaged using laser scanning confocal microscopy. At 3 ppm MeHg, 50% of ALS mice exhibited rotated test failure (a classical sign ALS) at ~98 days compared to 112 days for untreated G93A mice. When compared to wt mice, [Ca2+]i from untreated G93A mice was significantly (p<0.05) increased by KCl depolarization. In contrast, [Ca2+]i from MeHg-treated G93A slices was highly variable when depolarized by high [K+]o. [Ca2+]i was significantly (p<0.05) decreased by kainic acid in slices from untreated G93A mice when compared to untreated wt females. However, [Ca2+]i from MeHg-treated mice was potentiated by kainic acid, in contrast, AMPA reduced ([Ca2+]i). Additionally, there...
was no significant differences in [Ca2+]i when NAS [a GluR2 subunit lacking blocker] or CNQX (a blocker at AMPA and kainate receptors) was added. These data suggest that chronic exposure of G93A female mice to MeHg, hastens the onset of hind limb paralysis and changes the sensitivity and responsiveness of glutamate receptors. Supported by NIH grant 5T32 ES072759-19, R21ES14537 and R01ES05299.

2151 CRUCIFEROUS NUTRACEUTICAL 3/5-HT, 2-DITHIOLE-3-THOME PROTECTS HUMAN PRIMARY ASTROCYTES AGAINST NEUROTOXICITY ELICITED BY MPP+, 6-OHDA, ACROLEIN AND HNE.

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Astrocytes possess important roles in maintaining normal brain function and providing trophic support to the neurons. They are also chief target by a range of toxic insults of neurotoxins including 1-methyl-4-phenylpyridinium (MPP+), 6-hydroxydopamine (6-OHDA), acrolein and 4-hydroxy-2-nonenal (HNE). Recently, we have observed that the cellular antioxidants and phase 2 enzymes can be upregulated by 3-F,1,2-dithiole-3-thione (D3T), a cruciferous nutraceutical against many neurotoxins in human neuroblastoma cell lines. However, the regulation of the above cellular factors by D3T in astrocytes has not been investigated. In this study, we showed that incubation of human primary astrocytes with micromolar concentrations (5-100 microM) of D3T for 24 h resulted in significant increases in the levels of reduced glutathione (GSH), glutathione reductase (GR) and phase 2 enzyme NAD(P)H:quinone oxidoreductase 1 (NQO1). D3T treatment also caused time-dependent increases in mRNA expression of gamma-glutamylcysteine ligase catalytic subunit, GR and NQO1 in these cells. Incubation of the astrocytes with MPP+, 6-OHDA, acrolein and HNE led to concentration-dependent decreases in cell viability as detected by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. Pretreatment of human primary astrocytes with D3T was found to afford remarkable protection against the neurotoxicity elicited by the above neurotoxins. Taken together, this study demonstrates for the first time that cruciferous nutraceutical D3T potently induces cellular GSH system and phase 2 enzymes NQO1 in primary human astrocytes and D3T- upregulated cellular defenses are accompanied by markedly increased resistance to the above neurotoxins. The results of this study may have important implications for development of novel neuroprotective strategies.

2152 ACUTE METHAMPHETAMINE (METH)-INITIATED OXIDATIVE STRESS AND NEUROTOXICITY ARE NOT MODULATED BY NUCLEAR FACTOR-E2-RELATED FACTOR 2 (NRF2).

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Reactive oxygen species (ROS) can oxidatively damage cellular macromolecules, and/or activate redox-sensitive transcription factors such as Nrf2, leading to the transcription of genes that modulate toxicity. Whether METH-initiated oxidative stress can activate Nrf2 that modulates neurotoxicity is uncertain. We used Nrf2 wild-type and knockout mice to determine if Nrf2 plays a role in METH-initiated oxidative DNA damage, quantified by 8-oxo-2′-deoxyguanosine formation. Mice were administered 4 doses of METH (10 mg/kg p. i.) or saline, with a 2 hr interval between each dose. After 3 hr, METH treatment enhanced DNA oxidation 2-fold in the striatum of Nrf2 wild-type and Nrf2 knockout mice, and up to 15-20% in the cortex and midbrain, compared to saline controls (p<0.05). However, there was no difference between Nrf2 wild-type and Nrf2 knockout mice in either basal or METH-enhanced DNA oxidation at this dose and time. To further investigate the role of Nrf2 in METH-initiated neurotoxicity, mice were treated as described above, sacrificed 3 days after the last dose and analyzed for tyrosine hydroxylase (TH) and glial fibrillary acidic protein (GFAP) in the striatum. METH treatment decreased TH staining in the striatum of Nrf2 wild-type and Nrf2 knockout mice by an average of 50% compared to saline controls (p<0.05), indicating degeneration of dopaminergic nerve terminals. Similarly, METH increased striatal GFAP by 2.2-fold compared to saline controls (p<0.001), indicating neurodegenerative glial activation. Pretreatment with METH resulted in an apparent decrease in GFAP in METH-treated mice. Therefore, while Nrf2 may mitigate the degenerative effects of some ROS-enhancing neurotoxins administered chronically, it does not play a measurable role in oxidative stress and neurotoxicity caused by acute METH administration. [Support: CIHR, CIHR/Rx&D HRF].

2153 NEUROTOXICITY STUDIES OF ORTHO, META AND PARA-N-1-PHENYL SUBSTITUTED SPIROHYDANTOIN ANTICONVULSANT COMPOUNDS.

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Previously we reported the neurotoxicity of an initial series of N,N′-disubstituted-spirohydantoins that were prepared by reaction of the corresponding N,N′-disubstituted anilines with ninyhydrin, followed by treatment with aqueous sodium periodate. The spirohydantoins exhibited anticonvulsant activities in both the maximal electro-shock (MES) and pentylenetetrazole (PTZ) tests. Rotarod assay and righting reflex assays were done to determine the neurotoxicity of these compounds. The neurotoxicity of the spirohydantoins with different alkyl substituents in the para position of the N1-aryl ring did not show much difference until the chain length exceeds three carbons. Beyond that they showed a greater toxicity e.g.: butyl analog. In order to determine if the position of various groups at ortho, meta or para of the aryl group has any effect on neurotoxicity, a new series of compounds were prepared and tested. All the synthesized compounds passed the righting reflex assay. Compounds with the same group at the ortho or meta positions did not show much difference in their neurotoxicity in rotarod assay when compared to the para position of the aryl ring. This demonstrates that neurotoxicity of this class of compounds is dependent on the size of the substituent rather than its position on the aryl ring.

2154 THE HUNTINGTON’S DISEASE MUTATION LEADS TO AN ALTERATION IN MANGANESE TRANSPORT OR STORAGE.

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Huntington Disease (HD) is a neurodegenerative disorder caused by an expansion of a CAG repeat in the first coding exon of the Huntingtin gene. However, the mechanism by which the mutant HD protein causes pathology is unknown. Remarkably, many of the proposed mechanisms of HD are similar to those identified for neurotoxic metals and other toxins (e.g. mitochondrial dysfunction and oxidative stress). Therefore, we hypothesized that metals which impact pathophysiological mechanisms common HD will exhibit a gene-environment interaction. Using a cellular model of HD, we have found that expression of the mutant huntingtin protein induces a resistance to manganese (Mn) toxicity but not metals such as zinc, copper, or cobalt. In addition, the cells expressing the HD mutation have increased sensitivity to other toxins. Additionally, primary cultures from the YAC128 mouse model of HD recapitulate this Mn gene-environment interaction. To understand the cellular basis of this interaction we investigated the transport and storage of Mn. We find that net accumulation of Mn is significantly decreased in cells expressing mutant HD under both basal and Mn exposure conditions. Interestingly, the deficiency in basal Mn levels correlates with a substantial decrease in the levels of the Mn-dependent enzyme superoxide distumase 2 (SOD2), which plays a key role in limiting mitochondria oxidative stress. To explore the differences in Mn transport, we examined iron transport under basal and high iron exposure in mutant and wild-type cells. These data indicate that functional differences in the transferrin receptor pathway only partially contribute to the Mn-HD gene-environment interaction, implicating an additional unidentified Mn transport pathway. Thus, glutamine expanded HD causes a defect in Mn transport or storage, and leads to a loss of Mn-dependent enzymes such as SOD2.

2155 CUPRIZONE INDUCED-DEMYELINATION ALTERS THE EXPRESSION OF GENES INVOLVED IN ARACHIDONIC ACID METABOLISM IN THE MOUSE BRAIN.

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Chronic cuprizone exposure produces progressive demyelination in mice which reverses upon its removal from the diet. Although the mechanism of demyelination is unknown, activation of glia is integral to the process. Since metabolism of arachidonic acid (AA) is involved in glial activation, we hypothesized that cuprizone exposure would alter expression of AA cascade genes. Mice were exposed to cuprizone for 6 weeks after which they were returned to a normal diet. Histochemistry with the myelin stains Black Gold and Fluorosmyelin demonstrated that frank demyelination...
nation and influx of glial cells into the corpus callosum begins at week 3 and peaks at week 5. However, a decrease in myelin and oligodendrocyte markers, myelin basic protein (MBP) and 2, 3-cyclic nucleotide 3-phosphodiesterase (CNPyt), was evident at week one. Increased expression of CD11b and glial acidic fibrillary protein (GFAP), evidence of activated microglia and astrocytes, was also observed at week one. Coincident with these early changes, is an increase in cytochrome oxidase (COX)-2 and lipooxygenase (LOX)-15 expression, suggesting that these arachidonic acid metabolism genes are either involved in or respond to the earliest sign of demyelination. Expression of LOX-5 was not significantly changed during the early stages of demyelination but it peaked during week 5, when glial markers and frank demyelination also reached their peak of expression, suggesting that LOX-5 expression is a consequence of the massive influx of inflammatory cells into the area of demyelination. While expression of LOX-12 was not consistently increased during demyelination, increased LOX-12 expression was observed during the remyelination, suggesting a role for this isoform in the recovery process. Our study is the first to demonstrate that multiple enzymes involved in arachidonic acid metabolism are altered in the cuprizone model of demyelination and remyelination. These data may help to develop new therapeutic targets to treat demyelinating diseases, such as multiple sclerosis.

**2156** CORTICOSTERONE ATTENUATES HIPPOCAMPAL NEUROTOXICITY AND REACTIVE GLIOSIS THROUGH REGULATION OF THE BLOOD-BRAIN BARRIER IN C57BL/6J MICE TREATED WITH KAINIC ACID.

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High levels of stress or stress hormones have been reported to exacerbate human disorders of several physiological systems. As a model of stress, male mice were implanted with 0, 10, 30 or 100 mg/kg 212 labeled corticosterone (CORT) pellets (0, 19, 67, and 190 mg/kg/d). After 7d, mice were injected with saline or 25 mg/kg kainic acid (KA), were scored for seizures (Racine scale), and were allowed to recover for 12 or 24h (histology), 7d (ELISA for GFAP), or 1, 3, 6, or 12h (western blot for IgG). Tissue was prepared for histological analysis of neurogenesis by the cupric-silver stain; astroglia by ELISA and immunohistochemistry for GFAP; and microglial activation by Iba-1, CD11c, isoelectric, and reactive silver staining. Treatment of mice with CORT caused no neuronal death, and attenuated damage caused by KA. GFAP levels were elevated seven-fold in KA-treated mice. Increasing doses of CORT caused greater decreases in basal GFAP, and CORT pre-treatment attenuated KA-induced protein elevation. GFAP staining revealed hypertrophic astrocytes with thick processes following KA treatment. Astrocytic hypertrophy was attenuated by CORT pre-treatment. Iba-1 and silver staining revealed a population of resting microglia in all brain regions. Basal Iba-1 staining was attenuated by high doses of CORT. Lecitin and CD11c stained microglia were observed in regions that displayed KA-induced neurodegeneration and were rarely observed in control or CORT-treated animals. KA treatment caused a breach of the blood-brain barrier (BBB) and resulted in hippocampal levels of IgG that were increased by one hour. IgG levels were maximal at six hours post-treatment, and returned to baseline by 12 hours. CORT pre-treatment attenuated KA-induced BBB opening and IgG influx. Our data indicate CORT does not cause neuronal damage, and attenuates excitotoxic neurodegeneration and glial activation that has BBB disruption as a component of the pathological mechanism.

**2157** CYANIDE-MEDIATED INCREASE IN Ca2+ AND NITRIC OXIDE IS ASSOCIATED WITH CYTOTOXIC OXIDASE INHIBITION.

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Cyanide is a potent neurotoxicant that inhibits mitochondrial (mt) respiration at the level of cytochrome c oxidase (COX). Acute cyanide exposure causes dopaminergic cell death in the substantia nigra, which may lead to a delayed-onset Parkinson-like syndrome. Our studies have shown that nitric oxide (NO), produced by nitric oxide synthase (NOS), plays a role in cyanide inhibition of COX and the subsequent death of dopaminergic cells. Cyanide (5 μM) increased generation of mt NO (NOS) in mesencephalic cell culture. The rise in NOS could originate from cytosolic NOS or mt (mnNOS). In this study, we examined the mechanism by which cyanide increases NOmt in N27 rat dopaminergic cells. Since Ca2+-bound calmodulin (Ca2+-CAM) is an activator of NOS and cyanide increases cytosolic free Ca2+ (Ca2+cyt), we compared cyanide-induced changes in Ca2+cyt to NOmt. Cyanide-mediated changes in Ca2+cyt and Ca2+mt, were monitored in whole cells using the fluorescent probe Fluo-4 AM. It was noted that changes in Ca2+cyt did not correlate with changes in NOmt. Within the 1-10 μM KCN range, Ca2+cyt peaked at 8 μM KCN. In contrast, NOmt increased at 3 μM KCN, as determined by DAF-FM fluorescence. To further examine the mechanism of NOmt elevation, ruthenium red was employed to block Ca2+cyt uptake. Ruthenium red treatment enhanced NOmt by 50%, suggesting that elevated Ca2+cyt did not increase NOmt. Examination of Ca2+cyt showed a direct correlation between cyanide-mediated changes in NOmt and Ca2+cyt. Thus, it appears that cyanide increases NOmt primarily through activation of cytosolic NOS, not mtNOS. It is concluded that the cyanide-induced rise in Ca2+cyt stimulates to the cytosolic production of NO, which then enters mt to interact with COX, thereby enhancing cyanide inhibition of oxidase phosphorylation. (Supported in part by NIH Grant ES04140.)

**2158** INDUCIBLE EXPRESSION OF BNIP3 IN CLONAL MESENCEPHALIC CELLS LEADS TO NECROPTOTIC DEATH.

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BCL2/adenovirus E1B 19 kDa interacting protein (3BNIPI3) is a member of the BH3-only subfamily of pro-apoptotic Bcl-2 proteins and is associated with hypoxia-mediated cell death. To study involvement of BNIP3 in KCN-mediated cell death, a tetracycline (tet) inducible stable cell line was constructed. The cell line was prepared by stable transfection of the TetR plasmid in immortalized dopaminergic neuronal cells (N27), to obtain N27-TetR clones. The clones were stably transfected with expression plasmids containing BNIP3 gene downstream of cytochrome oxidase promoter carrying tetO sequences. In the cells BNIP3 expression increased in a dose-dependent and time-dependent manner following tet treatment. Both Propidium Iodide (PI) and trypan blue staining showed that minimal necrotic death occurred within 24 hrs of Tet-on (0.5ug/ml). However cell viability decreased with rising concentrations of tet (>0.5ug/ml). Cell death was detected by a fluorescence-activated cell sorting analysis (FACS). At higher concentrations of tet (0.5ug/ml) the DNA fragmentation was reduced and cell death shifted to necrosis as indicated by a smear on 1.5% agarose gel. Prolonged tet treatment for >48 hrs or treatment with cyanide (400 μM) shifted the mode of cell death to necrosis as confirmed by trypan blue and PI staining. Cell viability decreased following KCN treatment (400μM) as measured by trypan blue cell counting.

Neither the apoptosis nor necrotic death was associated with caspase 3 activity. It was concluded that following tet induced expression of BNIP3, a continuum of peripheral cytochrome c condensation. DNA laddering was detected. At higher concentrations of tet (0.5ug/ml) the DNA fragmentation was reduced. At higher concentrations of tet (0.5ug/ml) the DNA fragmentation was reduced and cell death shifted to necrosis as indicated by a smear on 1.5% agarose gel. Prolonged tet treatment for >48 hrs or treatment with cyanide (400μM) shifted the mode of cell death to necrosis as confirmed by trypan blue and PI staining. Cell viability decreased following KCN treatment (400μM) as measured by trypan blue cell counting. Prolonged tet treatment for >48 hrs or treatment with cyanide (400μM) shifted the mode of cell death to necrosis as confirmed by trypan blue and PI staining. Cell viability decreased following KCN treatment (400μM) as measured by trypan blue cell counting.
postnatal week. Histologically, these mice showed neuronal loss and glial proliferation in the cerebral cortex and hippocampus. The mortality in double-knockout mice was prevented by SA supplementation in the drinking water. Remarkably, when AA was stopped for 2 weeks, these rescued double-knockout mice developed epilepsy and similar pathological changes in brain; these mice died within 5 weeks of AA withdrawal. Thus, this lethal epileptic phenotype, associated with combined GSH and AA deficiency, implies that a significant and underestimated role of GSH-AA interaction occurs in the CNS under physiological conditions.

2160 OXIDATIVE STRESS PRODUCTS 4-HYDROXY-2-NONENAL AND MALONDIALDEHYDE ARE INHIBITORS OF CELLULAR ALDEHYDE DEHYDROGENASES AND REDUCTASES INVOLVED IN DOPAMINE CATABOLISM.

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Previous work demonstrated a role for oxidative stress in pathogenesis of Parkinson Disease (PD), and it has been proposed that a toxin endogenous to dopamine (DA) neurons yields selective neurodegeneration. In this study, it is hypothesized that oxidative stress products inhibit metabolism of a toxic and reactive intermediate of DA catabolism. Incubation of cellular aldehyde dehydrogenase (ALDH) and aldehyde reductase (AR) by oxidative stress products 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA) was studied in PC6-3 cells. The highly toxic product of DA catabolism, 3,4-dihydroxyphenylaldehyde (DOPAL) is generated from DA by mitochondrial monoamine oxidase and oxidized to 3,4-dihydroxyphenylacetic acid (DOPAC) or reduced 3,4-dihydroxyphenylethanol (DOPET) by ALDH and cytoplasmic AR, respectively. In this study, differentiated PC6-3 cells (6x10^5) were treated with various concentration of 4-HNE or MDA (0, 2, 10, 25 and 100 μM) in the presence of dopamine for 1 hr at 37°C and DA metabolites were analyzed using HPLC. Results showed concentration-dependent increase of DOPAL and decrease of DOPAC in both groups suggesting inhibition of ALDH activity by the lipid aldehydes. However DOPET was elevated only in 4-HNE group but not for MDA, suggesting MDA might be also an inhibitor of AR. To study the effect of MDA on AR, the cytosolic fraction (1mg/ml) containing AR from undifferentiated PC6-3 cells was incubated with DOPAC (10μM) and NADPH (1mM) for 1 hr at 37°C in the presence of MDA (0, 2, 10 and 25μM) or 4-HNE (25μM). The result showed concentration-dependent inhibition of enzyme activity in MDA-treated cells and effect was significant even at the lowest concentration of aldehyde tested (2μM) with 35% inhibition of AR but not by 4-HNE (25μM). In summary, these data demonstrated the dramatic effect of lipid peroxidation products, 4-HNE and MDA on DA catabolism as both aldehydes can generate aberrant level of endogenous toxin DOPAL, which may contribute to development of PD.

2161 UNCOUPLING PROTEIN 2 ENHANCEMENT OF CYANIDE-INDUCED NEUROTOXICITY: INVOLVEMENT OF MITOCHONDRIAL NITRIC OXIDE SYNTHASE.


Expression of uncoupling protein-2 (UCP-2) in brain is upregulated in a number of pathological conditions, including hypoxia, chemical-induced seizures and brain injury. In these conditions mitochondrial nitric oxide synthase (mtNOS) has been reported to be up-regulated. We have shown previously that UCP-2 up-regulation enhances cyanide-induced toxicity of dopaminergic cells. To elucidate the underlying mechanisms, a transgenic (tet)-inducible cell line was constructed in which UCP-2 can be overexpressed. After addition of tet, the protein level of UCP-2 was time and dose-dependently increased. This was accompanied by up-regulation of mtNOS as detected by Western blotting in isolated mitochondria. The mtNOS activity was determined by monitoring nitric oxide (NO) production and mitochondrial respiration. It is well known that NO can directly inhibit the respiratory chain (cytochrome c oxidase), resulting in decreased ATP generation and reduced membrane potential (ΔΨm). UCP-2 overexpression (tet-on) was accompanied by a marked increase of NO, which in turn reduced state 3 respiration. Activation of mtNOS was mediated by [Ca2+]i overload, since ruthenium red (RuRed), a specific inhibitor of the mitochondrial Ca2+ uniporter, blocked NO generation following induction of UCP-2. It was observed that UCP-2 overexpression cells were more sensitive to cyanide than non-induced cells, as reflected by mitochondrial dysfunction (decreased APM, ATP and mitochondrial swelling). RuRed and NO inhibitors (7-nitroindazole and L-NMMA) inhibited cyanide-induced cell death by blocking mtNOS activity and decreasing NO generation. It was concluded that mtNOS activation with NO generation plays an important role in mediating the enhanced sensitivity to cyanide in cells in which UCP-2 is overexpressed. (This study was supported by NIH grant ES04140).

2162 REACTIVE OXYGEN SPECIES PRODUCED BY MITOCHONDRIAL RESPIRATORY INHIBITORS POTENTIATE EXCITOTOXIC-INDUCED CELL DEATH IN ORGANOTYPIC HIPPOCAMPAL SLICE CULTURES.

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Two intensively investigated hypotheses of human neurodegenerative disease involve the role of mitochondrial dysfunction and the toxic actions of the excitatory neurotransmitter glutamate. We demonstrated increased cell death in organotypic rat hippocampal slices exposed for 24h to very low concentrations of NMDA plus rotenone, a broad-spectrum insecticide that inhibits complex I of the mitochondrial electron transport chain, (ETC). The increased cell death was synergistic at low NMDA concentrations and additive at higher concentrations and appeared to be receptor specific. Additionally, neuroprotection was obtained in the same culture system when mitochondrial toxins inhibiting ETC complexes II – IV were used concomitantly with NMDA. Inhibition of respiratory complexes within the ETC gives rise to oxidative stress and bioenergetic failure. Thus, in this study, we investigated the potential role of oxidative stress in the enhanced excitotoxicity of EUK 154, a salen-manganese complex that scavenges reactive oxygen species. Organotypic rat hippocampal slice cultures were exposed to rotenone (100 nM) concomitantly with NMDA (1 or 10 μM) in the presence of EUK 154 (20 μM) for 24h then assessed for cell death as determined by propidium iodide fluorescence. Separate experiments utilized antymycin A (100 nM, a complex III inhibitor) to investigate whether the response was complete I- or rotenone-specific. EUK 154 ameliorated cell death in slices exposed to ETC inhibitors plus NMDA. Complete protection was not observed suggesting that, although oxidative stress may contribute to cell death potentiation in this culture system, bioenergetic failure may also play a role. This further suggests that a combinatorial therapeutic approach may be required to ameliorate the potentiated cell death.

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2163 NEUROTOXIC IMPLICATION OF GLIAL INFLAMMATORY RESPONSE BY HISTONE DEACETYLASE INHIBITORS.


Histone deacetylase inhibitors (HDACi) have currently entered clinical trials for hyperproliferative disorders due to their pro-apoptotic and anti-inflammatory effects, but still very little is known about their activity towards nontumorous cells. In the present study we evaluated whether the HDACi, Trichostatin A (TSA), induces cytotoxicity and regulates the inflammatory response in a neutral model of inflammation in vitro. In this purpose, primary glial cells were exposed to lipopolysaccharide (LPS) 10 ng/ml with or without TSA. Cytokines release was measured by ELISA and/or by an appropriate biological assay. TSA did not affect glial viability at any of the concentrations used (0.01-200 nM), but it dose-dependently enhanced LPS-induced release of both Interleukin-1β (IL-1β) and Tumor necrosis factor-α (TNF-α) from glial cells, and significantly reduced the production of the anti-inflammatory cytokine interleukin-10 (IL-10). This unexpected effect occurred only in glial cells and was maximal at 10 nM, whereas TSA significantly reduced the release of pro-inflammatory cytokines from LPS-stimulated primary macrophages. The enhancement of TNF-α release from glia was maximal when LPS and TSA (0.1-100 nM) were applied simultaneously, but it was less pronounced if glia was pre-treated for 1h with TSA and TNF-α was measured by biological assay. Biological assays differ from ELISA, since they detect only the cytokines that have been processed to be biologically active. These results suggest that TSA enhances LPS-induced release of proinflammatory cytokines but also interferes with the pro-inflammatory cascade of pro-TNF-α to its mature form. The pro-inflammatory setting triggered by TSA is specific for glial cells, suggesting that histone acetylation may participate in sustaining the inflammatory response associated with a variety of neurodegenerative/neurotoxic conditions.

This work is supported by EC grant LSHM-CT-2006-037-498.
DIFERENTIAL RESPONSE OF BRAIN AND LIVER FREE FATTY ACIDS FOLLOWING ADMINISTRATION OF A SYNTHETIC ANALOG OF CERULENIN, C-75 IN CD-1 MICE.

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A potential drug for the treatment of obesity, C-75, has a stimulatory effect and as C-75-CoA derivative, an inhibitory effect on carnitine palmitoyltransferase I (CPT 1), the main regulatory enzyme in fatty acids oxidation. In rodents, C-75 has been shown to reduce body weight by inducing anorexia. The mechanisms involved in C-75 metabolic actions altering feeding behavior are unclear. Here, we examined the response of brain and liver free fatty acids (FFAs) in adult CD-1 mice injected i.p. either with saline (control) or C-75 at 20 mg/kg. Mice were sacrificed 1, 2, 4 and 24 hrs following the injection to harvest whole brain and liver. Long chain FFAs were extracted with chloroform and methanol (4:8 v/w) from tissue homogenates and the extracts were shaken, followed by centrifugation. The supernatants were reconstituted with ethanol (3:2, 4, v/w) and the chloroform was then evaporated under a stream of nitrogen. The residue was reconstituted with ether-hexane (50:50, v/v) and eluted by column chromatography on acid-washed Florisil. FFAs were derivatized with BF3/methanol and fatty acid methyl esters quantitated by gas chromatography. While concentrations of saturated FFAs in the liver and brain did not change following the C-75 injection, unsaturated brain FFAs were progressively increasing. The level of C-14:1 (myristoleic acid) near doubled from control level 0.63 ± 0.1 mg/g protein to 1.06 ± 0.1 mg/g protein, respectively, 24 hrs following treatment. Preliminary data suggest a regulatory role of brain unsaturated fatty acids in C-75-induced anorexia.

CONTINUOUS INTRAVENOUS INFUSION IN THE MOUSE: A SUITABLE ALTERNATIVE SAFETY ASSESSMENT MODEL TO AVOID HISTAMINERGIC REACTION IN THE RAT?

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It is known that certain peptides, proteins and macromolecules elicit a histaminergic response in vitro and in vivo. Numerous studies have documented the influence of infusion rate on the severity and probability of histamine release from mast cells in animal species and in humans. The need for improvements in the development of vascular infusion systems for rodents will continue to increase as the use of mouse models increases and more parenterally delivered protein and peptide therapeutics enter development. The rat is probably the most commonly used animal for pre-clinical continuous infusion. However, the rat is a particularly sensitive species with regard to histaminergic reactions. Additionally, repeat intravenous (iv) bolus administration of drugs which elicit histaminergic-type reactions pose a particular problem in rodents, as infiltration of an irritant solution around the tail vein (peri-vascular) can potentially lead to necrosis and make further injections difficult or impossible. A mouse continuous vascular infusion study was conducted, using a test article that was histaminergic in the rat. Pharmacokinetic and other data are presented from this study. The mouse model proved a viable alternative technique for avoiding histaminergic reaction in the rat.

HAEMATOLOGY AND SERUM CHEMISTRY PARAMETERS IN CYNO-MOLGUS MONKEYS (MACACA FASCICULARIS): COMPARISON BETWEEN PURPOSE-BRED AND CAPTURED ANIMALS.


Sponsor: M. Brighza.

Although the large majority of non-human primates used nowadays for biomedical research are purpose-bred, there might still be a few ‘nicks’ for which older and captured animals are required. Blood samples from purpose-bred and captured cynomolgus monkeys (Macaca fascicularis) originated from the same geographic area and kept under the same environmental and feeding conditions were collected, and standard hematologic and serum biochemistry parameters examined and compared. Statistically significant differences were observed in some parameters, particularly in eosinophils, total protein, globulins, alanine amino transferase and creatinine. Mean values for these parameters were always higher in captured animals than in purpose-bred monkeys. In addition eosinophils, large unstained cells, creatinine, globulins, alanine amino transferase, total proteins and globulins also showed significant differences between genders. Relevant differences present in both sexes, but without a statistical significance, were observed in basophils and monocytes. Fibrinogen, aspartate amino-transferase, alkaline phosphatase, g-glutamyl transferase, lactate dehydrogenase, a-hydroxybutyrate dehydrogenase, iron, potassium and phosphorus generally showed statistical significance differences only in one sex. From the analysis of this data it was concluded that significant differences in basal values might be observed in clinical pathology results in animals with different breeding background, and this should be carefully considered in pharmacototoxicological studies. Particularly, experimental data have to be compared from those obtained in the same animals before any pharmacological treatment, or with those filed in historical database and obtained from animals characterized by the same breeding background. After years of experience in performing preclinical studies in monkeys, Acceleras has build a robust database for the control of experimental data.
CONTINUOUS BLOOD PRESSURE MEASUREMENT IN CONSCIOUS, UNRESTRAINED CYTOMEGALOVIRUS MONKEYS VIA AN IMPLANTED PA-C10 (MOUSE) TRANSMITTER.

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Accurate determination of arterial blood pressure (BP) in conscious, unrestrained monkeys currently entails use of invasive telemetry techniques. In this study, the functionality and tolerability of a less invasive method was evaluated. A mouse BP transmitter (DMSH model PA-C10) was surgically implanted into a femoral artery of a male and a female cytomegalovirus monkey. BP and ECG data were collected continuously by jacketed external telemetry (JET) for at least 24 hours bi-weekly for 15 weeks. Evaluated were surgical implantation site, clinical pathology (CP) data, quality of BP signals, data dropout, systolic, diastolic, mean arterial pressure, and pulse rate. After completion of the collection period, animals were euthanized and the femoral arteries and portions of the quadriceps muscles were evaluated macroscopically and microscopically. The incision sites in the inguinal area healed uneventfully. CP results obtained on Days 48 and 93 indicated no health abnormalities. BP signals were of excellent quality during light/dark cycles and did not interfere with ECG signals. For BP data, 1-minute averages were calculated and were excluded from further analysis when signal dropout was evident in the respective minute. Average data dropout due to unfavorable positioning of the animal was 32%. No device-related macroscopic findings were noted. Expected microscopic findings consistent with the presence of an indwelling catheter, such as thickening of the intima and disruption of the internal elastic lamina of the arterial wall were found in both animals. No macroscopic or microscopic findings were noted in the muscle samples. In conclusion, implantation of a mouse PA-C10 BP transmitter using a minimally invasive technique provided reliable BP data for at least 24 hour intervals with insignificant data dropout in conscious, unrestrained non-human primates with no adverse histological findings. This novel technique could be a viable option for high quality ECG and BP measurements in toxicology studies up to 15 weeks in length.

AMIODARONE EXPOSURE DURING MODERATE INFLAMMATION INDUCES IDIOSYNCRASY-LIKE LIVER INJURY IN RATS.

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Idiosyncratic adverse drug reactions (IADRs) depend on sensitivity factors within the host. Amiodarone (AMD), a class III antiarrhythmic agent effective in treating atrial fibrillation, is known to cause idiosyncratic, hepatotoxic reactions in human patients. One hypothesis for the etiology of IADRs is that a concurrent inflammatory stress results in decreased threshold for drug toxicity, causing liver injury at an otherwise "safe" dose. To test this hypothesis, lipopolysaccharide (LPS), a cell wall component of Gram negative bacteria, was administrated to animals to induce low-level inflammation. Male rats were treated intravenously with nonhepatotoxic doses of LPS or its vehicle, and AMD or its vehicle. Liver injury, as marked by increases in serum alanine aminotransferase (ALT) activity and by midzonal hepatocellular necrosis in H&E stained liver sections, was observed only in groups of animals treated with both LPS and AMD. Dose-dependent mortality was observed in the LPS/AMD group with AMD doses greater than 60 mg/kg. The time of AMD administration relative to LPS was critical to the development of liver injury: AMD injected 2h after LPS resulted in a significant increase in ALT activity, whereas AMD injected 2 to 4h before or 4h after LPS failed to cause this response. These results demonstrate that AMD treatment during modest inflammation can induce severe hepatotoxicity in rats, a response resembling IADRs in human patients. (Supported by NIH GM075865.)

ROLE OF CANNABINOID RECEPTORS 1 AND 2 IN OVA-INDUCED ALLERGIC AIRWAY RESPONSES IN C57BL/6 MICE.

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In previous studies, mice deficient in cannabinoid receptors (CB1-/-CB2-/-) experienced exacerbated immune reactions in models of in vivo contact dermatitis as well as in response to influenza challenge. Thus we hypothesized that CB1-/-CB2-/- (KO) mice are more prone to develop an ovalbumin (OVA)-induced airway hypersensitivity reaction compared to WT mice. The purpose of this study was to assess whether KO mice are predisposed to develop allergic airway disease using a model of OVA-induced airway hypersensitivity. Female C57Bl/6 wild-type (WT) and KO mice with a CB1-/-CB2-/- background were intranasally sensitized with 30μl of 1% OVA in saline or saline alone (SAL) for 4 consecutive days. On days 14 and 21 after sensitization, mice were challenged with OVA or SAL. Mice were sacrificed 6 or 96 h after the last challenge. Blood and bronchoaveolar lavage fluid (BALF) were collected for IgE antibody and cellular analyses. Left lungs were fixed in 10% neutral buffered formalin and processed for microscopy. In response to OVA-instillation, an influx of neutrophils with a lesser number of eosinophils was observed 6 h post challenge in the BALF. An influx of eosinophils was further increased at 96 h, whereas neutrophil counts were decreased. Morphometric analysis of the interstitium revealed an increase of eosinophils in OVA-treated groups at 6 and 96 h, in both genotypes. Significant intraepithelial mucus production was detected at 96 h in OVA-treated mice. Also, elevated OVA-specific IgE was observed with no differences between OVA-instilled WT and KO mice, with the exception of lower plasma IgE levels in KO compared to WT mice. These findings suggest that the absence of CB1 and CB2 does not alter the response to OVA in a model of allergic airway disease. (Supported by MSU Respiratory Research Initiative, NIH: DA07908, NIH: T35RR017491.)

A TECHNIQUE FOR SUBRETINAL ADMINISTRATION IN RABBITS.

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Subretinal administration of therapeutic agents can be advantageous to localize exposure close to a discrete region within the retina, or when a local depot delivery is required for slow release in the eye. The subretinal injection procedure was optimized for dose volume/placement and minimal injury to the retina: 15 New Zealand White rabbits underwent subretinal injections with/or without partial vitrectomy. Animals were anesthetized with ketamine/xylazine and the eye exposed by a lateral canthotomy and pseudo-proptosis. A topical anesthetic and mydriatic were applied and the eye was hydrated throughout the procedure. Under a surgical ophthalmic microscope, a 25G fiberoptic light source was inserted for visualization of the retina. A 41G needle, microextension tube and 1cc syringe were used to administer the subretinal injection via a 20G sclerotomy. In some animals, prior to subretinal injection, a partial vitrectomy was performed with the integrity of the globe maintained by a BSS+ infusion. The injections were administered in one or two phases. A bleb was created by either a direct 50 to 150 μL injection of methylene blue (MB) solution (for visualization) to different regions of the retina, or a small saline bleb was created to cause the initial lift of the retina, followed by inflation of the bleb with MB solution. The most successful blebs were located in the vicinity of the optic disc or myelinated nerve fibers. Bleb creation was more difficult as distance from the optic disc increased. The vitrectomy did not provide any advantage for larger volumes of administration as blebs up to 150 μL were successfully created without it. Using saline to first lift the retina, provided some advantage against reflux that could occur during direct injections, however, on several occasions this method proved to be worse with the low viscosity solution used. The injected liquid resided within 1-2 days. Retinal histology of the bleb did not reveal any changes except at the discreet point of needle entry. Overall, the best technique was the direct injection without vitrectomy, near the optic disc.

CLINICAL PATHOLOGY REFERENCE INTERVALS IN CYTOMEGALOVIRUS MACAQUE (MACACA FASCICULARIS): ERYTHROCYTE INDICES DIFFER BETWEEN INDOENSIAN, INDOCHINESE AND MAURITIUS POPULATIONS.


The cynomolgus macaque accounts for the majority of nonhuman primate importations for biomedical, pharmacology and toxicology research. The species can be classified into three genetically diverging populations according to geographical region of origin: 1) Indochina (mainland), 2) Insular Asia (Philippines, Malaysia, and Indonesia), and 5) Mauritius. Phenotypic heterogeneity between populations has been documented and must be taken into consideration when designing and interpreting toxicology studies. Population differences have been noted for erythrocyte counts (RBC) and mean erythrocyte size (mean corpuscular volume, MCV), among others. However, biochemistry, hematology and hemostasis reference intervals for each subspecies have not been defined. The purpose of this study was to assess biological variation and establish reference intervals for hematologic, hemostasis, and chemical pathology parameters in cynomolgus monkeys imported from China, Vietnam, Cambodia, Indonesia, and Mauritius. Reference intervals and
**2174 TUMOR GROWTH OF NEGATIVE AND POSITIVE CONTROL CELL LINES FOR TUMORIGENICITY STUDIES IN IMMUNOCOMPROMISED MICE.**

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Tumorigenicity studies for cell lines used to produce biologic therapeutics are required by regulators to assess risks of cell therapies intended for human use. U.S. guidelines require the use of reference cell lines as positive controls to increase confidence in the validity of the results. Therefore, we investigated the tumorigenicity and tolerability of reference cell lines following administration to two strains of immunocompromised mice [NOD/NCr-Prkdcscid (NOD/SCID) and C57BL/6nu (nu/nu)]. Mice were given a single subcutaneous dose of positive control cell lines HT-1080 cells (1 x 10^6), PC-3 cells (2.5 x 10^6), or the negative control cell line MRC-5 (1 x 10^6). Viability, clinical observations, body weights, palpable mass observations, necropy evaluations and histopathology were performed; surviving mice were sacrificed on study day 61. The MRC-5 cells were not tumorigenic in either mouse strain, confirming the utility of this cell line as a negative control. No tumors were seen at the lowest dose of HT-1080 cells. At higher doses, HT-1080 cells caused mortality, tumors and additional signs of toxicity in NOD/SCID mice and mortality and tumors in nude mice. Neither dose of the PC-3 cells caused mortality or macroscopic changes in either mouse strain. However, both doses caused signs of toxicity in the NOD/SCID mice and tumors in both mouse strains. Both cell lines produced larger tumors at the primary site in NOD/SCID mice, as compared with nude mice at the same cell doses. The low dose of PC-3 cells induced only a single tumor in one nude mouse, but produced tumors in all NOD/SCID mice. The high doses of both HT-1080 and PC-3 cells consistently produced tumors in both mouse strains. In conclusion, MRC-5 cells can be used as a negative control and both HT-1080 and PC-3 cells can be used as positive controls in either mouse strain with careful consideration to study design, mouse strain and dose.

**2175 THE INFLUENCE OF A HIGH SALT DIET ON A RAT MODEL OF ISOPROTERENOL INDUCED HEART FAILURE.**


Salt models of heart failure (HF) share varied pathology and time to disease outcome, dependent on induction method. We found that subchronic (4 weeks) iso- proterenol (ISO) infusion exacerbated cardiomyopathy in Spontaneously Hypertensive Heart Failure (SHHF) rats. Others have shown that dietary salt loading induces HF in spontaneously hypertensive (SH) rats without acute injury. We hypothesized that ISO and salt combined would induce injury and more overt HF in SHHF rats than either treatment alone. Rats (male, 90d) were infused for 4 weeks with ISO (2.5mg/kg/d.s.c.) or saline via osmotic pump. Starting 2 weeks into the infusion regimen, rats were fed high-salt diets (4, 6, or 8% NaCl) for 6 weeks. We previously reported that relative to the controls, the 8% salt diet increased heart mass, heart failure markers (plasma B-type natriuretic peptide, IL-6), lung lymphocytes, and indicators of lung injury and edema (bronchoalveolar albumin and protein), while it increased urine pro-arrhythmic natriuretic peptide relative to control treatment. In the present study we examined the cardiac effects of ISO and salt diet on histopathology and markers of acute injury. ISO caused 29% lethality while high-salt diet had no effect on mortality. ISO-treated rats that died prematurely had elevated serum levels of heart fatty acid-binding protein (H-FABP) and cardiac troponin I (cTnI). At 4 weeks post-infusion, there was no effect of ISO and/or salt diet on cTnI or H-FABP. Preliminary histopathologic analysis indicates that high salt diet may have exacerbated cardiomyopathy in normal SHHF rats, but the effects of salt on ISO-treated SHHFs was unclear. Further examination of physiologic and histologic effects may conclusively determine if a high salt diet exacerbates ISO-induced heart failure in the SHHF rat. (Abstract does not reflect EPA policy. Supported by UNC/EPA CR8352601).

**2176 MOUSE MODEL FOR TREATING METASTATIC HUMAN OVARIAN CANCER WITH HYPERTHERMIC INTRAPERITONEAL CHEMOTHERAPY.**

V. A. States, J. H. Masters, C. S. Muensl, and J. Helm

Ovarian cancer is 4th leading cause of cancer death in women in the U.S. Diagnosis is usually at late stage when the cancer has metastasized within the peritoneal cavity. Frontline therapy consists of cytoreductive surgery followed by chemotherapy with platinum-based drug and a taxane. The newest modality of chemotherapy includes intraperitoneal lave with hyperthermic cisplatin solution. Platinum resistance limits the effectiveness of chemotherapy and results in recurrence of platinum resistant tumors in over half the patients. This platinum resistant disease eventually kills the patients. Hence, there is an urgent need for efficacious therapy and an in vivo model to test new therapeutic regimes. We have developed a mouse xenograft model for metastatic, platinum resistant human ovarian cancer. Methods for intraperitoneal injection of the cancer cells were refined to promote formation of multiple (<10) small tumors less than 5 mm in diameter. Mice were scanned by microCT to determine the size and location of the tumors which were confirmed upon autopsy. Tumor bearing mice were treated with a combination of cisplatin, arsenic, and hyperthermia for 60 min by continuous flow intraperitoneal chemotherapy. The tumors were collected immediately after and 24 h after treatment. Platinum accumulation and retention in the tumors was determined by ICP-MS. Preliminary data suggest that platinum is readily accumulated and retained in the tumors exposed to cisplatin either alone or in combination with arsenic and/or hyperthermia. Expression of P53 and several p53 regulated proteins involved in DNA damage response was determined by western blot of tumor extracts. These results suggest that the model will be useful for testing of new combination chemotherapies and for determining modulation of tumor responses related to cisplatin resistance. Supported in part by USPHS grants ES011314 and ES014443 and a grant from University of Louisville School of Medicine.

**2177 A RAT MODEL OF IDIOSYNCRATIC HEPATOTOXICITY: CONVERTING CARBAMAZEPINE TO A HEPATOTOXICANT.**


The liver is the most common serious target organ for drug-related idiosyncratic toxicities, which are currently difficult to predict and are the leading cause for black-boxed and withdrawn drugs. Oxidative stress/ reactive metabolite (OS/RM) - sensitive gene expression, together with covalent binding and glutathione conjugates, can be used to screen for OS/RM, which may be toxic in susceptible patients. Many of OS/RM compounds, such as carbamazepine, are safe at high doses preclinically (LD>4000 mg/kg). A hypothesis for idiosyncratic hepatotoxicity is that some patients to relatively safe drugs. Roth et al at Michigan State have used a low dose lipopolysaccharide (LPS) pretreatment to convert safe drugs to hepatotoxi- cants in rodents. Many classical hepatotoxicants and NSAIDs produce Kupffer cell immune cell activations, as occur in ongoing inflammation/ infection, sensitizes some patients to relatively safe drugs. In the hope of getting a more reproducible conversion of carbamazepine into a hepa- totoxicant, dextran sulfate sodium (DSS) was used to increase endogenous endo- toxin absorption from the gut, which potentiates direct hepatotoxic effects of OS/RM compounds. Intravenous low dose LPS converted carba- mazepine into a hepatotoxicant, but only in some rats. The sensitive rats showed gene responses suggesting that LPS blocks induction of many protective OS/RM-sensitive genes, as well as repressing several metabolism and transporter genes. In the hope of getting a more reproducible conversion of carbamazepine into a hepa- totoxicant, dextran sulfate sodium (DSS) was used to increase endogenous endo- toxin absorption from the colon. However, no conversion of carbamazepine was observed after DSS (24 hours or four days, 5% in drinking water), and instead there was a potentiation of the protective OS/RM gene responses, suggesting de- sensitization of Kupffer cells in this model, as reported previously. Further study is required to improve and understand conversion of non-toxic OS/RMs, such as carba- mazepine, into idiosyncratic hepatotoxicants.
2178 2-ACETYLMONOFUROENE EFFECTS ON CELL PROLIFERATION AND DNA ADDUCT FORMATION IN LIVERS OF LEAN AND DIET-INDUCED OBESE MICE.

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Non-alcoholic fatty liver is a risk factor for hepatocellular carcinoma. To explore possible mechanisms for this susceptibility, the effects of the activation-dependent DNA-reactive hepatocarcinogen, 2-acetylmofuorene (AAF) were studied in lean (conventional) C57BL/6 (LEAN) and diet-induced obese DIO C57BL/6 (DIO) male mice, 6 months old at study initiation. AAF was administered by gavage at 2.24 (LD) or 22.4 (HD) mg/kg (3 x week: Mon, Wed and Fri) for 31 days. Control male LEAN and DIO mice received 12450B (10% Kcal from fat) and 12492 (60% from fat) diets (Research Diets Inc.), respectively. The rate of hepatocellular proliferation (replicating fraction or RF) was assessed by scoring of the immunohistochemically stained nuclear DNA (improved MIB-1 antibody). The mean RF of the DIO control group (3.55 ± 0.65) was higher (p<0.05) compared to LEAN controls (2.72 ± 0.84), and the mean RF in both DIO (p<0.05) and LEAN (p=0.06) groups administered AAF was lower compared to respective controls, possibly reflecting the block to DNA synthesis produced by adduct formation. In the LEAN groups, the RF reduction was dose proportional (RF 2.21 in LD and 2.07 in HD), but not in the DIO AAF groups (RF 2.25 in LD and 2.48 in HD). AAF adducts were increased similarly and in a dose proportional manner in both LEAN and DIO AAF groups, reaching 10 adducts in LD and 218 in HD in DIO mice and 84 in LD and 189 in HD DIO mice. We interpret these findings to suggest that a factor in predisposing fatty livers to cancer development is increased cell proliferation, but that enhanced activation of a carcinogen may not be involved.

2179 GENDER-DEPENDANT Reactive oxygen species-mediated neurodegeneration in untreated aged acatalasemic mice.

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Oxidative stress occurs when the formation of reactive oxygen species (ROS) exceeds the detoxifying capacity of antioxidative enzymes like catalase, which metabolizes hydrogen peroxide. Neurodegenerative deficits may result from endogenous or xenobiotic-enhanced formation of ROS, which oxidatively damage cellular macromolecules and/or alter signal transduction. Reduced antioxidative activity, due either to a genetic deficiency and/or a progressive loss of antioxidative activity with age, may accordingly contribute to neurodegeneration associated with normal aging and various degenerative diseases. We have previously shown in utero that acatalasemic embryos are more susceptible to the pathogenic effects of endogenous ROS. Herein, we use aging catalase-deficient (C3Ga.Cg-Catb/J) acatalasemic mice and wild-type catalase-normal controls, aged 18 to 24 months, to determine what role any acatalasemic plays in preventing the brain from ROS-mediated, age-dependent motor coordination deficits quantified using a rotarod test. Aged female acatalasemic showed a substantial 64% loss in motor coordination compared to catalase-normal controls as demonstrated by decreased latency to fall (40 vs. 110 sec respectively, p<0.001). In contrast, aged male acatalasemic mice were not different from the male catalase-normal controls. This gender specificity is consistent with results from mice oxidatively stressed in utero with methamphetamine. These results provide the first evidence that catalase plays an important neuroprotective role with aging, and suggests that catalase deficiencies may constitute a risk factor for neurodegenerative outcomes.

2180 COMPARATIVE INTRAOCULAR PRESSURE MEASUREMENTS IN MULTIPLE SPECIES USING 2 HANDHELD TONOMETERS.

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Elevations or reductions in intraocular pressure (IOP) can play a significant role in ocular toxicity observed during ocular or systemic non-clinical toxicity studies. This effect has been well documented in glaucoma patients where control of IOP does not always prevent continued retinal degeneration. The data presented compares IOP measurements from 2 commonly used handheld devices, the Tonopen XL and the TonoVet, in the nasal cavity, esophagus, kidneys, spleen and trachea were considered due to any role catalase plays in protecting the brain from ROS-mediated, age-dependent neurodegenerative outcomes.

2181 TOXICITY OF ORALLY ADMINISTERED POLYETHYLENE GLYCOL (PEG-400) VEHICLE FORMULATIONS IN TRANSGENIC MICE.


The gavage route of administration of pharmaceuticals is commonly used in pre-clinical studies to mimic the intended clinical route and to not disturb the systemic exposure of the compound. Many pharmaceutical agents intended for oral administration require PEG-400 for solubilizing the drug but this may become problematic if the vehicle itself demonstrates toxicity. The toxicity of PEG-400 via the oral route is variably documented in the scientific literature. Characterizing these toxic effects will facilitate efficient interpretation of the toxicity data of formulations containing PEG-400. This study investigated the toxicity of PEG-400 Vehicles administered by gavage (10 mL/kg) daily for either 28 consecutive days in C57BL/6 and C57BL/6 wild-type mice (10 mice/sex/formulation) or for 26 weeks in p53+/−, C57BL/6 and Tg.rafH2 transgenic mice (25 mice/sex/formulation) that were used in short-term carcinogenicity studies. The Vehicle formulations contained between 10-100% PEG-400. Animals were observed during the course of the study for clinical sign, moribundity, mortality, body weights, food consumption, organ weight, histopathology. Daily administration of up to 100% PEG-400 for 28 days showed no significant toxicity in C57BL/6 or C57BL/6 mice. However, daily dosing of 20% and 100% PEG-400 Vehicles over 26 weeks led to 8-17% mortality in p53+/−, C57BL/6 and Tg.rafH2 mice. As compared to the sham control, in these animals there were significant decreases in body weights and food consumption. There were no treatment-related effects on organ weights. Microscopic abnormalities in the nasal cavity, esophagus, kidneys, spleen and trachea were considered due to PEG-400. In conclusion, oral administration of 26-100% PEG-400 formulations showed no adverse toxic effects when administered to mice daily for 28 days, however, it caused significant toxicity when administered for longer period in 26 week carcinogenicity studies using transgenic mice. There were no PEG-400 treatment-related neoplastic lesions noted in these studies.

2182 ADOPTIVE TRANSFER OF B-LYMPHOCYTES IN A MOUSE MODEL OF CHEMICAL-INDUCED ASTHMA.


To investigate the underlying mechanisms of an established model of chemical-induced asthma, we transferred B-lymphocytes from sensitized mice to naïve mice. BALB/c mice where dermally sensitized with 0.3% TDI (20 μl/ear), on day 1 and 8. On day 15, mice were sacrificed and the auricular lymph nodes isolated. B-lymphocytes (CD19+) where separated from the whole cell suspension and 175,000 cells where injected in the tail vein of naïve mice. Three days later, these mice received a single oropharyngeal challenge with 0.01% TDI (20 μl) or vehicle (acetone/olive oil) (controls). Airway reactivity to methacholine (resistance assessed by forced oscillation method, Flexivent) and total and differential cell counts in the broncho-alveolar lavage (BAL) fluid were measured 24 hours after challenge. Mice that received B-lymphocytes from TDI-sensitized mice showed a 3-fold increase in methacholine reactivity and an 8-fold increase in BAL neutrophils after challenge with TDI compared to those challenged with vehicle. Transfer of monkeys to the whole cell suspension without CD19+ lymphocytes (325,000 cells) resulted in a less pronounced airway reactivity to methacholine (1.5-fold) and a lower influx of neutrophils (1.5-fold) in the lungs. We were able to passively sensitize naïve mice with B-lymphocytes from sensitized mice. Following passive sensitization and an airway challenge, these mice showed an “asthmatic” response. These data suggest an important role for B-lymphocytes beside the already known important role of T-helper lymphocytes in chemical-induced asthma. In future experiments the role of other cell types will be determined.
2183 FEASIBILITY AND VARIABILITY OF TESTICULAR VOLUME MONITORING IN THE MARMOSET MONKEY (Callithrix jacchus).


Marmosets represent a New World primate species that is being used in preclinical safety evaluations including assessment of toxicity to male fertility. Although the andrological regulation of testicular function is different between Old World and New World monkeys, the histomorphology of the germinal epithelium is comparable to human testis including the arrangement of spermatogenic stages (2). Longitudinal testicular volume determination provides a convenient parameter for monitoring testicular toxicity. The present work evaluates the feasibility of testicular volume (TV) measurements in the marmoset model in the context of toxicity studies. TV was determined in sedated animals using calipers and the formula for a regular ellipsoid. Data for both tests were collected from 60 mature and naive animals, weighing 240-410 gram, on 2 or 3 occasions in weekly intervals. Average combined TV for all animals was 0.62 mL ± 0.13 mL (SD) without a statistically significant difference between left and right tests (P = 0.48). The intra-individual coefficient of variation of 2-3 determinations was 22 ± 0.05 % (range 14 – 35 %). In comparison, variability of this parameter in over 100 cynomolgus monkeys for 3 repeated caliper determinations was 15% (3). Comparison of baseline and terminal TV determinations in 13-week and 26-week toxicity studies did not yield significant differences. Testis volume and weight for 56 animals at necropsy was highly correlated (R < 0.0001, Pearson R = 0.68) but was markedly different regarding absolute values: 1.15 ± 0.18 gram vs 0.58 ± 0.15 mL. This data indicate a high precision but low accuracy of testicular volume determination with an approximate 50% underestimation of actual testicular weight by caliper-based testicular volume. Testicular weight correlation with body weight was weak (R-squared = 0.15). In conclusion, caliper-based determination of TV in the marmoset is feasible and can be applied in acute and chronic toxicity studies and male fertility studies. M. Luetjens et al. Biol Rev 80: 475 (2005); G. Weinbauer GF et al. Toxicol Sci 84: 194 (2005).

2184 EFFECTS OF ORAL ALUMINUM EXPOSURE ON NEUROGENESIS AND BEHAVIOR IN A MOUSE MODEL OF ALZHEIMER DISEASE.

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Although the neurotoxic effects of oral aluminum (Al) in humans and animals are well known, the relationship between Al and Alzheimer disease (AD) is still a controversial issue. In a previous study, we administered for 4 months low Al doses to wild and transgenic (Tg 2576) mice carrying the Swedish mutation for familial AD. An impairment in learning and memory in a complex water maze task was only observed in wild mice. It suggested Al impair cognition in non vulnerable subjects, but not an acceleration of AD at early stages. In the present study, we tested a higher Al dose for a more prolonged period of time (6 months). Tg 2576 and their respective wild type mice, were exposed through the diet to 0 and 11g/kg of Al lactate from 6 to 12 months of age. At 11 months of age, learning and memory were evaluated in the water maze task. After behavioral testing, mice were treated with bromodeoxyuridine (BrdU) to study cell proliferation survival and differentiation in the dentate gyrus of the hippocampus. At sacrifice, brain cortex and hippocampus samples were obtained to measure β-amloid concentrations in the cortex of these animals. In conclusion, exposure of Tg mice to Al lactate during 6 months impaired cell survival in hippocampus, increased β-amloid 1-40 levels, and impaired acquisition and retention of the water maze task. It indicates that exposure to high Al doses for a prolonged period of time can accelerate cognitive impairment in vulnerable subjects.

2185 THE USE OF THE TG.RASH2 MOUSE IN CARCINOGENICITY STUDIES.


The objective of the study was to evaluate the response of the Tg.rash2 mouse test system to two known carcinogens and to demonstrate its utility as a quicker and more cost-effective alternative to the conventional two-year mouse bioassay. Fifteen (15) male and 15 female Tg.rash2 mice were assigned to each of 3 dose groups. Animals of Group 1 received purified water by oral gavage once daily for 26 consecutive weeks. Animals of Group 2 received a total of 3 intraperitoneal injections of Urethane (1000 mg/kg each) at 2-day intervals, while animals of Group 3 received a single intraperitoneal injection of MN1 (MN1U - N-methyl-N-nitrosourea) at 75 mg/kg. Parameters monitored during the study included mortality, clinical observations, including examinations for the presence of palpable masses, body weights and food consumption. Upon completion of the 26-week treatment/holding period, all surviving animals were euthanized and subjected to a necropsy examination. Subsequently, tissues collected from all animals were examined histopathologically. A total of 2 Control, 28 Urethane-treated and 23 MN1-treated animals died or were preterminally euthanized due to poor clinical condition during the study. Bronchiolo-alveolar adenoma and/or carcinoma and hemangiosarcoma of the spleen were considered to be the cause of mortality/morbidity in the majority (26/28) of the Urethane-treated animals, while malignant lymphoma of the hemolymphoreticular tissue was considered to be the cause of mortality/morbidity in the in the majority (17/23) of the MN1-treated animals. In conclusion, the intraperitoneal administration of 2 known carcinogens (Urethane and MN1) to Tg.rash2 produced a clearly higher incidence of benign/malignant tumors resulting in significantly higher mortality rates among the animals treated with the carcinogens in comparison to Controls.

2186 HUMAN CONSTITUTIVE ANDROSTANE RECEPTOR/CARR SUPPORTS THE HYPERTROPHIC BUT NOT THE HYPERPLASTIC RESPONSE TO THE MURINE NON-GENOTOXIC CARCINOGEN PHENOBARBITAL (PB) IN VIVO.

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PB is a non-genotoxic carcinogen that in mice induces hepatomegaly (characterised by hypertrophy and hyperplasia) and, following long-term treatment, hepatocellular tumours, possibly due to its ability to increase cell proliferation. The relevance of these tumours to human health is controversial due to the lack of a clear molecular mechanism and suitable human-like models. PB has been shown to activate the murine and human constitutive androstane receptors (CAR) and pregnane X receptors (PXR). CAR knock-out mice cannot activate the Cyp2b10 gene in response to PB, nor do the hypertrophic and hyperplastic responses elicited by PB occur. PB (80mg/kg ip., 4 days) was administered to double “humanised” CAR and PXR (huPXR/huCAR) mice and wild type C57BL/6j mice to investigate whether hyperplastic responses to chemicals observed in rodents are relevant to man. Mice which were devoid of both receptors (PXRKO/CARKO) were used as controls. Mice were implanted with osmotic pumps containing BrdU to allow determination of replicative DNA synthesis Relative liver weights were increased (to about 120% of controls) by PB in the wild type (WT) mice and “humanised” mice but not in the PXRKO/CARKO animals. P450 induction was observed in WT and huPXR/huCAR mice but not in the PXRKO/CARKO mice, as determined by catalytic activity measurements and immunoblotting. The nuclear incorporation of BrdU was determined as a measure of cell proliferation. PB increased the hepato-cellular labelling index (S-phase) by approximately 5-fold in the WT mice. However, no change in S-phase was detected in either the huPXR/huCAR or PXRKO/CARKO mice following PB treatment. These data suggest that the human receptors are able to support the chemically-induced hypertrophic response but not the hyperplastic response. These data, obtained using novel human response models, suggest that it is unlikely that exposure to PB poses an hepatocarcinogenic hazard to humans. This work was supported by ITI Life Sciences, Scotland.

2187 SEROTONIN TRANSPORTER DENSITY AND 5HT1A FUNCTIONING IN BTBR MICE — A MODEL OF AUTISM.


Autism spectrum disorders (ASD) are developmental psychiatric disorders affecting roughly 1 in 150 children. ASDs are complex disorders, likely arising from multiple genetic and environmental factors. However, a common finding from ASD studies in humans is dysfunction of central serotonin systems. Until recently, no suitable model for ASD has been unavailable and this has hindered progress in identifying underlying neural mechanisms. Now, the BTBR T + tf/J (BTBR) inbred mouse emerges as a promising model for translational ASD studies because it parallels all three behavioral traits used as clinical diagnostic criteria (McFarlane et al., Genes

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Brain Behav. 2008;7:152-63). To assess how the tone of the serotonergic (5-HT) system may differ in BTBR mice, we examined the density of serotonin transporters (SERT), and functioning of G-protein coupled 5-HT1A receptors by autoradiography. The SERT terminates serotonergic neurotransmission by high affinity uptake of 5-HT; while 5-HT1A receptors are major modulators of 5-HT neurotransmission. Fresh frozen brains of four-month-old male BTBR and C5710/J mice were sectioned on a cryostat and incubated in 1nM [3H] cyanoimipramine to determine SERT density. Sections were also used to determine 5-HT1A receptor binding capacity by measuring [35S]GTPγS binding stimulated by the agonist 8-OH-DPAT. We found that SERT density is significantly reduced by 20-30% throughout the hippocampus (N=8, p<0.05). Also, 8-OH-DPAT stimulated [35S]GTPγS binding is 28% higher in the hippocampus of BTBR mice than in C5710/J mice, and this difference is significant (N=8, p<0.03). Thus, both the main regulator and a primary modulator of serotonin neurotransmission differ between BTBR and C5710/J control mice, indicating potential dysfunctions of the BTBR 5-HT system. Thus the BTBR model may be useful for examining potential underpinnings of the disease, and the potential for genetics to interact with environmental factors to determine ASD symptom severity.

2188 KANAMYCIN INDUCES CHANGES IN AUDITORY BRAINSTEM RESPONSE (ABR) IN CYNOMOLGUS MONKEYS.


There are only a few reports of auditory brainstem response (ABR) in monkeys. Therefore, using female cynomolgus monkeys (<5-year old), we first tried to collect physiological data on ABR (Exp. 1), and then, we examined time-course changes in ABR following a single intravenous or intramuscular administration of kanamycin (KM) (Exp. 2). In Exp. 1, ABR was recorded under ketamine and xylazine mixed-anesthesia in 17 animals. Acoustic stimulation was given through a headphone attached to the ears using clicks. The stimulation frequency was 10 Hz, and the average of electrical signals was 1000 sweeps. The stimulus intensity was between 20 and 90 dB sound pressure level (SPL). At the stimulus intensity of 20 dB SPL, all the animals exhibited the wave. At 30 dB SPL, the amplitude of each ABR component declined only in the left-ear side at 2 hours after dosing. In addition, the ABR threshold of the left ear rose up to 30 dB after 2 hours, but returned to normal 8 hours later. In this animal, 300mg/kg KM was administered intramuscularly once again 1 week later. Similar changes in ABR were observed only in the left-ear side from 6 to 7 hours after dosing. In addition, the ABR threshold of the left ear rose up to 20 dB, but returned to normal 8 hours later. Since it is generally said that drug-induced auditory disturbances develop bilaterally, the KM-induced unilateral auditory disturbance as detected in this study is interesting judging from the results of this study, ABR is considered to be a useful method to examine abnormalities in auditory function in monkeys.

2189 HAZARD IDENTIFICATION OF POTENT DERMAL SENSITIZERS.

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Dermal sensitivity is of particular concern in the workplace for the substances that are potent sensitizers. The Local Lymph Node Assay (LLNA) is a useful tool to identify dermal sensitization hazards and to determine the relative potency, which aids in selection of effective safe handling guidance. 29 LLNA studies conducted according to the OECD 429 method on pharmaceutical or chemical process compounds were identified as potent dermal sensitizers, EC5 < 1%. The EC5 is the concentration at which the stimulation index of the experiment is greater than the vehicle control group. Certain parameters were examined to determine if there was any predictability to identify potent dermal sensitizers. These included structural alerts, molecular weight (MW) as well as the usefulness of the log-linear extrapolation approach for estimation of EC5. A computer structure activity analysis was conducted using Derek for Windows version 10.0.2. (2006) identified 14 as negative and 15 as positive for structural alerts, the most common being “hydrazine or precursor.” This indicates that many of the structural moieties responsible for causing a sensitization reaction are not known.

The examination of the MW showed that all 29 molecules had MW less than 550d. For 21 of the compounds, a follow up study was needed to calculate the EC5. The datasets from primary and follow up experiments were used to compare the intercalated vs. extrapolated EC5 according to the equation published by Gerberick and Berntson (2005). Direct comparison of the two EC5 values identified 2/13 with an extrapolated EC5 in the correct order of magnitude, 8 incorrectly (5 were too high and 3 were too low) and 5 could not be calculated based on the experimental data. A discussion on the dose response and use of the extrapolation equation was made. This abstract emphasizes the use of a tiered approach and the importance of the LLNA to correctly identify potent dermal sensitizers. Determining an accurate potency is an important hazard identification step, supporting the development of worker protection from sensitization hazards.

2190 EFFECTS OF CONCENTRATED AMBIENT ULTRAFINE PARTICLE-CONTAINING AEROSOLS IN MICE WITH NEURONAL DYSFUNCTION.


Recent studies suggest that the central nervous system (CNS) is a site for accumulation of ultrafine particles (UFP, <100 nm) following respiratory tract exposure, with accompanying increases in inflammatory and oxidative stress markers, and that exposure may accelerate neurodegeneration. We hypothesized that UFP-containing aerosols can accelerate neuropathological changes and neuronal dysfunctions in mice that are prone to neuronal protein aggregation. We exposed transgenic (TG) R6/2 mice (model of Huntington’s disease) and their non-transgenic (Ntg) littermates to concentrated ambient UFP-containing aerosols (HUCAPS; 76.2 ± 8.5, 20.6 ± 10/cm² ± 0.7) for 4 hours/day for 6 weeks, starting at 1 week of age. Neurobehavioral function was measured at weekly intervals for the last 4 weeks of the exposure period. No alterations were observed in cell or biochemical parameters in lavey fluid that would indicate an exposure-or-genotype-related lung inflammation. As expected, locomotor function decreased over time in both groups. In comparison to air-exposed TG mice, the HUCAPS-exposed TG mice had slightly earlier locomotor functional decrements. A separate group of mice was exposed for 4 weeks to Ag UFP (22.4 ± 76.2 nm ± 13; 5.78 ± 10/cm² ± 0.57) to evaluate the CNS accumulation of model UFP in TG and Ntg R6/2 mice. Ag accumulated in all brain regions examined in the mice (olfactory bulb, striatum, cerebellum, cortex); however, TG mice consistently accumulated more Ag as compared to Ntg mice. These results indicate that ambient UFP accelerate pathology in a model that is prone to protein aggregation and neuronal dysfunction, possibly due to altered UFP translocation kinetics. This research was funded by NIHES Center grant P50 ES01247 and ROI CA1534218.

2191 DEVELOPMENT OF A MOUSE MODEL FOR SULFUR MUSTARD-INDUCED OCULAR INJURY.

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Ocular exposure to sulfur mustard (SM) can cause a severe acute injury that is characterized by inflammation and corneal edema. After an initial recovery phase, a delayed injury phase ensues which is characterized by corneal opacity, neovascularization, and erosion that severely compromise eye function. Little is known regarding the molecular events that lead to SM-induced ocular pathology. Knowledge of these mechanisms is necessary for the rational development of therapeutics. Though the clinical and histopathological aspects of ocular SM injury have been studied in rabbits, this animal model is not well suited for mechanistic molecular studies because the receptors necessary for molecular studies in rabbits are limited. In contrast, the mouse has been widely used for mechanistic studies of ocular injury. Many of the cellular and molecular events implicated in the pathogenesis of human eye disease/injury have correlated well to mouse models, and reagents are also widely available for the mouse. For these reasons we are developing a mouse model of SM-induced ocular injury. The right eyes of mice were exposed to neat SM vapor using a vapor cup. A dose response and time course study was conducted in which the time of exposure and the amount of SM liquid in the vapor cup reservoir of were varied. Ocular injuries were then evaluated over 28 days. Increasing the dose led to significant corneal and ocular involvement as well as marked ocular inflammation. Fluorescein staining revealed acute widespread destruction of the corneal epithelium. Re-epithelialization occurred 5-7 days after exposure, but was followed by the development of persistent epithelial defects, neovascularization, and chronic corneal edema. These injuries led to corneal clouding and eventual scarring. The development of this model will further our understanding of the molecular mechanisms of SM-induced ocular pathology and accelerate the development of therapeutics for this injury.
Carbon nanotubes (CNT) are nanomaterials with outstanding characteristics. Germany and Nanocyl S.A., Sambreville, Belgium.

When epithelioid granulomas and interstitial inflammation in the mouse lung were by alveolar macrophages, can migrate from the lung to the regional lymph nodes after 80 μg exposure. Subpleural lymphatics were also dilated in one mouse at 7 days post-exposure. In 4 mice, including both mice with subpleural lymphangiectasia, principal intracellular. At 56 days post-exposure, subpleural lymphatics were focally dilated in one mouse and peribronchiolar lymphatics were dilated in all 4 mice beyond the cytoplasmic margins of some macrophages, indicating incomplete phagocytosis or cytoplasmic penetration after phagocytosis. Airway epithelial changes included hypertrophy, cellular atypia and mucous metaplasia. Sirius Red staining demonstrated fibrosis of granulomas and alveolar septa by 7 days post-exposure. Activated caspase-3 and TUNEL assays of the 80 μg exposure group at 7 days post-exposure demonstrated increased apoptosis in alveolar macrophages. MWCNTs accumulated in the draining tracheobronchial lymph nodes and were principally intracellular. At 56 days post-exposure, subpleural lymphatics were focally dilated in one mouse and peribronchiolar lymphatics were dilated in all 4 mice in the 80 μg exposure group. Subpleural lymphatics were also dilated in one mouse at 7 days post-exposure. In 4 mice, including both mice with subpleural lymphangiectasia, MWCNTs appeared to penetrate the pleura. These findings demonstrate that MWCNTs cause persistent pulmonary inflammation, can be translocated within the lung by alveolar macrophages, can migrate from the lung to the regional lymph node and can penetrate the cytoplasm of macrophages.

Use of labeled single walled carbon nanotubes to study translocation from the lungs.

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Colloidal gold nanoparticles (10nm) were used to label single walled carbon nanotubes (SWCNT) in studies aimed at determining how SWCNT clear from the lungs. Gold labeled SWCNT were delivered to the lungs by pharyngeal aspiration to C57BL/6 mice. Neutron activation analysis (NAA) of lung, blood and other organs was carried out at various time points after aspiration to determine if the gold labeled SWCNT translocated out of the lungs. Five mice per group were studied at 1 hour, 1 day, 3, 7 and 28 days after exposure to a single dose (40μg) of gold labeled SWCNT. A phosphate buffered saline (PBS) aspiration group served as the negative control. At sacrifice, the lungs, GI tract, heart, brain, liver, kidneys, right cranial mediastinal lymph node and a blood sample were taken for analysis of gold content by NAA. Lungs from additional mice were fixed and sectioned for study of the gross distribution of gold labeled SWCNT in the lungs. Gold content of the PBS aspiration group was negligible. For labeled SWCNT, gold content was below detectable levels for all time points. Lymph node gold content was not significantly different from the PBS group at any time point. Only lung and GI tract had significant labeled SWCNT at any time point. Lung gold content was 0.18±0.008, 0.13±0.006, 0.089±0.014, 0.096±0.006 and 0.049±0.007 ug (means±SE) at 1 hr, 1 day, 3, 7 and 28 days, respectively. Initially there was a rapid decline of burden from the lungs due to mucociliary clearance which was correlated with GI tract content. Lung burden decreased by 69% between 7 and 28 days. Histological examination demonstrated significant gold labeled SWCNT still present in the airways at 7 and 28 days. While it is uncertain as to the mechanism of the slower phase clearance of lung burden between 7 and 28 days it does not appear to be due to transport to major organs.
7, 28 and 56 days post-exposure. In bronchoalveolar lavage (BAL) studies, poly-morphonuclear leukocytes (PMNs) were assessed to index pulmonary inflamma-
tion. BAL fluid lactate dehydrogenase (LDH) activity was measured as a marker of cytotoxicity, and BAL fluid albumin was measured as a marker of the lung air-
blood barrier integrity. MWCNT exposure induced dose- and time-dependent changes, with maximum changes occurring at 7 days post-exposure for all three BAL markers. At 7 days post-exposure, mice exposed to 40 μg/mouse MWCNT had increased BAL PMNs (724-fold), BAL fluid LDH activity (2.6-fold) and BAL fluid albumin (2.5-fold) versus vehicle-exposed controls. At 56 days post-exposure, mice exposed to 40 μg/mouse MWCNT still had increased BAL PMNs (22.3 fold), BAL fluid LDH activity (1.9-fold) and BAL fluid albumin (1.6-fold) versus vehicle-exposed controls. Thus, relative to 7 days post-exposure, these BAL markers had decreased, but were still significantly elevated above vehicle-exposed controls at 56 days post-exposure. At 28 and 56 days post-exposure, histopathology confirmed persistent interstitial inflammation and indicated fibrosis. In summary, these data indicate that exposure to MWCNT results in dose- and time-dependent changes in pulmonary inflammation and damage, suggesting that MWCNT may pose an occu-
pational health hazard.

We also found that the dispersed form of SWCNT was more effective in inducing cytotoxicity and lung fibrosis than the non-dispersed form, indicating the impor-
tance of nanoparticle dispersion on biological activities. Since Survanta® is com-
mercially available and its one step nanoparticle dispersion is simple and rapid, this method provides major advantages of ease of use and disper-
sion. Furthermore, our stability studies showed that Survanta®-dispersed nanopar-
ticles remain well dispersed for months and upon dilution with aqueous medium.

**2197 NEUROINFLAMMATION AND BLOOD-BRAIN BARRIER CHANGES FOLLOWING EXPOSURE TO ENGINEERED NANOMATERIALS.


The unique physico-chemical properties of engineered nanomaterials influence their ability to aerosolize. MWCNT exposure is of major occupational concern. Inhaled nanoparticles can potentially translocate to the brain via olfactory sensory neurons or through systemic circulation and cause irreversible damage to the nervous system. To determine if engineered nanomaterials pose a neurological risk, we evaluated the effects of multi-walled carbon nanotubes (MWCNT) in a murine model. Male C57Bl/6j mice were exposed to MWCNT either by pharyn-
geal aspiration (single dose of 10-40μg/mouse; 1, 7, 28, or 56 p/d) or whole-body inhalation (10mg/m3 x 5h/d x 2, 4, 8 or 12d; 1d post-exposure). MWCNT exposure induced (2 to 16-fold) the mRNA expression of several proinflammatory cytokines (Il-1β, Il-6, Il-10), selectins (SelectB, SelectD) and markers of cellular stress (HspB2, Mt1, Mt2). Exposure to MWCNT also decreased the expression of BBB-related markers (Etn2, Vegfα), suggestive of alter-
tations in BBB integrity. In the hippocampus, MWCNT altered the expression of certain Alzheimer’s-related genes (Applp2, Apbeta2, Apbeta3, Bace1, Bace2, Cx3, Cx4d), which interact with or are involved in the processing of amyloid-precursor protein. The neurotoxic responses were comparable between the two routes of expo-
sure and some of the effects persisted until 56d post-exposure. Taken together, our findings suggest that exposure to engineered nanomaterials, like MWCNT, can elicit neuroinflammation, disrupt BBB integrity and cause cell/molecular changes that could potentially culminate in neurodegeneration.

Managemetn of exposure to bioengineered single walled carbon nanotubes (SWCNT) is becoming a major environmental and health challenge. Because of unique characteristics, SWCNT have found increased applications in various fields of science and technology. With increased risk of human and environmental expo-
posure, strategies to degrade SWCNT are also garnering interest. Here, we show - for the first time - that two peroxidases, namely myeloperoxidase and horseradish per-
oxidase are effective in catalyzing the biodegradation of SWCNT. A systematic characterization of resulting products of degradation was performed using trans-
mission electron microscopy (TEM), dynamic light scattering (DLS), gel electro-
phoresis, mass spectrometry, UV-Vis-NIR spectroscopy and thermogravimetric analysis (TGA). We also demonstrate that unlike naïve non-biodegraded nanopar-
ticles, the peroxidase degraded particles did not elicit pulmonary inflammatory re-
ponse in mice as evidenced by the release of pro-inflammatory cytokines and the content of neutrophils (PMN) in bronchoalveolar lavage. These results mark a novel approach to employ peroxidase catalysis for directed biodegradation of carbon nanotubes in biofluids/tissues as well as in environmental settings. Supported by NIOSH OH08282, NORA 927000Y, National Heart, Lung and Blood Institute Grant HL-70755, The Swedish Research Council, the 7th Framework Program of the European Commission.

**2200 PULMONARY AND SYSTEMIC INHALATION TOXICITY OF MULTI-WALLED AND SINGLE WALLED CARBON NANOTUBES.

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Inhalation of multiwalled carbon nanotubes (MWcnts) and single walled carbon nanotubes (SWCNT) at particle concentrations up to 1 mg/m3 did not result in significant lung inflammation or tissue damage, but caused systemic immune func-
tion alterations. C57Bl/6 adult (10-12 week) male mice were exposed by whole-
body inhalation to control air or 0.5 or 1 mg/m3 respirable aggregates of MWCNTs or SWCNTs for 14 days, with either immediate sacrifice of mice or recovery group 30 days after the end of exposure. Histopathology of lungs from exposed animals showed alveolar macrophages containing significant amounts of black particles; however, there was minimal to no inflammation or tissue damage observed. Bronchial alveolar lavage fluid also demonstrated particle-laden macrophages; however, white blood cell counts were not increased compared to controls. Both types of carbon nanotubes caused systemic immunosuppression after 14 days and after recovery. Immunosuppression was characterized by reduced T-cell-dependent antibody response to sheep erythrocytes as well as T-cell prolifera-
tive ability in presence of mitogen, Concanavalin A (Con A).
properties which result from their fibrous shape and small size. The most common
technique for manufacturing SWCNT relies on the use of iron as a transition metal
and can result in the presence of up to 30% metal catalyst in raw SWCNT.
The low density of SWCNT may lead to inhalation as well as deposition on ex-
posed skin, which provides routes of exposure that are important to consider when
evaluating toxicity. We hypothesized that SWCNT are toxic to the skin, and this
toxicity is dependent on the ability of SWCNT to interact with the skin and initi-
ate oxidative stress, and the induction of transcription factors leading to inflamma-
tion. To test this hypothesis, the effects of SWCNT were assessed both in vivo and
in vitro. Engineered skin exposed to SWCNT showed increased epidermal thickness
and accumulation and activation of dermal fibroblasts, which resulted in increased
collagen as well as release of pro-inflammatory cytokines. Exposure of JBG cells
to unpurified SWCNT resulted in the production hydroxyl radicals as detected by
ESR and caused a significant size-dependent activation of AP-1 and NFκB, while
partially-purified SWCNT activated only NFκB. Topical exposure of SKH-1 mice
(5 days, 40, 80, 160 µg/mouse/day) to SWCNT caused oxidative stress, depletion
of GSH, oxidation of protein thiols and carbonyls, elevated MPO activity, and an
increase of dermal cell numbers resulting in skin thickening. Altogether, these data
indicated that topical exposure to unpurified SWCNT induced free radical genera-
tion, oxidative stress, and inflammation, leading to dermal toxicity.

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2202 PULMONARY EFFECTS OF SINGLE-WALLED CARBON
NANOTUBES: INHALATION VS ASPIRATION.

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Health effects and occupational risk of exposures associated with manufacturing and
application of nanoparticles are critical points for the safe and sustainable de-
velopment of nanotechnology. The toxic effects of nanoscale materials have not
been fully characterized and the limited in vitro studies indicate the urgent necessity
for further toxicological assessments of nanomaterials. Some argue that pharyngeal
aspiration – a single exposure to a bolus of SWCNT - is an artificial exposure where
the single large dose contributes to the pulmonary response. Moreover, aspiration
studies reported thus far have been relatively high dose exposures, which may not be
relevant to chronic lower dose seen in occupational settings. Inhalation of SWCNT
more closely mimics occupational and environmental venues than the above men-
tioned administrations providing more dispersed SWCNT structures while bolus
effects are avoided. By applying a new technique to aerosolize SWCNT, we obtained
stable and uniform SWCNT dispersions with a concentration of 5 mg/m3 and a
count mode aerodynamic diameter of 240 nm for the inhalation experiments. In the
current study, we utilized non-purified SWCNT containing up to 17.7% of iron for
both inhalation (5 mg/m3, 5 hrs/day for 4 days) and aspiration (varying doses of 5-
20 µg/mouse) exposures. Pathological events in both exposure routes were realized
through qualitatively similar synergized interactions of early inflammatory response
and oxidative stress culminating in the development of multifocal granulomatous
pneumonia and interstitial fibrosis. Quantitatively, SWCNT inhalation was more
effective than aspiration in causing inflammatory response, oxidative stress, collagen
deposition and fibrosis as well as mutations of K-ras gene locus in the lung of
C57BL/6 mice. Acknowledgements: supported by NIOSH OH008282, NIEH
HI70755, NORA 927000Y, and EC-FP-7-NANOMMUNE-214281

2203 SINGLE-WALLED CARBON NANOTUBES INDUCE
PULMONARY AND VASCULAR RESPONSE
FOLLOWING INTRATHORACIC INSTALLATION.

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Carbon-based nanotubes have been shown to induce varying degrees of pulmonary
response in rodents influenced by the dose, the extent of agglomeration, and the
functional groups. We hypothesized that low density or acid-functionalized (AF) single
walled carbon nanotubes (SWCNT) will cause dis-
tinct pulmonary and aortic effects on markers of inflammation, coagulation, vaso-
construction following pulmonary exposure. Male Wistar Kyoto rats (12 week old)
were intratracheally instilled with pre-characterized freshly sonicated suspensions of
SWCNT or AF-SWCNT at 0, 100 or 500 µg/kg in saline. Pulmonary injury and
inflammatory effects were small as determined by bronchoalveolar lavage fluid
(BALF) analysis and were concentration-dependent. Small increases in BALF pro-
tein were noted with high dose of both types of tubes while albumin increased only

with AF-SWCNT. Surprisingly, small but significant increases in BALF γ-glutamyl
transferase activity (marker of cell membrane damage) were noted only with high
concentrations of SWCNT but not AF-SWCNT. LDH activity was increased 4-d
at high concentration of both types of SWCNT. Both types of nanotubes moder-
ately increased BALF neutrophils at high concentration while no increase in
macrophages occurred (1-d>4-d). Real-time PCR for mRNA markers of oxidative
stress, inflammation, vasoconstriction, thrombosis and cell filamentous
components in rats exposed to AF-SWCNT revealed pulmonary induction of HO-1,
MMP-2, endothelin-1, PAI-1 and β-catenin at both time points (1-d>4-d). However,
mRNA expression for any of the biomarkers was not altered in the aorta at
either time points. Pulmonary instillation of SWCNT produces acute pul-
monary inflammatory, vasoconstrictive and prothrombotic effects in rats, whereas
these effects are not evident within 4 days in the aorta. (Does not reflect US EPA

2204 PULMONARY EFFECTS FROM ACUTE EXPOSURE
TO AEROSOLIZED SINGLE-WALLED CARBON
NANOTUBES.

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Although nanotechnology is still an emerging field and the enthusiasm for the poten-
tial societal benefits of engineered nanomaterials continues, concerns are being
raised about whether our knowledge of possible health risks is keeping pace with
products going to market. Single-walled carbon nanotubes (SWCNTs) have gained
notable attention for application in a number of industries because of their unique
electronic, optical, mechanical, chemical, or even biological properties. Due to the
potential for human exposure, toxicological studies are needed to understand the
potential health hazards of these nanomaterials. To better understand the biological
responses associated with acute SWCNT exposure, Sprague Dawley rats were ex-
posed to either aerosolized SWCNTs (300 or 1000 µg/m3, raw [Fe3SWCNT or pu-
rified SWCNT]) or fresh air via nose-only inhalation for 6 hours for 1 day.

Cytotoxicity markers (total protein, lactate dehydrogenase [LDH], and gamma-
 glutamyl transferase [GGT]) in bronchoalveolar lavage and mucin and collagen
staining in lung tissue were used as a means to assess immediate and persistent (0,
1, 3, 7 and 28 d post exposure [PE]) effects of acute exposure to SWCNTs. Results
showed that markers for cytotoxicity (LDH, GGT) were acutely increased up to 7
d PE, whereas mucin staining in proximal airways initially increased and then de-
creased at 7 and 28 d PE, and collagen staining in the alveoli increased at 7 and 2 PE.
Effects in all cases were transient, influenced by dose and particle composition (e.g.,
iron content), and generally were resolved by 7-28 d PE. While these data suggest
that exposure to aerosolized SWCNTs may induce cytotoxic and structural re-
sponses in the lungs, further research is needed to evaluate whether these changes
are suggestive of precursor events to pathological changes or lung remodeling that
might develop under more severe or prolonged exposure conditions and may have
implications for human health risk for persons potentially exposed to airborne
SWCNTs.

2205 INHALED MULTI-WALLED CARBON NANOTUBES
STIMULATE A PLEURAL INFLAMMATORY RESPONSE
IN THE LUNGS OF MICE.

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Multi-walled carbon nanotubes have recently been reported to have asbestos-like properties
since they stimulate the formation of pleural granulomas when injected into the ab-
dominal cavity of mice. This has raised legitimate concerns over the safety of nan-
otubes because mesothelial granulomas that form on the pleural surface have the
potential to develop into mesothelioma, a type of cancer associated with the inhala-
tion of asbestos fibers. Here we report that male C57BL/6 mice that inhaled an
aerosol of multi-walled carbon nanotubes (6-hr exposure at 100 mg/m3) developed
inflammatory foci on the pleural surface of the lung, even though very little inflam-
mation or fibrosis was observed within the lung parenchyma. These foci were pri-
marily mononuclear and persisted at 14 days post-exposure. We observed nanotubes
dispersed throughout the lung at 1 day post-exposure with some embedded within
the pleural wall. Most of the carbon nanotubes (~90%) were contained within
macrophages with the larger particles concentrated at the base of the alveoli. This
may be due to the route of occupational exposure to carbon nanotubes, and our findings are the first
to demonstrate that inhaled carbon nanotubes cause pleural inflammation. We
emphasize that further work is urgently needed to assess whether longer term, lower
concentration nanotube exposures cause similar pleural responses as seen with this
single high exposure concentration and to assess whether these pleural lesions are likely to progress to neoplasia. (Funded by The Hamner Institutes for Health Sciences and North Carolina State University College of Agricultural and Life Sciences).

2206 COMPARATIVE PROTEOMICS, GENOMICS AND PULMONARY TOXICITY OF INSTILLED SINGLE WALLED CARBON NANOTUBES, CROCIDOLITE ASBESTOS AND ULTRAFINE CARBON BLACK IN MICE.

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CNT have emerged as a nanomaterial of considerable concern to the occupational and environmental health and safety communities. Early, consistent reports from high-dose, short-term screening studies in rodents suggest a sequence of biological events and pulmonary pathology similar to that caused by asbestos and synthetic vitreous fibers (SVF). Whether CNT strictly adhere to the asbestos paradigm of pulmonary toxicity is a question of critical importance. The pulmonary response of mice exposed repeatedly (4 doses, 28 days, pharyngeal aspiration) to single-walled CNT (SWCNT), crocidolite asbestos (AS) and ultrafine carbon black (CB) was assessed by global proteomics and genomics of lung tissue and ELISA protein microarray of bronchial lavage fluid. Three cytokines, IL-6 and IL-12 and Mip-1g were considerably higher in SWCNT and AS treated animals compared to CB. The T cell chemotactic protein MDC was present in high levels in SWCNT and AS groups, but not detectable in control or CB treated groups. Two sentences on similarities and differences via proteomics. Microarray analysis of lung tissue revealed over 3000 genes significantly changed by SWCNT exposure, compared to only 469 genes.

2207 PERMEABILITY OF THE BLOOD-CSF BARRIER IN THE CHOROID PLEXUS AS EXPOSED BY SINGLE-WALLED CARBON NANOTUBES, IN VITRO.

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Nanotechnology is an emerging field and has made great advances in production and product integration. Nanoparticle use in medical imaging, diagnosis and drug delivery vectors has created novel exposure routes, raising concerns about biological fate and susceptible organs. The blood and the brain are separated by two distinct barrier systems: the blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCB). The BBB is formed by the choroidal plexus, a tissue with high blood flow and large surface area. The BBB plays a key role in maintaining the homeostasis of internal milieu and protecting the brain against toxic insults in the blood. Improper function of the BBB has been linked to a variety of neurodegenerative diseases. The hypothesis tested was that exposure to single-walled carbon nanotubes (SWCNT) may damage the structure and function of the BBB. A BBB model utilizing an immortalized Z101 choroidal epithelial cell line was used to assess the effect of SWCNT exposure. Cell viability and ROS generation were determined with the colorimetric MTS and fluorometric DCF assays, respectively. A confluent cell monolayer grown on a permeable membrane in the Transwell inserts was used to estimate the para-cellular diffusion of C14 labeled sucrose, a parameter reflecting the leakage of the barrier. Following 24-hr incubation with SWCNT, the cell viability decreased 6% (p<0.05) and 17% (p<0.01) at the dose of 10 and 50 μg/mL, respectively. More drastic alterations were observed with 72-hr treatments where 10 and 50 μg/mL caused a 16% (p<0.05) and 27% (p<0.01) loss in cell viability. A concentration of SWCNT as low as 1 μg/mL created a significant increase in ROS generation after a 24-hr exposure. The diffusion rate of sucrose across the cell monolayer after treatment with 10 μg/mL SWCNT for 48 h was assessed, but did not reach a statistical significance (p<0.0585). Taken together, our data suggest that SWCNT exposure produces a negative effect on BBB function. Additional studies are warranted to better characterize nanoparticle toxicity on brain barrier systems.

2208 ENHANCED OCCUPATIONAL EXPOSURE TO NANOMATERIALS WHEN MIXED IN ENVIRONMENTALLY-RELEVANT MATRICES.

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The goal of this research was to assess the release of engineered nanomaterials into the laboratory when handling and preparing nanomaterials for mixing into environmentally-relevant matrices. Air-borne fullerene (C60), undervatized multiwalled carbon nanotubes (Raw MWNT), hydroxylated MWNT (MWNT-OH), and carbon black (CB) were measured as the nanomaterials were weighed and transferred to beakers filled with water, as well as when sonicated in de-ionized water and reconstituted freshwater with natural organic matter (100 ppm). Air-borne nanomaterials were measured for 20 minutes using a handheld condensation particle counter, confirmed by transmission electron microscopy, and expressed as total particles per cubic centimeter of sampled air within six specific size ranges from 300–10,000 nm. After adjustment for background particle number concentrations, it was evident that increases in air-borne particle concentrations occurred for each nanomaterial except CB during weighing. Air-borne particle concentra- tions were inversely correlated with particle size. Sonication nanomaterial-spiked water resulted in increased air-borne nanomaterials. The increase in air-borne nanomaterials after sonication was most evident with MWNT-OH, the more hydrophobic form of MWNT, in water with natural organic matter and CB, a natural functionalized carbon-based NM. In conclusion, engineered nanomaterials, especially when functionalized or in water containing natural organic matter, can become air-borne when mixed in solution by sonication, putting workers at increased risk of occupational exposure to air-borne nanomaterials.

2209 IN VITRO BIOCOMPATIBILITY OF SILVER NANOPARTICLES ANCHORED ON MULTI-WALLED CARBON NANOTUBES.

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Silver nanoparticles (Ag NPs) have a variety of applications due to their high extinction coefficient and surface plasmon resonance emission. By manipulating the surface and the core-shell structure of Ag NPs, different functionalities can be engineered to explore the molecular behaviors in living cells. This study investigates if Ag NPs anchored to different types of multi-walled carbon nanotubes (MWCNTs) show biocompatibility. Ag NPs were anchored via a novel chemical route involving silver nitrate, nanotubes and a reducing agent. Scanning electron microscopy (SEM), X-ray powder diffraction, and transmission electron microscopy (TEM) were performed to characterize the nanomaterials. The human keratinocyte cell line (HaCaT) was exposed to the nanomaterials at various concentrations and time points and biocompatibility was evaluated using mitochondrial function and morphology. MWCNTs correspond to pure MWCNTs, COx-MWCNTs to carbonyl doped and CNx-MWCNT to N-doped. The chemical reactivity of the Ag NPs on the nanotubes was MWCNTs< COx-MWCNTs< CNx-MWCNT. The average Ag NP size on the nanotubes was MWNTs 6.64nm +/- 2.25nm; CNx-MWCNTs, 13.24nm +/- 3.94nm, and COx-MWCNTs/Ag were 11.75nm +/- 4.65nm. The X-ray diffraction patterns show peaks corresponding to graphite and silver. Cryoviva enhanced light microscopy imaging revealed nuclear binding for all of the materials. After 48 hour exposure, there was disruption of the actin filaments and continuous nuclear localization and binding. The MTS assay did not show a reduction in mitochondrial function, indicating potential for biocompatibility. The lack of toxicity combined with NPs binding to the nucleus indicates potential for a novel method of delivering Ag or other nanoparticles to the nucleus. Further studies are in progress to demonstrate biocompatibility and to deliver modified Ag NPs in a non-invasive way to other specific regions within cells.

2210 IL-1β REGULATES THE EXPRESSION OF MIP-1α AND MIP-1β INDUCED BY CARBON NANOTUBES IN A MULTIPLE CELLULAR CO-CULTURE SYSTEM.

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In vitro models are very useful to understand the cellular mechanisms related to particulate matter (PM) toxicity. The use of single cell cultures, or even co-cultures of two different cells may overlook the important interactions with other cell types and the communication with distant cells, such as the possible cross talk between the lung epithelium and the vascular endothelium. In the present study, we evaluated the proinflammatory effect of single wall carbon nanotubes (SWCNT) on cultures of multiple human cells that have been used successfully to evaluate urban PM METHODS. Single cultures (pseudocyesms, A549; macrophages, THP-1; mast cells, HMC-1). Bicultures (A549+THP-1; A549+HMC-1; THP-1+HMC-1) and Tricultures (A549+THP-1+HMC-1) were exposed to 0, 1, 3, 10 or 30 μg/cm2 of SWCNT. Inserts containing endothelial cells (EAHY926) were introduced to some Tricultures after 12 h of exposure. TNFα, IL-1β, IL-6, IL-8, MIP-1α, MIP-1β and G-CSF concentrations were quantified in all the cultures after 24 h of ex-
Exposure. Antibodies against TNFα, IL-1β and IL-6 were added together with the SWCNT to evaluate its role in the expression of MIP-1α and MIP-1β in Trilocules+EAHY926. The results were expressed as fold changes compared to unexposed cultures. RESULTS. Single cultures: IL-1β was induced in THP-1 cultures (5.5 fold) while IL-8 was induced in HMC-1 cultures (3.9 fold). Biculures: Increases of TNFα (4 fold), IL-1β (2.5 fold) and IL-6 (2.5 fold) were observed in A549/THP-1 Biculures. Trilocules: No changes in any of the evaluated cytokines were observed when Trilocules were exposed, but increases in MIP-1α (1.5 fold) and MIP-1β (2 fold) were found when EAHY926 cells were introduced in the system. Anti IL-1β enhanced the expression of MIP-1α (3 fold) and MIP-1β (20 fold). CONCLUSIONS. IL-1β seems to play an important role in regulating the expression of MIP-1α and MIP-1β when pneumocytes+macrophages+ mast cells exposed to SWCNT are in communication with endothelial cells.

2213 A COMPARATIVE STUDY OF EFFECTS OF SINGLE-WALL CARBON NANOTUBES AND CROCIDOLITE ASBESTOS IN HUMAN BEAS-2B CELLS.

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Single-wall carbon nanotubes (SWCNT) are of great interest to many aspects of industry including, but not limited to, electronics and pharmaceutics. As SWCNT-manufactured goods are being introduced into market, concerns regarding their potential for pulmonary and other human health effects have been raised. In the present study, we investigated SWCNT effects on the human bronchial epithelial cells (BEAS-2B). Exposure of BEAS-2B cells to SWCNT resulted in dose-dependent loss of cell viability and oxidative stress by means of dose-dependent increase in OH radical production and superoxide dismutase activity (SOD). Furthermore, SWCNT induced time- and dose-dependent apoptosis and phosphorylation of histone γ-H2AX, a variant of histone H2A that is activated following DNA damage. Whether SWCNT have the potential to induce apoptosis, BEAS-2B cells were exposed for different time points to SWCNT (50 μg/cm2), and analyzed for PARP activation, as a molecular marker of apoptosis. The level of cleaved PARP increased after 18h of exposure. This level decreased when cells were allowed to recover for 48 h in normal growth media. We also examined the ability of SWCNT to induce the transactivation of activator protein-1 (AP-1) and nuclear factor-kappaB (NF-κB), transcription factors that are important members of signal transduction pathways. SWCNT induced greater activation of AP-1 and NF-κB at lower doses as compared to higher doses. These effects were diminished by PD98059, an inhibitor MAP kinase. Crocidolite asbestos was used as a control along with SWCNT, and the effects of crocidolite were far greater as compared to SWCNT. Considering the role of airway epithelium as a barrier and its role in pulmonary functioning, the results of the present study suggest that interaction of SWCNT with airway cells may cause adverse biological responses that may initiate respiratory diseases.

Key words: Apoptosis, asbestos, carbon nano-particles, cell toxicity, oxidative stress, inflammatory pathways.

2214 AEROSOLIZATION DELIVERY SYSTEM OF AIRBORNE NANOPARTICLES FOR NOSE-ONLY INHALATION STUDIES.


Understanding human health risks associated with engineered nanoparticles is particularly challenging because of the wide range of plausible exposure scenarios. 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. For hazard assessment of inhaled nanoparticles, it is critical to have a means to deliver respirable airborne nanoparticles for experimental animal studies. An aerosolization system was developed to administer nanomaterials from a dry bulk media into respirable airborne particles for delivery into a nose-only inhalation system. Utilization of a cannabis-based feed system, diamond grinding wheel, cyclone-type conditioning chamber, and Krypton-85 source (charge neutralization) allows for efficient delivery of otherwise difficult to produce respirable-size particles. Different nanomaterials (e.g., single-wall carbon nanotubes, ultrafine carbon black) were tested with the aerosolization system and aerosolized particles were characterized by size, mass, and number distribution using a gravimetric filter analysis, inertial cascade impactor, and scanning mobility particle sizer with a condensation particle counter, as well as by particle morphology using transmission electron microscopy. Aerosolized particles represented a wide range of size and morphological characteristics with particles spanning the fine (0.1-2.5 μm) and ultrafine (<0.1 μm) size range mostly in an agglomerated state. An advantage that this system offers over other aerosol-generating systems is that it utilizes relatively small amounts of dry material (<0.5 g) to generate respirable particle concentrations up to 1 mg/m3 continuously over a 6-hr period. Relating exposure characteristics of airborne particles in experimental studies to those in human exposure settings will be important for establishing exposure/dose-response relationships and standards to protect human health.

2215 COMPARISON OF CARBON NANOTUBE-INDUCED CYTOTOXICITY IN A549 AND NORMAL HUMAN BRONCHIAL EPITHELIAL (NHBE) CELLS.

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The most attractive features of nanomaterials including their small size, large surface area, and reactivity might also be the main factors for their toxicity. Pulmonary bronchial epithelial cells are a potential target for toxicity during respiratory exposure. The tumor cell line A549, a pulmonary human type II like epithelial cells, is
widely used for evaluation of particle toxicity. We hypothesized that A549 cells are more resistant to toxicity compared to the normal epithelial cells. To test this hypothesis we examined the dose-dependent cytotoxicity effects of single-walled (SWCNT) and multi-walled (MWCNT) carbon nanotubes on NHBE and A549 cells using lactate dehydrogenase (LDH) release and the cell proliferation/viability WST-1 assays. We found that NHBE exhibited marked cytotoxicity, similarly to that observed with hydrogen peroxide treatment (positive control), consistently by 24 hours post-exposure to high concentrations [50-150μg/106 cells (1.5-4.5μg/ml)] of CNT. In contrast, exposure of A549 cells to similar concentrations of CNT resulted in only a milder response. Overall, the results presented here indicate that SWCNT or MWCNT induce cytotoxicity in human lung epithelial cells in a time and dose-dependent manner. Furthermore, we demonstrate a large variation between the particle-induced cytotoxic responses in A549 cells and normal primary epithelial cells.

**2216 CELLULAR AND MOLECULAR MECHANISMS OF BROMATE-INDUCED TOXICITY IN RAT AND HUMAN KIDNEY CELLS.**

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The cellular and molecular mechanisms of bromate-induced toxicity in normal rat kidney (HEK-293) and human embryonic kidney cell lines (HEK293) after acute exposures were investigated. Bromate (added as KBrO3 at 0.8 μM/ml) exposure decreased MTT staining. These data demonstrated the bromate-induced G2/M arrest in both cell lines. Further, bromate treatment induced both time- and concentration-dependent increases in the activity of the mitogen activated protein kinases (MAPK) p38 and ERK1/2. The activity of p38 and ERK1/2 increased after just 15 min of bromate exposure, prior to any increase in cell death or cell cycle arrest. Inhibition of p38 and ERK1/2 using SB202190 and PD98059, respectively increased bromate-induced decreases in MTT staining. Interestingly, treatment of cells with SB202190 inhibited the ability of BEL to activate p53 and p21 in LNCaP cells, and reversed BEL-induced G2/M arrest in PC-3 cells. In contrast, inhibition of ERK1/2 using PD98059 only slightly altered p53 activation in LNCaP cells. Interestingly, iPLA2 inhibition significantly increased the activity of epidermal growth factor receptors (EGFR) in PC-3 cells, but not LNCaP cells. Treatment of PC-3 cells with the EGFR inhibitor AG1478 prevented BEL-induced ERK1/2 activation. Similar results were seen using the matrix metalloproteinases inhibitor GM6001. Neither of these inhibitors altered BEL-induced p38 activation. Inhibition of ERK1/2 prior to treatment with BEL decreased PC-3 cell growth in comparison to cells exposed to BEL alone. Similar results were seen in LNCaP cells where, again, BEL was inhibited. In contrast, inhibition of p38 in PC-3 cells protected against BEL-induced cytotoxicity. These data demonstrate the novel findings that iPLA2 inhibition differentially activates p38 and ERK1/2 in prostate cancer cells, and further suggest that these signaling kinases have differential roles in prostate cancer cell growth.

**2217 THE NOVEL TUMOR PROMOTER PALYTOXIN ACTIVATES EXTRACELLULAR SIGNAL REGULATED KINASE 5 THOUGH A Na+, K+-ATPase-DEPENDENT PATHWAY.**

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Palytoxin is classified as a non-12-O-tetradecanoylphorbol-13-acetate (TPA)-type tumor promoter because it does not bind to or activate protein kinase C. The novel characteristics of palytoxin led us to investigate how it affects cellular signal transduction pathways. We previously showed that palytoxin stimulates the activation of three major members of the mitogen activated protein kinase (MAPK) family, c-Jun N-terminal kinase (JNK), p38, and extracellular signal regulated kinase (ERK) 1/2. Here we report palytoxin also activates ERK5, another MAPK family member, in mouse keratinocytes derived from initiated mouse skin (308 cells) and HeLa cells. By contrast, palytoxin does not activate ERK5s in these cell lines. The putative cell surface receptor for palytoxin is the Na+, K+-ATPase. Accordingly, ouabain blocked the ability of palytoxin to activate ERK5. We did not detect the activation of ERK5 by ouabain alone, however, indicating a divergence in the signaling pathways activated by these two ligands for the Na+, K+-ATPase. The activation of ERK5 by palytoxin was not mimicked by cycloheximide, okadaic acid, or sodium orthovanadate. These results indicate that the stimulation of ERK5 by palytoxin is not simply due to its ability to inhibit protein synthesis or to inhibit tyrosine or serine/threonine phosphatases. Thus palytoxin appears to activate ERK5 through a mechanism that differs significantly from the mechanisms by which it activates JNK, p38, and ERK1/2. The observation that ERK5 is involved in the regulation of cell proliferation and survival suggests that ERK5 may be an important target of palytoxin action.

**2218 INHIBITION OF CALCIUM-INDEPENDENT PHOSPHOLIPASE A2 ACTIVATES MAP KINASE SIGNALING PATHWAYS DURING CYTOTOXICITY IN PROSTATE CANCER CELLS.**

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The p53-dependent and -independent signaling pathways activated during cytotoxicity induced by Ca2+-independent phospholipase A2 (iPLA2) inhibitors in prostate cancer cells were investigated. iPLA2, inhibition using siRNA, or the selective iPLA2 inhibitor, have been shown to decrease growth in LNCaP (p38 positive) and PC-3 (p53 negative) human prostate cancer cells. Decreased cell growth correlated to time- and concentration-dependent activation of the mitogen activated protein kinase p38 and ERK1/2. p38 was activated in both cell lines, while ERK1/2 was transiently activated in only PC-3 cells. Inhibition of p38 using SB202190 inhibited the ability of BEL to activate p53 and p21 in LNCaP cells, and reversed BEL-induced G2/M arrest in PC-3 cells. In contrast, inhibition of ERK1/2 using PD98059 only slightly altered p53 activation in LNCaP cells. Interestingly, iPLA2, inhibition significantly increased the activity of epidermal growth factor receptors (EGFR) in PC-3 cells, but not LNCaP cells. Treatment of PC-3 cells with the EGFR inhibitor AG1478 prevented BEL-induced ERK1/2 activation. Similar results were seen using the matrix metalloproteinases inhibitor GM6001. Neither of these inhibitors altered BEL-induced p38 activation. Inhibition of ERK1/2 prior to treatment with BEL decreased PC-3 cell growth in comparison to cells exposed to BEL alone. Similar results were seen in LNCaP cells where, again, BEL was inhibited. In contrast, inhibition of p38 in PC-3 cells protected against BEL-induced cytotoxicity. These data demonstrate the novel findings that iPLA2 inhibition differentially activates p38 and ERK1/2 in prostate cancer cells, and further suggest that these signaling kinases have differential roles in prostate cancer cell growth.

**2219 THE TUMOR SUPPRESSOR GENE TSC-2 MODULATES TRANSLATION INITIATION OF CYCLIN D1 THROUGH ERK CROSSTALK WITH 4EBP1.**


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The mTOR and MAPK signaling cascades have been implicated in a number of human cancers. The tumor suppressor gene tuberous sclerosis-2 (Tsc-2) functions as a negative regulator of mTOR. Critical proteins in both pathways are activated following treatment of Eker rats (Tsc-2−/−) with 2,3,5-tris-(glutathion-S-yl) hydroquinone (TGHQ), which also results in loss of the wild-type allele of Tsc-2 in renal preneoplastic lesions and tumors. Western blot analysis of kidney tumors formed following 8-months of TGHQ treatment of Tsc-2−/− rats revealed increases in B-Raf, C-Raf, p-ERK, cyclin D1, 4EBP1, and p-4EBP1-Thr70, -Ser65, and -Thr37/46 expression. Concomitant with increases in expression of these proteins in TGHQ-induced tumors, similar changes are observed following TGHQ transformation of primary renal epithelial cells derived from Tsc-2−/− rats (QTRRE cells). QTRRE cells are null for tuberin due to loss of heterozygosity at the Tsc-2 allele and exhibit high ERK, B-Raf and C-Raf kinase activity, and increased expression of cyclin D1, 4EBP1, and p-4EBP1-Thr70. Following siRNA knockdown of C-Raf, QTRRE cells are null for tuberin due to loss of heterozygosity at the Tsc-2 allele and exhibit high ERK, B-Raf and C-Raf kinase activity, and increased expression of cyclin D1, 4EBP1, and p-4EBP1-Thr70. Following siRNA knockdown of C-Raf, QTRRE cells are null for tuberin due to loss of heterozygosity at the Tsc-2 allele and exhibit high ERK, B-Raf and C-Raf kinase activity, and increased expression of cyclin D1, 4EBP1, and p-4EBP1-Thr70. Following siRNA knockdown of C-Raf, QTRRE cells are null for tuberin due to loss of heterozygosity at the Tsc-2 allele and exhibit high ERK, B-Raf and C-Raf kinase activity, and increased expression of cyclin D1, 4EBP1, and p-4EBP1-Thr70. Western blot analysis revealed a significant decrease in C-Raf, cyclin D1, and all p-4EBP1 forms noted above. In contrast, siRNA knockdown of B-Raf resulted in a nominal change in these proteins. Furthermore, treatment of the QTRRE cells with the Raf kinase inhibitor Sorafenib (therapeutic anti-cancer agent) and the MEK1/2 kinase inhibitor PD 98059, both produced a significant decrease in the protein expression of cyclin D1 and all p-4EBP1s. Western analyses revealed three distinct species of 4EBP1, with the lowest molecular weight band lacking phospho-rylations on Thr65 and Thr70. Moreover, treatment with both inhibitors caused an increase in the relative abundance of all three bands. The data indicate that Raf-MEK-ERK participates in crosstalk with 4EBP1, which represents a novel pathway interaction leading to increased protein synthesis, cell growth, and kidney tumor formation. (GM39338, ES007091, ES06694).

**2220 OVERLAPPING SIGNAL SEQUENCES AND PHOSPHORYLATION CONTROL NUCLEAR LOCALIZATION AND ENDOPLASMIC RETICULUM RETENTION OF GLUCOSE REGULATORY PROTEIN GRP58.**


Glucose regulatory protein (GRP58), has two thioridoxin-like domains and is known to function as thiol-dependent oxidoreductase. GRP58 is present in cytosol, nucleus and endoplasmic reticulum (ER). GRP58, retained in ER, specifically in-
teracts with glycoproteins such as calnexin and calreticulin, and acts as a molecular chaperone during glycoprotein biosynthesis and folding. We investigated the sig-

nals that control localization of GRP58 in the cytosol, nucleus and ER for specific functions. Proxite search revealed that GRP58 contains putative nuclear localiza-
tion (494KKPPKKKK300) and ER retention (502QEDL505) signals. Deletion/mutation of nuclear localization signal (NLS) abruptly reduced import of GRP58. NLS attached to EGFP localized EGFP in the nucleus. However, dele-
tion/mutation of putative ER retention signal alone did not alter ER retention of GRP58. Interestingly, a combined deletion/mutation of NLS and ER retention sig-

nals blocked the GRP58 retention in the ER. These results concluded that overlap-

ping NLS and ER retention sequence signals regulate nuclear localization and ER retention of GRP58. Further studies demonstrated that thioltransfer domains do not play a role in subcellular localization of GRP58. We also investigated the role of phosphorylation in subcellular distribution of GRP58. GRP58 immunoprecipitated from cytosolic, nuclear and ER compartments all were phosphorylated at seri-

ne residues. However, GRP58 from cytosol and nucleus but not from ER were also phosphorylated at tyrosine residues. Reverse IP experiments showed similar results.

Nerphp and Prosite search have shown presence of putative PKC and tyrosine ki-

nase sites. These sites have been mutated in GRP58 to determine the role of specific site(s) of phosphorylation in subcellular distribution of GRP58. The mutated sites also be used in identifying whether GRP58 play a role in translocation of other pro-

teins such as STAT3 to the nucleus which are found associated with GRP58 in cell membrane, ER and nucleus.

2222 KEY ROLES OF ANNEXINS AND PHOSPHOLIPASES IN TOXICANT-INDUCED REGULATION OF CELL SIGNALING RELEVANT TO CANCER.

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Lower molecular weight polymeric aromatic hydrocarbons (PAHs) with specific structural features are potent in vitro inhibitors of gap junctional intercellular com-

munication (GJIC) and activators of mitogen-activated protein kinase pathways, two cellular events linked with tumor promotion and other pathologies. We found that inhibition of GJIC and activation of MAPKs are induced in a rat liver epithelial cell line (WB-F344) within several minutes by a model representative of lower molecular weight PAHs, 1-methylnaphacene (1-MeA), and these effects are preceded by the activation of phosphatidylinositol-specific phospholipase C (PI-PLC) and the release of arachidonic acid also suggesting an activation of phospholipase A2 (PLA2). We used advanced proteomics techniques (SILAC – Stable Isotopes Labeling with Amino Acids in Cell Culture, ZOOM® ionoelectric fractionation, 1-

DE, 2-DE and mass spectroscopic identification) to further identify early upstream biochemical signaling events. Among the proteins specifically affected within a 5 min exposure time to 1-MeA, annexins A1, A3 and A5 showed significant re-
sponses characterized by their disappearance from plasma membrane and reciprocal increases in the fraction of soluble proteins. Western blotting and immunostaining experiments on annexin A3 indicates a translocation from the plasma membrane within 30 s of exposure followed by reintegration back into the plasma membrane after 60 min. Interestingly, removal of annexin A3 from plasma membrane was ef-

fectively prevented by pre-treatment of the cells with PC-PLC inhibitor, D609. We hypothesize that annexins closely interact with phospholipases in the plasma membrane until removed from the membrane in response to 1-MeA, and the subsequent phospholipase-induced events then regulate GJIC and MAPK. Support: NIEHS grant #R01 ES015268-01A2 to Uppham.

2223 HEMATOPOETIC CELL KINASE (HCK) ACTIVATES P38 VIA APOTOPSIS SIGNALING KINASE 1 (ASK1) AND MAPK KINASE KINASE 6 (MKK6) DURING THE DEOXYNIVALENOL-INDUCED RIBOTOXIC STRESS RESPONSE.

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Although the trichothecene mycotoxin deoxynivalenol (DON) is believed to act via a ribotoxic stress response, the signaling mechanisms remain incompletely charac-
terized. The goal of this study was to (1) verify the role of hematopoietic cell ki-

nase(Hck) in p38 activation and proinflammatory gene expression in vitro and (2) identify downstream kinases involved in DON-mediated Hck activation to p38 in vitro. Peritoneal macrophages elicited from wild-type (WT) and homozygous Hck knockout (KO) mice were compared relative to their capacity to respond to DON. Incubation of WT macrophages with DON (500 ng/ml) induced robust p38 acti-

vation and proinflammatory gene expression, however, these responses were markedly reduced in Hck-KO mice. A similar trend toward reduced proinflammas-

tory gene expression was also observed in spleen and Peyer's patch samples obtained from orally gavaged (5 mg/kg body weight DON) Hck-KO mice as compared to

DON-treated WT mice. When DON-induced Hck signaling pathways leading to p38 activation were investigated in RAW 264.7 macrophages, the Hck inhibitor PP2, was found to inhibit activation of ASK1 and MKK3/6 which are known to be upstream of p38. Immunoprecipitation revealed that phosphorylated MKK3/6 was shown to interact with the Hck within 5 min after DON treatment. Activated Hck was transiently detectable by Western blotting in ribosomal fractions within 5 min, and concurrent p38 interaction observed. MKK6 but not MKK3 was likewise found to transiently interact with the ribosome. These data suggest that rapid sequential activation of Hck-ASK1-MKK6-p38 via the ribosome is a critical signaling pathway for mediating DON-induced ribotoxic stress response in murine macrophages.

2224 NON-COPLANAR PCBs INDUCE APOPTOSIS RELATED GENES AND INHIBIT CELL PROLIFERATION IN NORMAL LIVER EPITHELIAL CELLS BUT PROMOTE FOCI FORMATION IN INITIATED LIVER EPITHELIAL CELLS.


Polychlorinated biphenyl (PCB) mixtures are widespread environmental contami-

nants. The coplanar isomers of these mixtures are ligands for the Ah receptor; the non-coplanar (NC) isomers that are the major components of PCB mixtures are poor ligands for the AhR. Both types of PCB isomers were shown to promote carci-

nogenesis through non-mutagenic pathways. The mechanisms by which the NC-

PCBs promote neoplastic growth are not clear. Since mitogenic signaling pathways play a central role in cell growth and proliferation we examined the activation of ERK signaling pathway in a rat liver epithelial cell line W6 by 2,2’4,4’-tetrachlorobiphenyl (TCB) as a prototype of NC-PCBs. At micromolar concentrations TCB caused activation of ERK. The activation of ERK did not cause proliferation of the liver cells. Instead in cells treated with 20.0 µM TCB cell proliferation was inhibited and apoptotic changes ensued. Flow Cytometric analysis revealed that there was no increase in cells undergoing transition from G1 to S phase of cell cycle. In contrast, treatment with transforming growth factor-β (10.0 ng/ml) that also ac-

tivated ERK there was significant increase in the number of cells undergoing G1 to

S phase transition. Microarray analysis of gene expression profiles of TCB treated

cells showed elevated expression of apoptosis related genes such as Bax in these cells. There was no increase in the number of transformed foci formed in the culture suggest-

ing that TCB exerted tumor promoting effect in the initiated cells. Taken together

to activation of extracellular-signal-regulated protein kinase (ERK), a MAPK re-

lated to cell proliferation and survival. MAPK cascade expression and activation

were measured using microarray and Bioplex technologies, respectively. Our mi-

croarray data suggests APAP treatment up-regulates gene expression at multiple lev-

els of the JNK cascade including a JNK-specific scaffold protein. Expression data was confirmed by Bioplex, which measures MAPK phosphorylation ratios. APAP

treatment led to a significant activation of JNK compared to AMAP. In contrast,

microarray analysis on the AMAP treated dataset shows an up-regulation of ERK
gen expression, whereas showed AMAP treatment led to significant ERK phosphorylation compared to APAP. Cell viability assays confirmed that activ-

ation of JNK by APAP was related to higher cell death, whereas activation of ERK by AMAP may be cytoprotective.
our results suggest that activation of ERK does not necessarily exert proliferative ef-
fect in normal cells but following initiation with a carcinogenic agent NC-PCBs exert non-genotoxic effects to promote the growth of initiated cells (supported by grants from Philip Morris External Research Program and NIH ES-04911).

**2225 Cytotoxicity, cell death and activation of MAP kinases pathways in primary hepatocytes in response to aflatoxin B1 and ochratoxin A. Taken individually or combined.**

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Aflatoxin B1 (AFB1) and Ochratoxin A (OTA) are two common contaminating mycotoxins which can occur jointly in a wide range of food commodities. Both myco-
toxins have several toxic effects but share a significant carcinogenic potential. Considering the concomitant production of AFB1 and OTA, it is very likely that humans are always exposed to the mixture rather than to individual compounds. The aim of the present study was to investigate the effect of AFB1 and OTA taken individually or combined on cytotoxicity, cell death (apoptosis/necrosis) and on mito-
gen-activated protein kinases (MAPK) cascades including ERKs, SAP/JNK and p38 which are major MAP kinases pathways. The study was conducted using pri-
mary rat hepatocytes, recognized as the most appropriate in vitro system which takes into account xenobiotic metabolism and biontransformation. Our results clearly showed that both AFB1 and OTA induced hepatocytes cell death with IC50 about 0.1μM and 1μM, respectively. In addition, examination of the kind of cell death showed that both mycotoxins induced, in a dose dependent manner, apop-
tosis and necrosis. Furthermore, AFB1 and OTA significantly increased phosphory-
lated Raf, MEK 1/2, ERK 1/2 and e-Jun in rat hepatocytes but had a slight effect on p38 MAPK activation. A selective phosphorylation of ERK proteins and of their substrate ELK1/2 and p90RSK was observed. Altogether, our study showed that AFB1 and OTA combination effects are clearly synergistic. The present studies pro-
vided evidence of a direct association between cell survival, cell death and cancer development. The understanding of the role of this signalling cascade could be rel-
evant to explain the molecular basis of liver diseases and tumorigenesis in-
duced by naturally occurring mycotoxins.

**2226 O,P’-DDT induces cyclooxygenase-2 gene expression in murine microphages: role of AP-1 and CRE promoter elements and p33 kinase/Akt/MAPK signaling pathways.**

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Dichlorodiphenyltrichloroethane (DDT) has been used as an insecticide to prevent the devastation of malaria in tropical zones. However, many reports suggest that DDT may act as an endocrine disruptor and may have possible carcinogenic ef-
fects. Cyclooxygenase-2 (COX-2) acts as a link between inflammation and carcino-
genesis through its involvement in tumor promotion. In the present study, we ex-
amined the effect of o,p’-DDT on COX-2 gene expression and analyzed the molecular mechanism of its activity in murine RAW 267.4 macrophages. Exposure to o,p’-DDT markedly enhanced the production of prostaglandin E2 (PGE2), a major COX-2 metabolite, in murine macrophages. Furthermore, o,p’-DDT dose dependently increased the levels of COX-2 protein and mRNA. Transfection with the major COX-2 metabolite, in murine macrophages. Furthermore, o,p’-DDT dose
dependently increased the levels of COX-2 protein and mRNA. Transfection with
the activator protein 1 (AP-1) and cyclic AMP response element (CRE) sites, but DNA-affinity protein-binding assay experiments revealed that o,p’-DDT activated
on p38 MAPK activation. A selective phosphorylation of ERK proteins and of their
substrate ELK1/2 and p90RSK was observed. Altogether, our study showed that
FB1 and OTA combination effects are clearly synergistic. The present study pro-
vided evidence of a direct association between cell survival, cell death and cancer development. The understanding of the role of this signalling cascade could be rel-
phant. The pathological role of HDAC2 is a key regulator of HDAC2 enzyme activity in response to inflammation and lung injury. Thus, in-
hibition of CK2 on HDAC2 may reverse steroid resistance in response to envi-
ronmental stimuli.

**2227 Cigarette smoke regulates HDAC2 via serine phosphorylation in macrophages and lung epithelial cells: implications in steroid resistance.**


Rationale: Loss of HDAC2 is implicated in an impaired response to glucocorti-
coids in patients with chronic obstructive pulmonary disease (COPD). We recently found that HDAC2 is a target of cigarette smoke (CS)-induced serine phosphory-
lation however the mechanism is unknown. We hypothesize that CS-mediated phosphorylation of HDAC2 occurs on specific serine sites through a CK2-medi-
ated mechanism and regulates enzyme activity. Methods: Monoacyl/macrophage (MM6), bronchial epithelial (H292) and primary airway epithelial (SAEC) cells were exposed to cigarette smoke extract (CSE). Mice were exposed to subchronic CS. Serine phosphorylation of HDAC2 from lung tissue and cell extracts was deter-
ted. To inhibit CSE-induced phosphorylation, H292 cells were pretreated with 4,5,6,7 tetrabromobenzotriazole (TBB), a specific CK2 inhibitor. Results: HDAC2 was phosphorylated on serine residues in MM6, H292 and SAEC cells in response to CSE and in lung tissue HDAC2 from mice exposed to CS. Inhibition of phosphorylation, observed with the CK2x inhibitor (TBB), and co-immuno-
precipitation of PCK2x/CK2x with HDAC2 indicated a prominent role for the CK2 x-holoenzyme in CSE-induced HDAC2 phosphorylation.Phosphorylation of an HDAC2 88-amino acid c-terminal deletion mutant indicated that S422, S425, S452, S465 and S471 were not required for HDAC2 phosphorylation. Increased phospho-
rylation of HDAC2 was associated with transient formation of deacetylase co-re-
pressor complex with HDAC1 in response to CSE. H292 phosphorylation had no effect on nucleo-cyttoplasmic shuttling either with CSE or by increasing intra-
cellular phosphorylation with a PP2A phosphatase inhibitor, okadaic acid. Conclusion: CK2-mediated serine phosphorylation of HDAC2 is a key regulator of HDAC2 enzyme activity in response to inflammation and lung injury. Thus, in-
hibition of CK2x on HDAC2 may reverse steroid resistance in response to environ-
mental stimuli.

**2228 Heat shock proteins and the toxicological response.**

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Heat shock proteins (HSPs) are protein chaperones that facilitate protein folding and function. HSPs are induced by heat stress, oxidative stress and multiple classes of
tonics. In order to fully understand HSP, it is important to review the latest research on HSP biology in inflammation, metal toxicity, cardiac physiology, neu-
roderegeneration, and endoplasmic reticulum stress. During inflammation, HSP70 is important in antigen presentation. HSP70 interacts with lipids and proteins and
binds to its own message to change the mRNA stability to modulate inflammatory response. During metal toxicity, signal transduction pathways induced by metals are tightly linked to protective heat shock protein pathways. HSP gene expression can be induced pharmacologically by novel agents currently being tested to induce protection against toxicities. For cancer therapy, HSP90 inhibitors are currently being tested in clinical trials. In cancer cells, inhibition of HSP90 is attractive since HSP90 chaperones multiple proteins including erbB2 and Akt, telomerase, en-
thodelial nitric oxide synthase, channels. hormone receptors like glucocorticoid re-
ceptor, and transcription factors like HIF1α and Akir. It is not yet known whether
HSP90 inhibitors will induce sufficient cancer cell death without inducing toxic
effects. Conversely, HSP90 inhibitors are proposed to reduce neurodegenera-
tion. A, TAKEN INDIVIDUALLY OR COMBINED. IN RESPONSE TO AFLATOXIN B1 AND OCHRATOXIN

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Heat shock proteins (hsp) are intracellular chaperones that are involved in the fold-
ing and assembly of newly synthesized proteins. In conditions of stress, hsp play a key role in the refolding of denatured proteins, in the recovery from the stress, and in protection from subsequent insults. Recently, hsp have also been found in the extracellular medium after an array of insults. Extracellular hsp act as ‘danger’ sig-
als and are capable of activating the immune system. The mechanism involved in hps release is controversial since this protein does not present a consensual secre-
tory signal. Hsp70, which is the major inducible form of hps, has been observed in the plasma membrane of stressed as well as transformed cells, exposing the C-terminal of the molecule to the extracellular environment. Moreover, Hsp70 can inte-
grate into artificial lipid bilayer openings of ion conductance pathways. The lipid
specificity of Hsp70 is almost exclusive for phosphatidyl serine (PS). However, the interaction of Hsp70 with membranes could be enhanced by the presence of cholesterol and sphingolipids, which are components of detergent resistant membranes (DRM) of lipid rafts. In fact, Hsp70 has been detected in DRM fractions isolated from cells. Surface Hsp70 is released into the extracellular environment still associated with membranes, which have been named export cellular vesicles (ECV). The Hsp70 present in the membrane of ECV shares the same characteristics of this protein in the plasma membrane. ECV containing Hsp70 displayed a robust capacity for activating macrophages. We proposed that ECV are part of a natural mechanism for the communication between epithelial and immune cells, which has been coined “the health surveillance system.” Consequently, the release of ECV containing Hsp70 could alter the immune system when an injury occurs and activate the body’s machinery to combat further propagation of the insult.

**2230 HEAT SHOCK PROTEIN 90 EXPRESSION AND FUNCTION IN NEURODENERATION AND CARDIAC DEGENERATION: CLINICAL IMPLICATIONS OF HSP90 INHIBITORS.**

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HSP90 is a chaperone protein for hundred of proteins including erbB2 and Akt, telomerase, endothelial nitric oxide synthase, various channels, hormone receptors such as glucocorticoid receptor, and transcription factors such as HIF1α and AhR. Up-regulation of HSP90 in the heart is observed in diabetes, doxorubicin-induced cardiac toxicity and cyclosporine A-induced cardiac toxicity. It is currently not known if an up-regulation of HSP90 in the heart is protective or whether this up-regulation predisposes the heart to dysfunction. In the brain, HSP90 may play a role in neurodegeneration. As such, HSP90 inhibition may be beneficial and inhibitors are proposed to treat neurodegenerative diseases. HSP90 inhibitors are also currently being tested in clinical trials for cancer therapy. In this setting, HSP90 inhibitors induce the degradation of the client proteins of HSP90 with reduction of cancer cell volume. This presentation will review some of the latest findings relevant to the clinical use of HSP90 inhibitors, potential side-effects or potential benefits. A novel transgenic mouse with cardiac specific HSP90 over-expression will also be discussed.

**2231 UNFOLDED PROTEIN RESPONSE: ROLE OF ENDOPLASMIC RETICULUM AND NUCLEAR SIGNALING PATHWAYS IN TOXICITIES.**

L. Hendershot4, K. J. Gabrielson1, A. De Maio1, and Y. Kang1. Surgery, University of California, San Diego, CA, 1Medicine, University of Louisville, Louisville, KY, 1Molecular and Comparative Pathobiology, Johns Hopkins University, Baltimore, MD and 4Genetics / Tumor Cell Biology, St. Jude’s Childrens Hospital, Memphis, TN.

The endoplasmic reticulum (ER) is the major site of synthesis of secretory and membrane proteins. In order to properly prepare these nascent proteins for an extracellular fate, the ER lumen possesses a unique environment that is specialized for high fidelity protein folding and assembly. It contains high concentrations of molecular chaperones, folding enzymes, and ATP which aid proper maturation of proteins. However, it also possesses an oxidizing environment, which favors intra- and inter-molecular disulfide bond formation, and mM concentrations of Ca2+ that cause unusual complications for folding. Only proteins that fulfill quality control standards are allowed to exit ER and travel further along the secretory pathway toward their final destinations. If the extracellular environment is altered through changes in normal physiology or the presence of toxic compounds, unfolded proteins will accumulate in the ER. This upsets the normal ER homeostasis and induces a signaling pathway called the unfolded protein response (UPR), which serves to alleviate the stress. In cases of prolonged or extreme stress, apoptotic pathways are activated to eliminate the affected cells in order to protect the organism. The initial phase of the UPR is cytoprotective. First, ER chaperones and folding enzymes are upregulated to cope with large numbers of unfolded proteins. Second, protein translation is transiently attenuated to diminish the load of unfolded proteins. And third, the degradative capacity of the cell is enhanced to dispose of unfolded proteins. However, if ER stress is not resolved, the UPR switches gears and guides the cell toward death in order to protect the organism. The balance between cytoprotective and cytodestructive components of the UPR is not well understood and appears to vary greatly by tissue type. In many cases, the cytodestructive part of the UPR is responsible for the pathologies associated with various diseases and toxicities. The components of the UPR that sense ER stress and initiate the response as well as the downstream effectors will be discussed along with the role of the UPR in disease.

**2232 CROSS TALK OF HEAT SHOCK AND HEAVY METAL REGULATORY PATHWAYS.**

Y. Kang4, K. J. Gabrielson1, A. De Maio1 and L. Hendershot4. Surgery, University of California, San Diego, CA, 1Medicine, University of Louisville, Louisville, KY, 1Molecular and Comparative Pathobiology, Johns Hopkins University, Baltimore, MD and 4Genetics/Tumor Cell Biology, St. Jude’s Childrens Hospital, Memphis, TN.

Toxic metal exposure activates several adaptive signaling pathways as well as the expression of heat shock proteins (HSPs) in mammalian system. The cross talk between the adaptive signaling pathways and HSPs constitutes a complementary protective mechanism against metal toxic insult. This cross talk involves regulation by HSPs of adaptive signaling pathways and HSP inhibition of detrimental pathways such as apoptosis. On the other hand, heat shock causes activation of signaling pathways leading to HSPs production, which requires the participation of metals such as zinc in the process. In particular, the final common pathway involved in the expression of HSPs is controlled by heat shock factors (HSFs), the function of which is zinc-dependent. Pharmaceutical agents can stimulate HSPs production, which prepares mammalian cells for protection against subsequent toxic metal exposure. Therefore, the co-existence and cross talk between the metal-induced signaling pathways and the HSPs production make mammalian system well prepared for toxicological exposure, however, disturbance of the cross talk results in diseases.

**2233 BIOMARKERS FOR ASSESSING THE SYSTEMIC INFLAMMATORY RESPONSE SYNDROME IN TOXICOLOGY STUDIES.**

C. Louden, Pathology, Johnson & Johnson, Alderley Park, United Kingdom.

Systemic inflammatory response syndrome (SIRS) is a state of whole body inflammation that can result in multiple organ dysfunction (MOD), circulatory collapse and even death. SIRS is considered as a self-defense mechanism to non-specific insults that arise from chemical, necrotic (as a result of tissue damage), ischemic, or infectious stimuli that induce organ pathology. SIRS and MOD are complex processes that involve hemodynamic, humoral and cellular responses, complement activation and cytokine cascade. SIRS and MOD develops in stages that are mediated in part through acute phase proteins, cytokine dysregulation and hemodynamic events. In toxicology studies, safety evaluation of xenobiotics, pharmacologically active immune stimulants and immunosuppressants can induce SIRS and MOD in rats, dogs and non-human primates and therefore measurement of circulating mediators as biomarkers of SIRS and MOD will enable clinicians to avoid a potentially severe catastrophic event. Furthermore chemically-induced pathology resulting from events such as ischemia, extensive tissue damage and necrosis, activation and release of stress hormones all can induce SIRS and MOD as a secondary toxic response. Whole body SIRS is considered an adverse finding in toxicology studies. Therefore, knowledge and use of the appropriate inflammatory biomarkers that reflects this pathological process is quite useful both preclinically and clinically. Additionally, pre-clinical and clinical monitoring of biomarkers that are early predictors or reporters of SIRS are valuable to the toxicologist in hazard identification and risk assessment of novel therapeutics with the potential to cause a pro-inflammatory response. To adequately explore these ideas, we will highlight the progress and challenges of SIRS.

**2234 CURRENT CONCEPTS AND AN OVERVIEW OF THE PATHO-PHYSIOLOGY OF CYTOKINE STORM, SIRS AND MOD.**

S. D. Burdert1 and C. Louden2. 1Wright State University, Dayton, OH and 2Pathology, Johnson & Johnson, Raritan, NJ.

Systemic inflammatory response syndrome (SIRS) can be caused by ischemia, inflammation, trauma, infection or combined insults. Multi-organ dysfunction syndrome (MODS) is a state of physiologic derangement in organ dysfunction caused by hypoperfusion or hypotension. Independent of etiology SIRS, has the same pathophysiologic properties and many consider the syndrome a self-defense mechanism. Inflammatory cascade is complex and involves humoral and cellular responses, complementation, and cytokines. TNF-α, IL-1, IL-6, and IL-8 are the primary cytokines in the cascade. SIRS is best summarized as a 3-stage process; but, a multi-hit theory involving infectious stimuli that induce organ pathology. SIRS and MOD are complex processes that involve hemodynamic, humoral and cellular responses, complement activation and cytokine cascade. SIRS and MOD develops in stages that are mediated in part through acute phase proteins, cytokine dysregulation and hemodynamic events. In toxicology studies, safety evaluation of xenobiotics, pharmacologically active immune stimulants and immunosuppressants can induce SIRS and MOD in rats, dogs and non-human primates and therefore measurement of circulating mediators as biomarkers of SIRS and MOD will enable clinicians to avoid a potentially severe catastrophic event. Furthermore chemically-induced pathology resulting from events such as ischemia, extensive tissue damage and necrosis, activation and release of stress hormones all can induce SIRS and MOD as a secondary toxic response. Whole body SIRS is considered an adverse finding in toxicology studies. Therefore, knowledge and use of the appropriate inflammatory biomarkers that reflects this pathological process is quite useful both preclinically and clinically. Additionally, pre-clinical and clinical monitoring of biomarkers that are early predictors or reporters of SIRS are valuable to the toxicologist in hazard identification and risk assessment of novel therapeutics with the potential to cause a pro-inflammatory response. To adequately explore these ideas, we will highlight the progress and challenges of SIRS.
either bind the cytokine or block the receptors. The balance of SIRS and CARs determines prognosis after an insult. Evidence suggests, because of CARs, many of the new medications meant to inhibit proinflammatory mediators may lead to deleterious immunosuppression. Studies have been designed to help differentiate causes of SIRS by evaluating acute-phase reactants. C-reactive protein (PCT), interleukin-6 and other acute phase reactants were evaluated. PCT was a consistent useful biomarker in differentiating SIRS from sepsis. Unfortunately, PCT and newer acute-phase reactants are not available commercially and the impact on clinical care is not maximized. However, PCT and many newer acute phase reactants may provide insight into the inflammatory response to either underlying diseases or reactions to experimental medications.

### 2235 Diagnosis of Systemic Inflammatory Response in Preclinical Drug Development

W. Shao and C. Louden, 1Safety Assessment, Merck & Co., West Point, PA and 2Toxicologic Pathology, Johnson & Johnson, Raritan, NJ.

Preclinical drug development can be complicated by the challenge of precisely diagnosing systemic inflammatory response or differentiating between toxicity and exaggerated immunopharmacology. The systemic inflammatory response is an innate systemic inflammatory reaction in response to diverse tissue injury or infection. During the response, the concentrations of some serum proteins called acute phase proteins (APPs) elevate or decrease significantly. The analysis of these acute phase proteins as inflammatory biomarkers has shown great diagnostic values of detecting and monitoring diseases and is thus increasingly used in human and veterinary medicine. However, the application of APPs in preclinical drug development is still underdeveloped. In this presentation, we will discuss our advances in optimizing the diagnosis of systemic inflammatory response in preclinical safety assessment by using these acute phase proteins.

### 2236 In Vitro Approaches to Investigating Cytokine Release Following Potential Agonist Immunostimulation

C. J. Betts, 1M. Jacobsen and C. Louden, 1AstraZeneca Safety Assessment, Macclesfield, United Kingdom and 2Pathology, Johnson & Johnson, Raritan, NJ.

To develop safer and more effective drugs for diseases, development of agonists/agonist pairs that elicit therapeutic responses in preclinical and clinical development, there is a need for accurate prediction of potential cytokine dysregulation, as seen with TGN1412. Adverse reactions to TGN1412 occurred despite appropriate in vivo preclinical testing, raising the question whether pertinent in vivo data was overlooked, the correct end points assessed or that in vivo studies lacked the sensitivity or specificity that could have predicted the clinical response. There is a growing need for in vitro approaches to address cytokine dysregulation. In vitro, the testing of human peripheral blood cells by free, cross-linked or plate bound biologics is one option that could provide answers to the problem of species-specificity. Similarly, stimulation of blood-derived dendritic cells or other innate immune system components may provide information on the action of agonists on bacterial or viral antigen pattern recognition receptors. However, design of such in vitro studies needs to be carefully considered to ensure that conditions closely match those in vivo. Alternatively, ‘cell chips’ are designed to give not only an indication that a perturbation of the immune system occurs in response to a compound, but also elucidate the potential immunological mechanism involved. The induction of cytokine promoter-regulated GFP expression in different functional cell lines is available for the mouse and may be extended to address cytokine dysregulation. In vitro, the testing of human peripheral blood cells by free, cross-linked or plate bound biologics is one option that could provide answers to the problem of species-specificity. Similarly, stimulation of blood-derived dendritic cells or other innate immune system components may provide information on the action of agonists on bacterial or viral antigen pattern recognition receptors. However, design of such in vitro studies needs to be carefully considered to ensure that conditions closely match those in vivo. Alternatively, ‘cell chips’ are designed to give not only an indication that a perturbation of the immune system occurs in response to a compound, but also elucidate the potential immunological mechanism involved. The induction of cytokine promoter-regulated GFP expression in different functional cell lines is available for the mouse and may be extended to address cytokine dysregulation.

### 2237 Monoclonal Antibody Induced Cytokine Release Syndrome: Preclinical Screening

P. J. Busfeld, 1Pathology, Johnson & Johnson, Alderley Park, United Kingdom and 2CenBio, RDG, Radnor, PA.

Monoclonal antibodies (mAb) are widely used in anti-inflammatory and tumor therapy. They are highly efficient in certain diseases, but can cause a variety of adverse effects. Toxicity may result from the expected pharmacological effects of the antibody and from interaction with antigen expressed on tissues other than the intended target. This can result in mild infusion reactions or cytokine release syndrome (CRS). A number of mAbs are associated with CRS. These include: alemtuzumab; LO-CD2a/BI-322; rituximab; CP-870,893; birtumomab and TGN1412. In this talk, the cytokines involved, the mechanisms mediating cytokine release and the role of Fc receptors in CRS associated with mAbs will be reviewed briefly. The poor predictive value of studies in animals, the promise of in vitro screens with human cells and approaches to supporting first in human studies will be the major focus of the presentation.

### 2238 Opportunities and Challenges for Safety Evaluation of Small and Large Molecule High Risk Targets

H. Parmar and C. Louden, 1AstraZeneca Pharmaceuticals, Longborough, Leicestershire, United Kingdom and 2Pathology, Johnson & Johnson, Raritan, NJ.

The human genome readout identified a number of potential drug targets. Target knowledge are rudimentary and speculative. Scientific research is needed to understand biology, efficacy and safety of these targets. In-vitro and in-vivo models not always predict and this is exacerbated when quantitative and qualitative species differences exist. For example, the same receptor may produce opposing responses in two species. This may be due to different homology, polymorphisms and ligand activity on the same receptors. Evaluation of CD-28 (TGN1412T) in non-human primate at doses 500-fold greater than the first human dose did not elevate cytokines TNF, IFN alpha, IL-6 etc. However, massive increase (>2000-fold) in these cytokines caused serious adverse effects after a single dose in volunteers. These findings can impact development of monoclonal antibodies, vaccines etc. The biology of these targets need to be understood, special techniques need to be employed to assess the spectrum of effects anticipated upon human administration. Apart from the traditional toxicology studies, new methodologies need to be developed to model effects in humans that take into account in vitro and in vivo effects in different species. Restricted early Phase 0 clinical studies using micro dosing, microdialysis and other ex-vivo techniques using human tissue samples for exploratory analyses can give a better framework of understanding about how such targets will behave in humans upon first administration. Other PK/PD modeling approaches need to be developed to select first dose in man that takes in account receptor occupancy, MTD, PD effect size, target organ tissue effects and down stream up or down regulation of certain mechanisms. Some of these approaches will be described in this presentation. A holistic model will be described that can be applied to such complex targets in a systemic manner so as to obtain the best quality information before selecting the first doses in man.

### 2239 Workshop Introduction

J. DeWitt: Pharmacology and Toxicology, East Carolina University, Greenville, NC.

Perfluorooalkyl acids (PFAAs) used to manufacture myriad consumer products and are present in the environment, humans and wildlife. PFAAs undergo degradation to a limited number of extremely stable products, including PFOA and PFOS; both are reported to alter immune function. Human immunologic and body burden data from a highly exposed population will provide context for the discussions of rodent data that follow. The animal data will address propose modes of action for PFOA and PFOS, and body burdens associated with altered immune function. This workshop will appeal to a broad range of meeting attendees, including immunotoxicologists, risk assessors, and molecular, and regulatory toxicologists. The immunomodulation of PFOA and PFOS reported in rodent models has increased the level of regulatory concern regarding these chemicals. The doses of PFOA or PFOS administered in rodent studies result in serum concentrations that are 100-200 fold greater or slightly below, respectively, the amounts reported in serum of occupationally exposed humans. The corresponding suppression in antibody responses of the dosed rodents suggests that PFOA or PFOS may be immunomodulatory in highly-exposed humans.

### 2240 Immune Status in a Community Exposed to PFOA: Findings from the C8 Science Panel Study

T. Fletcher, G. Leonard, B. Genser and M. Luster, 1PEHRU, London School of Hygiene and Tropical Medicine, London, United Kingdom, 2BGSTATS, Graz, Austria and 3Luster Assoc., Morgantown, WV.

New findings are presented on the relationship between PFOA and immune biomarkers in a very large cross-sectional study conducted in a community of 69,000 children and adults in the mid-Ohio Valley exposed via drinking water to PFOA from a nearby industrial source. This population has significant
higher exposure than national background: serum analyses of several PFAs found a range of concentrations up to 22000 μg/l of PFOA in serum, with a median of 28.2 (interquartile range 13.4-70.6 μg/l). PFOS was closer to normal values (median 20.2, interquartile range 13.9-29.0 μg/l). The dependence of a number of immune and inflammation biomarkers (including total IgA, IgM, IgG, IgE, ANA and CRP), on PFOA and PFOS is investigated by multivariate regression models with adjustment for potential confounders including age, smoking and BMI.

2241 PFOA-INDUCED IMMUNOMODULATION IN MICE: AN OVERVIEW.
J. DeWitt1 and R. Lucke.1, 2Pharmacology and Toxicology, East Carolina University, Greenville, NC and 1ORDINHEELETT/DTR, U.S. EPA, RTP, NC.
PFOA suppresses T-dependent antibody responses (TDAR) in C57BL/6 mice, at a benchmark dose of 3 mg/kg/d. This presentation will discuss possible modes of PFOA action for TDAR suppression, including corticosterone production and per-oxisome proliferator activated receptor alpha (PPARα) activation. The data indicate that suppression of TDAR is not the product of generalized toxicity as evalu-ated by corticosterone production or clinical blood chemistries and not exclusively dependent on the presence of PPARα.

2242 ADJUVANCY AND IMMUNOSUPPRESSION: MECHANISMS OF IMMUNOMODULATION FOLLOWING DERMAL EXPOSURE TO PFOA IN MICE.
S. Anderson, L. Butterworth, L. G. Jackson, F. Frasch, J. Franko and B. L. Meade. NIOSH, Morgantown, WV.
The majority of investigations into the immunotoxic effects of perfluoroalkyl acids have focused on immunosuppression following the oral route of exposure. The poten-tial for dermal exposure exists not only in the manufacturing of products and re-formulations but also in use of end products such as fire-retardants. Recent studies have demonstrated that as compared to oral dosing, dermal exposure results in qualitatively similar immunosuppressive effects and additionally, that while not al-lergenic itself, dermal exposure to PFOA simultaneously with exposure to a respira- tory allergen augments the IgE response to that allergen. This presentation will dis-cuss the modulation of immune related genes following PFOA exposure, helping to explain the reciprocal relationship between the mechanisms governing immune suppression and augmentation of IgE-mediated hypersensitivity and demonstrating the kinetics of absorption and penetration of PFOA though human and mouse skin. Genetic diversity in these immune responses was also demonstrated between Th1 and Th2 strains of mice.

2243 SUPPRESSION OF IMMUNE FUNCTION IN MICE AFTER DEVELOPMENTAL EXPOSURE TO PFOS.
M. Peden-Adams. Department of Pediatrics and Marine Biomedicine and Environmental Science Center, Medical University of South Carolina, Charleston, SC.
Maternal PFOS exposure caused no significant dose-responsive changes in matern-al or pup body weights, cell phenotypes, or macrophage function in B6C3F1 pups. Suppression of NK cell function and IgM production were documented but were not evident until 8 weeks of age, at lower doses in male than female pups; NOAEL and LOAEL values were 0.1 and 1.0 mg/kg/d (males only) following maternal PFOS exposure, respectively. This study establishes that the developing immune system is sensitive to PFOS and results in functional deficits in innate and humoral immunity detectable at adulthood.

2244 EVALUATION OF THE IMMUNE SYSTEM IN RATS AND MICE ADMINISTERED AMMONIUM PERFLUOROOCTANOATE (APFO).
S. E. Lovelace. DuPont Haskell Global Centers for Health and Environmental Sciences, Newark, DE.
Relatively few studies have examined effects on the immune system after exposure to APFO or PFOA. In earlier studies in rats or monkeys exposed to APFO from one month to two years, nothing remarkable was observed in microscopic examina-tions of spleen, thymus and mesenteric lymph nodes at any dose, including doses that resulted in significant increases in liver weights. More recently, repeated high doses of APFO for about two weeks have been reported to affect immune system function in mice. To examine dose response characteristics in both rats and mice, animals were dosed by oral gavage with 0.3 to 30 mg/kg/day of linear ammonium perfluorooctanoate (APFO) for 29 days following USEPA 870.7800 immunotoxicity guidelines. Anti-sheep red blood cell (SRBC) IgM levels, clinical signs, body and liver weights, spleen and thymus weights and cell number, selected histopathol-ogy, and cytokine concentrations were evaluated (IL1b, TNFα, IFNγ, IL4 and CRP, ANA and ESR). APFO had no effect on the immune system in rats and mice at the NOAEL or dosages (2000 μg/kg/d). This is the first reported study of the long-term effects of APFO exposure on the immune system in rats and mice. The data suggest that APFO does not affect the immune system in rats or mice at doses up to 2000 μg/kg/d.
within protein catalytic triads. As discussed in subsequent presentations, this unified adduct-based mechanism for type-2 alkenes has significant implications for the pathogenesis of human diseases and environmentally acquired toxicities.

**2247 Atherogenic Effects of Enals.**

S. Srinivasan and D. J. Conklin. Cardiovascular Medicine, University of Louisville, Louisville, KY.

Enals are alpha,beta-unsaturated aldehydes that are ubiquitous pollutants in air, water and food and are generated endogenously by lipid peroxidation and during the metabolism of xenobiotics. Acrolein (C-3 alkene) and crotonaldehyde (C-4 alkene) are major constituents of automobile exhaust and cigarette smoke. Moreover, acrolein is generated endogenously by oxidation of lipids, myeloperoxidase activity, and from allylamine and cyclophosphamide metabolism. Hexenal (C-6 alkene) is abundant in fruits and is used as a flavoring agent. Additionally, 4-hydroxynonenal (HNE; C-9 alkene) is the most abundant aldehyde generated during the oxidation of lipids. These enals exert a variety of atherogenic effects including endothelial activation, smooth muscle cell proliferation, foam cell formation and enhanced cytokine production. Moreover, acrolein and HNE modify several proteins in vascular cells in vitro. MALDI-TOF analysis and confocal imaging show that most of these adducts are localized in the endoplasmic reticulum (ER). In addition, enals also cause the induction of ER-chaperon proteins and activate both the alarm and adaptive phases of the unfolded protein response (UPR). Chemical chaperones of protein-folding inhibit enal-induced UPR, inflammatory signaling and endothelial activation, and genetic ablation of the alarm phase gene, activating transcription factor 3 (ATF3), enhances inflammatory signaling. Moreover, enals (e.g., acrolein) exposure in vivo causes dyslipidemia in C57BL/6 mice and exacerbates atherogenic lesion formation in apoE-null mice. This presentation will discuss the molecular mechanisms by which enal-induced ER-stress affects cardiovascular cell signaling and UPR, and how regulation of enal metabolism modulates ER-stress and atherogenesis.

**2248 Overview of Protein Targets Modified by the α,β-Unsaturated Aldehyde 4-Hydroxynonenal: Insights into Molecular Mechanisms Predisposing Proteins to Modification.**

D. R. Peterson. Toxicology, University of Colorado, Denver, CO.

A number of chronic diseases are associated with significant inflammation resulting in oxidative stress, which is a documented stimulus for the peroxidation of cellular lipids. Among the end products of lipid peroxidation is the type-2 alkene, 4-hydroxynonenal (4-HNE) proposed to be involved in both survival and the cytotoxic responses of cells to oxidative stress. Whereas many of the cellular mechanisms involved in the 4-HNE responses are presumed to involve protein modification, identification of the specific nucleophilic amino acid targets of this type-2 alkene is under intense investigation. To date, the primary amino acid residue identified is the soft nucleophile, cysteine. This presentation will provide an overview of these studies with special emphasis on cellular factors, the structural properties of target proteins as well as the cellular consequences associated with protein modifications by type-2 alkenes.

**2249 Type-2 Alkenes Produce Nerve Terminal Damage: Relevance to Neurotoxicity and Neurodegenerative Diseases.**

R. M. LoPachin. Anesthesiology, Albert Einstein College of Medicine, Bronx, NY.

Synaptic dysfunction appears to be an early pathogenic event in Alzheimer’s disease, amyotrophic lateral sclerosis and Parkinson’s disease. Although the molecular mechanism of this synaptotoxicity is not known, evidence suggests that these diseases are characterized by a common pathophysiological cascade involving oxidative stress, lipid peroxidation and the subsequent liberation of α,β-unsaturated aldehydes such as acrolein and 4-hydroxy-2-nonenal (HNE). A diverse body of in vivo and in vitro data have shown that these soft electrophilic chemicals can cause nerve terminal damage by forming Michael-type adducts with nucleophilic sulfhydryl groups on presynaptic proteins. Therefore, the endogenous generation of acrolein and HNE in oxidatively stressed neurons of certain brain regions might be mechanistically related to the synaptotoxicity associated with neurodegenerative conditions. In addition, acrolein and HNE are members of a large class of structurally related chemicals known as the type-2 alkenes. Chemicals in this class (e.g., acrylamide, methylvinyl ketone, methyl acrylate) are pervasive environmental pollutants and new research has shown that these α,β-unsaturated carbonyl derivatives are also toxic to nerve terminals. In this talk, we provide evidence that the regional synaptotoxicity, which develops during the early stages of many neurodegenerative diseases, is mediated by endogenous generation of acrolein and HNE. Based on a presumed common nerve terminal site of action, we propose that the onset and progression of this neuropathogenic process is accelerated by environmental exposure to other type-2 alkenes and/or electrophilic chemicals.

**2250 Using Carbonyl Scavengers to Probe the Toxicological Significance of Acrolein-Mediated Protein Adduction in Lung Cells.**

P. C. Burcham. Pharmacology, University of Western Australia, Nedlands, WA, Australia.

Acrolein and related carbonyl compounds are important toxic by-products of organic matter (e.g., plastics, wood and tobacco) combustion. Due to its strong electrophilic character and the readiness with which it attacks cell proteins, acrolein is a key mediator of the life-threatening damage to lung epithelium that accompanies smoke inhalation injury in fire victims. Since therapeutic options during the management of smoke inhalation injury are limited, our laboratory has explored the use of nucleophilic drugs to block the contribution of carbonyl compounds to smoke toxicity. Testing a range of putative protectants during in vitro experiments using lung-derived cell lines, we have demonstrated that diverse carbonyl scavengers effectively block toxicity elicited by smoke released during polyethylene combustion. However, intriguing differences emerge between individual carbonyl scavengers when the molecular mechanisms underlying their cytoprotective actions are studied. Whereas some scavengers protect key cellular agents such as intermediate filaments against adduction and cross-linking, and attenuate transcriptional responses to acrolein, others have little effect on these end-points. In this presentation, we propose that careful attention to the chemistry underlying protein adduction during acrolein toxicity, together with the use of appropriate nucleophilic reagents will provide helpful new insights into the toxicological events that mediate acutely smoke-poisoned lung.
**Deadline for Proposals for SOT 2010**

**Annual Meeting Sessions:** April 30, 2009

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

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

**2010 Thematic Approach**

The Scientific Program Committee will continue the thematic approach for the 2010 Annual Meeting. All proposal submissions will be reviewed for their relevance under the following themes—Cell Signaling, Gene-Environment Interactions, Metabolic Disease, Mitochondrial Basis of Disease, Toxicity Testing in the 21st Century, and Translational Toxicology for the 2010 meeting. Please note that while we are actively soliciting proposals for the themes listed above, all proposal submissions will be reviewed under the current criteria for their timeliness and relevance to the field of toxicology.

Please refer to the SOT 2009 Program, Scientific Program Overview on the fold-out cover for a list of 2009 sessions highlighted under the thematic approach.

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